Génétique, acides gras oméga-3 et facteurs de risque des maladies cardiovasculaires

Thèse

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Résumé

Les maladies chroniques telles que les maladies cardiovasculaires (MCV) sont complexes et leur risque est modulé par de nombreux facteurs tels que le bagage génétique et l'alimentation. Dans le cadre de ce projet, une cohorte de 210 participants a été recrutée. Deux profils alimentaires ont été identifiés, le profil Prudent caractérisé par une consommation plus élevée de légumes, fruits, produits céréaliers à grains entiers et de gras non-hydrogénés et le profil Western caractérisé par une consommation plus élevée de produits céréaliers raffinés, desserts, sucreries et viandes transformées. Ces deux profils alimentaires modulaient l'expression de gènes impliqués dans le système immunitaire, la réponse inflammatoire, le cancer et/ou les MCV. Le profil alimentaire Western était également associé à un profil de métabolites constitué de concentrations plus élevées de certains acides aminés et d'acylcarnitines à courtes chaînes. Afin d'étudier la variabilité interindividuelle dans la réponse à un nutriment, une supplémentation de 6 semaines en huile de poisson a été réalisée chez les 210 participants. Des SNPs de gènes reliés aux voies de la lipogénèse de novo et la bêta-oxydation des acides gras (*ACLY*, *ACACA*, *GCK*, *RXRA*, *ACOX1*) affectaient seuls, ou en interaction avec la diète, la réponse des triglycérides (TG) plasmatiques suite à la supplémentation en huile de poisson. La variabilité génétique présente dans le gène *sterol regulatory element binding transcription factor 1* (*SREBF1*) expliquait une partie des différences interindividuelles observées dans la réponse des concentrations d'insuline suite à la supplémentation en huile de poisson. Dans une seconde cohorte de 691 individus, des associations entre des SNPs, identifiés à l'aide d'un GWAS réalisé sur la cohorte des 210 individus ayant pris la supplémentation en huile de poisson, et les concentrations de TG et d'acides gras dans les phospholipides plasmatiques ont été observées. Cette thèse comprend un volet d'application des connaissances où l'attitude a été identifiée comme le principal déterminant de l'intention des diététistes de discuter de nutrigénétique avec leurs patients/clients. Globalement, ces résultats démontrent que les profils alimentaires influencent le métabolisme à différents niveaux et que la réponse à l'huile de poisson peut être variable tout dépendamment du bagage génétique et de l'alimentation.

Abstract

Chronic diseases such as cardiovascular diseases (CVD) are complex and their risk factors are regulated by many factors, for example the genetic background and dietary intakes. In this project, 210 participants were recruited. Two dietary factors were identified, the Prudent dietary pattern which was characterised by higher intakes of vegetables, fruits, whole grain products and non-hydrogenated fats and the Western dietary pattern, characterised by higher intakes of refined grain products, desserts, sweets and processed meats. Both dietary patterns modulated the expression of genes related to the immune system, inflammatory response, cancer and/or CVD. The Western dietary pattern was also associated with a metabolite profile which comprised greater concentrations of certain amino acids as well as small chain acylcartinines. To examine the interindividual variability in the response to a nutrient, a 6 week fish oil supplementation was conducted among the 210 participants. SNPs related to genes involved in de novo lipogenesis and fatty acid beta-oxidation (*ACLY*, *ACACA*, *GCK*, *RXRA*, *ACOX1*) were associated alone or in an interaction effect with dietary intakes with the plasma triglyceride (TG) response to the fish oil supplementation. The genetic variability within *sterol regulatory element binding transcription factor 1* (*SREBF1*) gene was associated with differences in the response of insulin concentrations following fish oil supplementation. In a second cohort of 691 participants, associations between SNPs, identified in a previous GWAS conducted among the 210 participants supplemented with fish oil, and TG as well as plasma phospholipid fatty acid concentrations were observed. This thesis also comprises a knowledge transfer section where the attitude was identified as the main determinant of the intention of dietitians to discuss nutrigenetics with their patients/clients. Globally, these results demonstrate that dietary patterns modulate the metabolism at several levels and that the response to fish oil is variable depending upon genetic profile and dietary intakes.

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Liste des abréviations

AA : *amino acid*
ABCD2 : *ATP-binding cassette, sub-family D (ALD)*
AC : *acylcarnitine*
ACAA1 : *acetyl-CoA acyltransferase 1*
ACAA2 : *acetyl-CoA acyltransferase 2*
ACACA : *acetyl-CoA carboxylase alpha*
ACADVL : *acyl-coa dehydrogenase very long chain*
ACLY : *ATP citrate lyase*
ACOX1 : *acyl-CoA oxidase 1, palmitoyl*
ACP : analyse en composante principale
ADH : acide docosahexaénoïque
ADP : acide docosapentaénoïque
AEP : acide eicosapentaénoïque
AGPI : acide gras polyinsaturé
AHA : *American Heart Association*
ANOVA : *analyses of variance*
apoB : *apolipoprotein B*
APOC3 : *apolipoprotein C3*
APOE : *apolipoprotéine E*
BCAA : *branched-chain amino acid*
BCKD : *branched-chain α-ketoacid dehydrogenase*
BMI : *body mass index*
C : *cholesterol* (cholestérol)
C3 : propionylcarnitine
C3 : *complement C3*
C5 : isovaleryl carnitine
CD36 : *CD36 molecule (thrombospondin receptor)*
CD68 : *CD68 molecule*
CHD : *coronary heart disease*
CHO : carbohydrate
ChREBP : *carbohydrate responsive element-binding protein*
CPT : *Cell Preparation Tube*
CPT1A : *carnitine palmitoyltransferase 1A*
CPT1B : *carnitine palmitoyltransferase 1B*
cRNA : *complimentary RNA*
CRP : *C-reactive protein* (protéine C-réactive)
CVD : *cardiovascular disease*
CXCL8 : *chemokine (C-X-C motif) ligand 8*
DASH : *Dietary Approach to Stop Hypertension*
DBP : *diastolic blood pressure*
DHA : *docosahexaenoic acid*
DGAT1 : *diacylglycerol O-acyltransferase 1*
DTC : *direct-to-consumer* (disponible directement au consommateur)
EPA : *eicosapentaenoic acid*
ERK : *extracellular-signal-regulated kinase*

FA : *fatty acid*
FAS : *Genes, Omega-3 Fatty Acids and Cardiovascular Disease Risk Factors*
FASN : *fatty acid synthase*
FDR : *false discovery rate*
FFQ : *food frequency questionnaire*
FOXO1 : *forkhead box O1*
FOXO3 : *forkhead box O3*
GCK : *glucokinase*
GISSI : *Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico*
GLM : *General Linear Model*
GPAM : *glycerol-3-phosphate acyltransferase, mitochondrial*
GOLDN : *Genetics of Lipid Lowering Drugs and Diet Network*
GWAS *genome-wide association study*
HCTZ : *hydrochlorothiazide*
HDL : *high-density lipoprotein* (lipoprotéine de haute densité)
HOMA : *homeostasis model assessment*
HNF4A : *hepatocyte nuclear factor 4 alpha*
IDL : lipoprotéine de densité intermédiaire
IL6 : *interleukin 6*
IPA : *Ingenuity Pathway Analysis*
IMC : indice de masse corporelle
INAF : Institut sur la nutrition et les aliments fonctionnels
INSIG1 : *insulin induced gene 1*
IQCJ-SCHIP1 : *IQ Motif Containing J-Schwannomin Interacting Protein 1 Fusion Protein*
IR : *insulin resistance*
IRS2 : *insulin receptor substrate 2*
IS : *insulin sensitivity*
JADE1 : *Jade family PHD finger 1*
JELIS : *Prevenzione*, *Japan EPA Lipid Intervention Study*
LD : *linkage disequilibrium*
LDL : *low-density lipoprotein* (lipoprotéine de basse densité)
LPL : lipoprotéine lipase
LXR : *liver X receptor*
MAF : *minor allele frequency*
MCV : maladie cardiovasculaire
miARN : micro ARN
MMP9 : *matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)*
MSC : *mesenchymal stem cell*
MLXIPL : *MLX interacting protein-like*
MYB : *V-myb avian myeloblastosis viral oncogene homolog*
n-3 : oméga-3
n-6 : oméga-6
NCBI : *National Center for Biotechnology Information*
NCC : *Nutrition Coordinating Center*
NELL1 : *NEL-like 1 (chicken)*
NF2 : *neurofibromatosis type 2*

NFAT : *Nuclear Factor of Activated T cells*
NFKB : *nuclear factor kappa B*
NR1H4 : *nuclear receptor subfamily 1, group H, member 4*
NXPH1 : *Neurexophilin 1*
OGTT : test oral de tolérance au glucose
OMS : Organisation mondiale de la Santé
OPDQ : Ordre professionnel des diététistes du Québec
PA : *phosphatidic acid*
PBMCs : *peripheral blood mononuclear cells* (cellules mononuclées périphériques sanguines)
PCA : *principal component analysis*
PDK4 : *pyruvate dehydrogenase kinase*
PKC : *protein kinase C*
PKU : *phenylketonuria*
PL : *plasma lipid*
PPARA : *peroxisome proliferator-activated receptor alpha*
PPARD : *peroxisome proliferator-activated receptor delta*
PPARG : *peroxisome proliferator-activated receptor gamma*
PPRE : *peroxisome proliferator response element*
PREDIMED : *Prevencion Con Dieta Mediterranea*
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)
QUICKI : *quantitative insulin sensitivity check index*
RD : *registered dietitian*
RIN : *RNA integrity number*
RXRA : *retinoid X receptor alpha*
S1P : *site-1 protease*
SAM : *Significant Analysis of Microarray*
SAS : *Statistical Analysis Software*
SBP : *systolic blood pressure*
SCAP : *SREBP cleavage-activating protein*
SCD : *stearoyl-CoA desaturase*
SD : *standard deviation*
SERPINF1: *serpin peptidase inhibitor, clade F*
SFA : *saturated fatty acid*
SLIT2 : *Slit homolog 2 (Drosophila)*
SNP : *single-nucleotide polymorphism* (polymorphisme nucléotidique simple)
SPARC : *secreted protein, acidic, cysteine-rich*
SRE : *sterol regulatory element*
SREBF1 : *sterol regulatory element binding transcription factor 1*
SREBP1 : *sterol regulatory element-binding protein 1*
TC : *total cholesterol*
TCP : Théorie du comportement planifié
TG : *triglyceride* (triglycéride)
TLRs : *Toll-like receptors*

TNFA : *tumor necrosis factor alpha*
TPB : *Theory of planned behavior*
tSNP : *tag SNP*
UCP2 : *uncoupling protein-2*
UTR : *untranslated region*
VLDL : *very-low-density lipoprotein* (lipoprotéine de très basse densité)

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Avant-propos

Plusieurs personnes ont contribué aux travaux présentés dans cette thèse. D'abord, ma directrice de recherche **Dr Marie-Claude Vohl**, professeur au Département des sciences des aliments et de nutrition 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, à l'approbation et à la révision de chacun des articles. Le **Dr Simone Lemieux**, ma co-directrice de recherche est co-auteure des travaux présentés aux chapitres 3, 4, 5, 6, 7 et 8 de cette thèse. Celle-ci a contribué à la planification du projet de recherche dans lequel s'insère ce projet de doctorat (cohorte FAS (*Genes, Omega-3 Fatty Acids and Cardiovascular Disease Risk Factors*)). Cette dernière détient une expertise en regard du métabolisme des lipides et des lipoprotéines ce qui lui a également permis de réviser et de commenter ces travaux. **Dr Ann-Marie Paradis** a contribué à l'article présenté au chapitre 3, de par son expertise en regard de l'analyse des profils alimentaires et par son importante contribution à la révision de l'article. Le Dr Ann-Marie Paradis a également contribué à la coordination du volet clinique de l'étude FAS. **Dr Iwona Rudkowska** a contribué à la planification du projet de recherche dans lequel s'inscrivait ce projet de doctorat (cohorte FAS), cette dernière a participé à la révision de même qu'à certaines analyses statistiques pour les articles présentés aux chapitres 3, 4, 5, 6, 7 et 8. Le **Dr Patrick Couture** détient une expertise relative au diabète et à l'alimentation de même que les maladies cardiovasculaires, ce dernier a participé à l'élaboration et à la révision des travaux présentés aux chapitres 3, 4, 5, 6, 7 et 8. Le **Dr Frédéric Guénard** détient une expertise en lien avec les études d'associations sur le génome entier (GWAS) qui a rendu possible les travaux décrits au chapitre 9. Ce dernier a également contribué à réviser les travaux décrit au chapitre 9. Le **Dr Louis Pérusse** a contribué à la planification du projet dans lequel s'inscrit le chapitre 9 et a participé à la révision de l'article qui y est présenté. L'expertise du Dr Louis Pérusse en alimentation et génétique a grandement contribué à la qualité de ces travaux. Le **Dr Sophie Desroches** a été une coauteure essentielle pour les travaux décrits au chapitre 10. Cette dernière a contribué à toutes les étapes de ce projet, de la planification, à la rédaction du projet et des questionnaires jusqu'à l'analyse des données et

la révision de l'article. Finalement, le **Dr Julie Robitaille** a contribué aux travaux présentés au chapitre 10. Cette dernière ayant une expertise en nutrigénomique et santé publique a participé à l'élaboration et à la révision de l'article scientifique.

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 5, 6, 7, et 8), de la conception des questionnaires (chapitre 10), 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 la première auteure 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 ci-bas.

Chapitre 3

Bouchard-Mercier A, Paradis AM, Rudkowska I, Lemieux S, Couture P, Vohl MC. Associations between dietary patterns and gene expression profiles of healthy men and women: a cross-sectional study. Nutr J 2013;12:24.

Chapitre 4

Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC. The metabolic signature associated with the Western dietary pattern: a cross-sectional study. Nutr J 2013;12:158.

Chapitre 5

Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC. Polymorphisms, de novo lipogenesis, and plasma triglyceride response following fish oil supplementation. J Lipid Res 2013;54:2866-73.

Chapitre 6

Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC. Polymorphisms in Genes Involved in Fatty Acid beta-Oxidation Interact with Dietary Fat Intakes to Modulate the Plasma TG Response to a Fish Oil Supplementation. Nutrients 2014;6:1145-63.

Chapitre 7

Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC. An interaction effect between glucokinase gene variation and carbohydrate intakes modulates the plasma triglyceride response to a fish oil supplementation. Genes Nutr 2014;9:395.

Chapitre 8

Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Pérusse L, Vohl MC. SREBF1 gene variations modulate insulin sensitivity in response to a fish oil supplementation. Lipids Health Dis 2014;13:152.

Chapitre 9

Bouchard-Mercier A, Guénard F, Pérusse L, Vohl MC. Polymorphisms, gene-diet interaction effects on plasma triglyceride and plasma phospholipid fatty acid concentrations : a cross-sectional study. Soumis à Journal of Lipid Research.

Chapitre 10

Bouchard-Mercier A, Desroches S, Robitaille J, Vohl MC. Do registered dietitians intend to or actually discuss nutrigenetics with their patients/clients? An application of the Theory of planned behavior. Soumis à Journal of Nutrition Education and Behavior.

Introduction générale

Selon l'Organisation mondiale de la santé (OMS), les maladies chroniques (maladies cardiovasculaires (MCV), cancers, diabètes et maladies pulmonaires) étaient responsables en 2008, de 63% des décès à travers le monde [1]. L'OMS estimait en 2008 que 17,3 millions de personnes à travers le monde étaient décédées d'une MCV tandis que 8,2 millions de personnes à travers le monde étaient décédées d'un cancer en 2012 [2-4]. En 2030, il est prédit que 23,3 millions de personnes décéderont de MCV et que ce type de maladie demeurerait la plus importante cause de mortalité à travers le monde [3,5,6]. Au Canada, la mortalité attribuable aux MCV a diminué depuis quelques années. Toutefois, les MCV représentaient toujours 27% en 2009 de toutes les causes de décès ce qui les plaçait au deuxième rang des causes de mortalité chez les adultes canadiens après le cancer [7]. Le cancer quand à lui représentait 30% de l'ensemble des décès [8]. Étant donné l'ampleur de la prévalence de ces maladies, il est primordial d'améliorer notre compréhension des mécanismes reliés au développement de ces maladies afin de développer des interventions efficaces.

Tout au long de la vie, plusieurs facteurs peuvent moduler le risque de maladies chroniques d'une personne. Les facteurs génétiques, les comportements liés à la santé et l'environnement interagissent ensemble et influencent le risque d'une personne [9]. Certains facteurs de risque sont modifiables tels que le tabagisme, le niveau d'activité physique, la consommation de légumes et de fruits, le surplus de poids ou l'obésité, le stress, l'hypertension artérielle et le diabète de type 2 tandis que d'autres sont non-modifiables tels que l'âge, le sexe, les antécédents familiaux, l'origine ethnique et les antécédents d'incidents cardiovasculaires [9]. La *World Heart Federation* ajoute également le fait de consommer une diète riche en gras saturés comme facteur de risque modifiable [10]. D'ailleurs, il est estimé qu'une alimentation riche en gras saturés serait responsable de 31% des maladies coronariennes et des infarctus du myocarde [10].

L'alimentation a un impact primordial sur le risque de développer une maladie chronique telle une MCV. L'alimentation d'un individu est constituée d'une combinaison complexe de nutriments [11]. L'étude des profils alimentaires permet de tenir compte des effets d'interaction présents entre les différents aliments et nutriments composants l'alimentation d'une personne. Les profils alimentaires sont associés entre autres à l'obésité, aux concentrations de lipides plasmatiques et au cancer [12-18]. Toutefois, l'étude des profils alimentaires permet difficilement de comprendre les mécanismes physiologiques exacts impliqués dans l'effet d'un certain profil alimentaire sur le risque de maladies chroniques étant donné la complexité des interactions entre les nutriments. Une approche systémique par le biais de l'étude par exemple des profils d'expression et des profils de métabolites pourrait potentiellement aider à mieux comprendre les voies métaboliques régulées par les profils alimentaires. La science nommée la transcriptomique est l'étude des molécules d'ARN produites par une cellule ou une population de cellules d'un type particulier qui permet l'étude des profils d'expression génique. L'alimentation peut influencer les concentrations d'ARN par différents mécanismes. Par exemple, certains nutriments sont des ligands naturels de facteur de transcription qui affecteront ensuite l'expression de plusieurs gènes. Aussi, l'alimentation peut avoir un effet sur les niveaux de méthylation qui à leur tour moduleront l'expression des gènes [19]. Une autre science, la métabolomique étudie les métabolites qui sont des composés organiques intermédiaires entre deux réactions ou issus du métabolisme. L'alimentation affecte les profils de métabolites selon différents mécanismes, d'abord elle peut directement contribuer à la présence de certains métabolites, par exemple, les consommateurs de citrons ont des concentrations de stachydrine plus élevées ou encore les consommateurs d'arachides ont des concentrations plus élevées de bétaïne tryptophane [20]. Ensuite, le type d'alimentation peut également affecter le métabolisme, par exemple, via la modification de l'expression génique, et ainsi modifier le profil de métabolites d'un individu [21].

Malgré que l'étude de l'effet des profils alimentaires est essentielle, il est également intéressant d'évaluer l'effet des aliments ou des nutriments de façon isolée afin d'améliorer notre compréhension des mécanismes physiologiques sous-jacents. Un des nutriments les plus étudiés et dont les effets ne sont pas encore entièrement élucidés, est l'acide gras

polyinsaturé (AGPI) oméga-3 (n-3) d'origine marine. Dans les études précédentes de notre groupe de recherche, il a été observé que la présence du polymorphisme nucléotidique rendue la simple (SNP) L162V du gène *peroxisome proliferator activated receptor alpha* (*PPARA*) peut affecter la réponse à une supplémentation en huile de poisson [22]. Cette variabilité dans la réponse à une supplémentation en huile de poisson a également été observée dans une cohorte finlandaise constituée de 312 participants [23]. Dans cette étude, les auteurs ont observé que 31% des participants n'avaient pas diminué leurs concentrations de triglycérides plasmatiques (TG) suite à la prise d'huile de poisson durant 8 semaines [23,24]. Les AGPI n-3 sont des ligands naturels de différents facteurs de transcription, par exemple PPARA. De plus, ils affectent également l'expression du gène *sterol regulatory element binding transcription factor 1* (*SREBF1*) [25-27]. Grâce à ces effets, les AGPI n-3 contenus dans l'huile de poisson constituent un modèle intéressant afin de mieux comprendre les effets d'interaction gène-diète sur les facteurs de risque des MCV, principalement dû à leur effet hypotriglycéridémiant qui est d'ordre pharmacologique. D'ailleurs, la prise d'un supplément de 2 à 4g d'AGPI n-3 (acide eicosapentaénoïque (AEP) et acide docosahexaénoïque (ADH)) est recommandée par l'*American Heart Association* (AHA) chez les individus hypertriglycéridémiques [28]. Conséquemment, il est essentiel de pouvoir cibler adéquatement les individus qui abaisseront réellement leurs concentrations de TG suite à la prise d'AGPI n-3 (AEP + ADH).

Le dernier volet de cette thèse porte sur l'application des connaissances en lien avec la nutrigénétique chez les diététistes membre de l'Ordre professionnel des diététistes du Québec (OPDQ). Au Canada, il y a quelques tests de nutrigénétique disponibles directement aux consommateurs. Il existe également un test qui est distribué via un diététiste et qui offre différentes recommandations nutritionnelles en fonction du profil génétique suite au génotypage de certains SNPs. Malgré que ces tests soit déjà disponibles, il a été observé que les diététistes de même que les autres professionnels de la santé ne sont pas prêts à les utiliser avec leurs patients/clients. Le niveau de connaissances est relativement faible en ce qui a trait à la nutrigénomique chez les professionnels de la santé dont les diététistes [29-32]. Conséquemment, afin de former adéquatement les diététistes, des formations et des outils cliniques devront être créés et délivrés. L'utilisation d'un cadre

théorique pourrait permettre la détermination des éléments clés d'une formation ou encore d'un outil clinique utile pour les diététistes. La Théorie du comportement planifiée (TCP) a été employée dans de nombreuses études afin d'étudier les déterminants de l'intention ou d'un comportement chez les professionnels de la santé [33].

Cette thèse se distingue par la présentation de trois cohortes. La première où 210 participants ont suivi un protocole de supplémentation en huile de poisson durant six semaines (cohorte FAS). Celle-ci étudiait principalement la variabilité interindividuelle dans la réponse des concentrations de TG suite à la supplémentation en huile de poisson. Pour cette cohorte, plusieurs données ont été mesurées, entre autres, des données de transcriptomique et de métabolomique. La deuxième étude était transversale avec un nombre considérable de participants soit 691 (cohorte INFOGENE). Dans cette étude, la mesure des acides gras dans les phospholipides du plasma et dans les érythrocytes était disponible. Finalement, la troisième étude est une cohorte de 141 diététistes ayant complété un questionnaire composé de 47 questions basées sur la TCP évaluant les déterminants de l'intention et du comportement pour le diététiste de discuter de nutrigénétique avec ses patients/clients.

Cette thèse se subdivise en onze chapitres. Le premier chapitre, chapitre 1, présente une revue de littérature portant sur les profils alimentaires et leurs liens avec le risque de MCV et de cancer, l'expression génique de même qu'avec les profils de métabolites. Ce premier chapitre traite également des AGPI n-3 (AEP+ADH) ou de l'huile de poisson et leur effet sur le risque de MCV, ses facteurs de risque et la présence d'une variabilité interindividuelle dans la réponse suite à une supplémentation en huile de poisson. Le chapitre 1 se termine par une revue de littérature portant sur l'application des connaissances, plus spécifiquement dans le domaine de la nutrigénomique suivie de l'utilisation de la TCP chez les professionnels de la santé. Le chapitre 2 présente les objectifs et les hypothèses de la présente thèse. Le chapitre 3 se constitue d'un article scientifique s'intitulant «*Associations between dietary patterns and gene expression profiles of healthy men and women : a cross-sectional study*» qui a été publié dans la revue *Nutrition Journal* en 2013. Le chapitre 4 se constitue d'un article scientifique s'intitulant

«*The metabolic signature associated with the Western dietary pattern : a cross-sectional study*» qui a été publié dans la revue *Nutrition Journal* en 2013. Le chapitre 5 se constitue d'un article scientifique s'intitulant «*Polymorphisms, de novo lipogenesis, and plasma triglyceride response following fish oil supplementation*» qui a été publié dans la revue *Journal of Lipid Research* en 2013. Le chapitre 6 se constitue d'un article scientifique s'intitulant «*Polymorphisms in genes involved in fatty acid β-oxidation interact with dietary fat intakes to modulate the plasma TG response to a fish oil supplementation*» qui a été publié dans la revue *Nutrients* en 2014. Le chapitre 7 se constitue d'un article scientifique s'intitulant «*An interaction effect between glucokinase gene variation and carbohydrate intakes modulates the plasma triglyceride response to a fish oil supplementation*» qui a été publié dans la revue *Genes and Nutrition* en 2014. Le chapitre 8 se constitue d'un article scientifique s'intitulant «*SREBF1 gene variations modulate insulin sensitivity in response to a fish oil supplementation*» qui a été publié dans la revue *Lipids in Health and Disease* en 2014. Le chapitre 9 se constitue d'un article scientifique s'intitulant «*Polymorphisms, gene-diet interaction effects on plasma triglyceride and plasma phospholipid fatty acid concentrations : a cross-sectional study*» qui a été soumis à la revue *Journal of Lipid Research*. Le chapitre 10 se constitue d'un article scientifique s'intitulant «*Do registered dietitians intend to or actually discuss nutrigenetics with their patients/clients? An application of the Theory of planned behavior*» qui a été soumis à la revue *Journal of Nutrition Education and Behavior* en 2014. Finalement, le chapitre 11 conclut cette thèse en intégrant les résultats présentés aux chapitres 3 à 10 et en présentant certaines pistes à explorer afin de poursuivre ces recherches dans le futur.

Chapitre 1 :
Revue de la littérature

1.1 Profils alimentaires

Les approches plus traditionnelles observent l'effet sur la santé d'un seul nutriment ou de quelques nutriments [34]. Bien qu'utile, ce type d'approche n'inclut pas les effets d'interaction ou synergiques entre les nutriments. Dans un contexte de «vraie vie», les individus consomment des combinaisons complexes d'aliments que l'on peut regrouper en profils alimentaires. Par exemple, une consommation élevée en fibres alimentaires est souvent associée à des apports plus élevés en légumes et en fruits ou encore des apports plus faibles en lipides totaux sont associés à une consommation plus élevée de légumes, de fruits, de fibres alimentaires, d'acide folique et de produits céréaliers à grains entiers [35,36]. De plus, lors de l'étude de maladies complexes telles que les MCV ou le cancer, il est fort probable que l'interaction de plusieurs nutriments module le risque à long terme de développer ces maladies [37]. L'étude des profils alimentaires s'avère pertinente dans ce contexte puisque ces derniers sont élaborés afin de tenir compte des combinaisons d'aliments qui sont consommées ensembles et de la colinéarité existant entre les nutriments [38,39]. Finalement, il est possible que l'effet de nutriments isolés peut être trop faible pour être observé tandis que l'effet d'un profil alimentaire pourrait être plus facilement détectable [40]. La section 1.1 présente diverses méthodes afin de déterminer des profils alimentaires.

1.1.1 La méthodologie pour dériver les profils alimentaires

Différentes méthodes permettent de créer des profils alimentaires [38]. Il existe trois principaux types d'approches soient l'approche théorique où des scores sont définis par le chercheur, l'approche empirique qui est basée sur l'information issue des données nutritionnelles et finalement, une approche qui combine les deux premières tenant compte des voies biologiques et de l'information issue des données nutritionnelles [41]. Ces trois types d'approche sont décrits plus en détails dans les prochaines sections.

L'approche théorique

Brièvement, l'approche théorique est basée sur l'établissement à priori d'index de qualité de l'alimentation [38]. Le *Healthy Eating Index* [42] en est un exemple. Ces index de qualité de l'alimentation sont basés sur les connaissances actuelles, sur les lignes directrices établies et sur la théorie [38]. Les apports alimentaires sont préalablement évalués à l'aide d'un outil d'évaluation alimentaire. Les individus reçoivent ensuite un «score» qui indique leur degré d'adhésion à l'index de qualité de l'alimentation.

L'approche empirique

La deuxième approche est l'approche empirique. Cette méthode permet d'identifier des profils alimentaires à postériori grâce à des analyses statistiques réalisées sur les données nutritionnelles récoltées. Conséquemment, cette approche ne dépend pas de la vision de la saine alimentation des auteurs. Deux méthodes sont fréquemment utilisées en épidémiologie nutritionnelle, l'analyse par groupe et l'analyse factorielle. Les prochaines sections décriront brièvement ces deux méthodes avec une attention particulière à l'analyse en composantes principales, un type d'analyse factorielle, qui est la méthode employée pour les études présentées dans cette thèse.

L'analyse par groupe

L'analyse par groupe est une méthode fréquemment utilisée lors de l'analyse de données nutritionnelles [38]. Cette méthode permet de déterminer les groupes d'individus d'une population donnée pour lesquels plusieurs composantes de l'alimentation sont similaires. D'abord, les apports alimentaires sont évalués à l'aide d'un questionnaire alimentaire tel qu'un questionnaire de fréquence alimentaire (QFA) ou un journal alimentaire. Les aliments consommés par chacun des individus sont classifiés dans des groupes préalablement identifiés par les auteurs du projet de recherche. Ces groupes peuvent être déterminés en fonction de la valeur nutritive des aliments ou encore des groupes alimentaires auxquels ils appartiennent. Finalement, les groupes d'individus identifiés statistiquement (par exemple selon les méthodes de *K-Means* ou *Ward*) sont nommés en fonction des groupes d'aliments qui sont les plus consommés chez ces derniers. Par

exemple, le groupe nommé «*Healthy*» ou «*Healthier*», relatif à une saine alimentation est fréquemment retrouvé dans la littérature [38].

L'analyse factorielle

L'analyse factorielle est la méthode la plus utilisée afin de générer des profils alimentaires [38,43]. Celle-ci permet de déterminer les aliments ou les groupes d'aliments qui sont corrélés entre eux et donne ensuite un score à chaque individu pour chacun des profils alimentaires identifiés [44]. Selon l'analyse de Newby et collaborateurs [38] portant sur les méthodes utilisées afin d'évaluer les apports alimentaires, les QFA et les journaux alimentaires étaient les deux méthodes les plus fréquemment employées. Les apports alimentaires sont parfois mesurés selon la fréquence de consommation, le poids ou le pourcentage d'énergie quotidien. Ensuite, il est possible de réaliser l'analyse en composantes principales (ACP) en utilisant les macronutriments et/ou les micronutriments, les aliments ou encore des groupes d'aliments générés par les auteurs. Comme pour l'analyse par groupe, les groupes d'aliments peuvent être créés en fonction de la valeur nutritive ou encore des différents usages culinaires. Afin de dériver les profils alimentaires, l'analyse factorielle est fréquemment réalisée avec l'ACP suivie d'une rotation orthogonale en ne conservant que les profils alimentaires répondant à certains critères. Les critères employés sont généralement, le test d'accumulation de variance (*scree test*) et les valeurs propres >1 à >1,25 [45]. L'ACP consiste à compresser les données en moyennes pondérées d'un plus petit nombre de profils (ou facteurs) qui sont générés selon la matrice de corrélation des variables originales. Dans les études, le nombre de facteurs (profils alimentaires) varient, allant de 2 à 25 facteurs différents [38]. Ensuite, les noms des profils alimentaires sont attribués en fonction des macronutriments et/ou micronutriments, des aliments ou des groupes d'aliments s'y retrouvant [38]. Les profils alimentaires Prudent et Western sont des profils retrouvés très fréquemment dans la littérature [38,43,45].

L'approche hybride : combinaison des approches théoriques et empiriques

Les deux prochaines méthodes décrites sont des méthodes hybrides qui sont les plus récentes dans le domaine de la détermination de profils alimentaires [41,46,47].

Reduced rank regression

Le *reduced rank regression* est l'approche hybride la plus fréquemment utilisée. Cette méthode, permet de déterminer les combinaisons linéaires d'aliments expliquant le plus de variance dans les apports en nutriments ou des marqueurs de maladies désignées [41]. Les variables prédites doivent être continues [41]. Pour que cette approche soit la plus performante possible, Kroke [48] décrit deux composantes essentielles, premièrement, le mécanisme biologique reliant des nutriments ou des profils alimentaires à une maladie spécifique doit être clair; deuxièmement, des données précises sur la composition nutritionnelle de l'aliment ou du profil alimentaire doivent être disponibles. Kroke [48] mentionne également que sans ces deux conditions, la méthodes du *reduced rank regression* n'est pas plus utile qu'un profil alimentaire défini à priori, c'est-à-dire théoriquement.

L'analyse treelet transform

L'analyse *treelet transform* est le deuxième type d'approche hybride utilisé afin de générer des profils alimentaires. Un des désavantages mentionné dans la littérature de l'utilisation de l'analyse factorielle pour générer des profils alimentaires est la difficulté de son interprétation [46]. C'est pourquoi l'analyse *treelet transform* a été proposée par Lee et collaborateurs [49]. Ce type d'analyse permet de combiner les capacités d'extraction de l'ACP et l'interprétation plus facile de l'analyse par groupe. La principale différence avec l'ACP est que les facteurs générés par l'analyse *treelet transform* ne sont constitués que d'un nombre restreint de variables et que les autres variables sont exclues du facteur en leur allouant un poids de 0 [46]. Une autre différence est que l'analyse *treelet transform* produit une structure de regroupement hiérarchique qui est visualisable sous la forme d'un arbre de regroupements [46]. Très récemment, Schoenaker et collaborateurs [50] ont comparé l'analyse factorielle et l'analyse *treelet transform* afin de dériver des profils alimentaires et

d'étudier les associations avec le diabète. Ils ont observé que seul le profil alimentaire Western dérivé de l'analyse factorielle était associé avec l'incidence de diabète de type 2 [50]. Ces auteurs mentionnent que l'analyse *treelet transform* produit des profils alimentaires éparses qui ne tiennent pas comptent de tous les aliments ou groupes d'aliments consommés par un individu (en raison du poids de 0 alloué à certains aliments) ce qui pourrait expliquer l'absence de relation avec l'incidence de diabète de type 2 dans leur étude [50].

1.1.2 Profils alimentaires et facteurs de risque des maladies cardiovasculaires et cancers

L'objectif de cette section est de présenter une revue de la littérature non-exhaustive des plus récentes études, principalement les revues systématiques de la littérature (2010 à aujourd'hui) portant sur les profils alimentaires, les MCV et le cancer ou encore sur leurs facteurs de risque chez les adultes.

Profils alimentaires et maladies cardiovasculaires

Deux profils alimentaires ressortent fréquemment dans les études dérivant les profils alimentaires par analyse factorielle, le profil Prudent/*Healthy* caractérisé par une consommation plus élevée de légumes, fruits, produits céréaliers à grains entiers, légumineuses et poissons et le profil Western/*Unhealthy* caractérisé par une consommation plus élevée de viandes rouges et transformées, produits céréaliers raffinés et sucreries [51]. Des profils alimentaires tels que le profil Prudent/*Healthy*, la diète DASH (*Dietary Approach to Stop Hypertension*) ou encore la diète méditerranéenne sont associés avec le risque de MCV [51-54]. Williams et collaborateurs [54] ont examiné des études prospectives et ont observé que chez les individus adhérant aux profils alimentaires *Healthy*, Prudent, Méditerranéen ou DASH la réduction du risque de MCV était de l'ordre de 10 à 60%. Une réduction du risque d'accidents cérébrovasculaires avec la consommation des profils Prudent, DASH ou Méditerranéen a également été observée, tandis qu'une adhésion élevée au profil alimentaire Western en augmentait le risque [51]. Chez une cohorte de 30 239 adultes âgés de 45 ans et plus, un profil *Plant-based* caractérisé entre

autres par une consommation plus élevée de légumes, fruits, légumineuses et produits céréaliers à grains entiers, était associé avec une diminution du risque d'accidents cérébrovasculaires [55]. En 2010, Kant et collaborateurs [53] avaient constaté que les individus ayant une alimentation de type *Healthy* avait un risque réduit de 15% à 30% de la mortalité toute causes confondues et aussi due à une maladie coronarienne. En 2014, dans une cohorte de 7 216 participants à risque cardiovasculaire élevé, un profil alimentaire de type *Provegetarian* caractérisé par une consommation plus élevée de légumes, fruits, noix, céréales, légumineuses, huile d'olive et pommes de terre, était associé avec un plus faible taux de mortalité toutes causes confondues (0.59; 95% IC : 0.40-0.88) [52]. Il a également été rapporté qu'une augmentation de deux points du score à la diète Méditerranéenne (*Alternate Mediterranean Diet* (aMED)) était associé à une réduction de 7% du risque de mortalité toutes causes confondues. Cette observation a aussi été décrite chez 16 008 Espagnols où le risque de mortalité toute causes confondues était de 0,53 (95% IC : 0,34-0,84) chez les individus ayant les scores les plus élevés comparativement aux individus ayant les scores les plus bas au profil alimentaire Méditerranéen [56].

Profils alimentaires et diabète de type 2

Les profils alimentaires ont aussi été associés au risque de diabète de type 2 [57,58]. Une récente méta-analyse a observé qu'un score élevé à un profil alimentaire *Healthy* (alimentation contenant plus de légumes, fruits, produits céréaliers à grains entiers, poissons, produits laitiers faible en gras, produits du soya, poulet et/ou une consommation modérée d'alcool) diminuait le risque de diabète de type 2, tandis que les individus avec un score élevé au profil *Unhealthy* (consommation élevée de viandes rouges et/ou transformées, produits céréaliers raffinés, frites, produits laitiers riches en gras, sucreries, desserts, boissons sucrées, grignotines) augmentaient leur risque de diabète de type 2 [57]. De plus, un profil alimentaire caractérisé par des apports plus importants en légumes, fruits, produits laitiers, huile d'olive, pâtes alimentaires et pain a été associé avec une meilleure sensibilité à l'insuline dans une cohorte de 507 adultes en santé [58].

Profils alimentaires et poids corporel

Dans une cohorte de 1 070 adultes asiatiques âgés de 50 ans et plus, les individus ayant des scores élevés au profil alimentaire Western avaient davantage de risque d'être obèses, hypertendus et d'avoir le syndrome métabolique [59]. Mu et collaborateurs [60] ont également observé une association entre le profil alimentaire «Western» et le risque de présenter un surplus de poids ou de l'obésité chez 1 319 étudiants âgés en moyenne d'environ 18 ans. Le profil alimentaire Western a aussi été associé positivement au tour de taille et au ratio tour de taille/tour de hanche [61]. Quand à eux, Tucker et collaborateur [62] ont observé chez 281 femmes en santé, une association positive entre le profil alimentaire *Meat*, caractérisé par une consommation plus élevée de viandes maigres et d'autres viandes, et le pourcentage de tissu adipeux de même que l'indice de masse corporelle (IMC) tandis qu'une association inverse avait été observée pour les profils alimentaire *Low-fat milk* et Prudent. Dans une autre étude menée auprès de 780 pompiers de sexe masculin, les individus obèses avaient des scores plus faibles à la diète Méditerranéenne et consommaient davantage de *fast-food* et de boissons gazeuses [63]. Un profil alimentaire caractérisé par une consommation élevée d'alcool, boissons gazeuses, viandes, café et thé était associé à un risque augmenté de stéatose hépatique [64].

Profils alimentaires et cancer

Les profils alimentaires sont associés avec le risque de différents types de cancer tels que le cancer de la bouche, du larynx et du pharynx, le carcinome épidermoïde de l'œsophage, le cancer de l'estomac, le cancer colorectal et le cancer du sein [65-78]. Par exemple, une augmentation d'une unité du score à la diète Méditerranéenne (*MedDietScore*) est associée avec une diminution de 9% des risques de cancer du sein [72]. Cette observation a été rapportée en 2014 par Albuquerque et collaborateurs [66] dans une revue systématique de la littérature comprenant 26 études. Mis à part la diète Méditerranéenne, le profil Prudent est aussi associé à une diminution du risque de cancer du sein tandis que les profils caractérisés par une consommation plus élevée d'alcool étaient associés à un risque plus élevé du cancer du sein [71]. Les profils alimentaires Méditerranéen, Prudent, caractérisés par une consommation élevée de légumes et de fruits étaient associés avec une diminution

du risque de cancer touchant le tube digestif (cancer colorectal, cancer rectal, cancer de l'estomac, carcinome épidermoïde de l'œsophage et cancer du pharynx) [65,67-70,73,75-77] tandis que les profils Western ou *Animal products* avec un risque plus élevé de cancer colorectal, cancer de l'estomac, cancer du larynx, cancer du pharynx et cancer de la prostate [67-70,74,77,78].

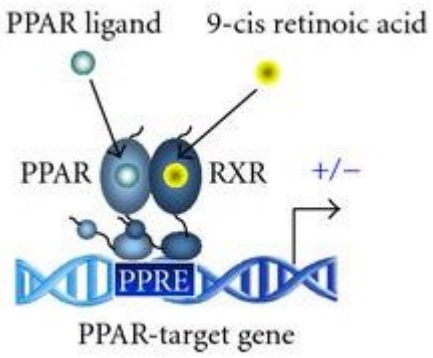
1.1.3 Profils alimentaires et expression génique

Selon nos connaissances, aucune étude à l'exception des travaux rapportés dans le cadre de cette thèse ne s'est penchée spécifiquement sur les associations entre des profils alimentaires, à l'exception de la diète Méditerranéenne, et la transcriptomique (expression génique). Conséquemment, les données rapportées ci-dessous sont majoritairement issues des études ayant observé l'effet d'un ou de quelques nutriments de même que la restriction calorique et les profils d'expression génique ou encore l'expression de quelques gènes candidats. Les prochaines sections traitent d'abord de quelques facteurs pouvant moduler l'expression génique et ensuite de l'impact de l'alimentation sur l'expression génique.

Facteurs affectant l'expression génique

Différents mécanismes ont été mis de l'avant afin d'expliquer l'impact de l'alimentation sur les niveaux d'expression génique. D'abord, les nutriments peuvent, en se liant à des facteurs de transcription, moduler les niveaux d'expression de plusieurs gènes. Par exemple, les facteurs de transcription encodés par les gènes *PPARs* ont pour ligands exogènes naturels les AGPI. Tel que présenté à la figure 1.1, PPARA, PPAR gamma (G) et PPAR delta (D) se lient ensuite aux éléments de réponse nommés *peroxisome proliferator response elements* (PPREs) localisés sur de nombreux gènes cibles et modulent ainsi l'expression de gènes impliqués dans différentes voies métaboliques telles que le métabolisme des lipides, la prolifération cellulaire, la différenciation cellulaire de même que les réactions immunitaires et inflammatoires [79,80].

Figure 1.1 La liaison de l'hétérodimère PPAR-RXR sur le PPRE d'un gène cible. [81]



L'alimentation peut aussi avoir un impact sur l'expression génique via des modifications épigénétiques [82]. Supic et collaborateurs [82] en 2013 ont décrit que les apports alimentaires entre autres en folate, polyphénols et isoflavones affectent la méthylation de l'ADN, la modification des histones et les micro-ARN (miARN). Toutes ces modifications sont ce que l'on appelle des modifications épigénétiques, c'est-à-dire des modifications qui affectent le génome et la régulation de l'expression des gènes sans modifier la séquence d'ADN [82].

Impact de l'alimentation sur l'expression génique

Konstantinidou et collaborateurs [83] ont réalisé en 2013 une revue de la littérature portant sur l'effet de la diète méditerranéenne ou encore l'ajout d'huile d'olive à l'alimentation sur l'expression génique. Ces derniers ont rapporté les résultats de quatorze études. D'abord, l'expression des gènes était principalement mesurée dans les cellules mononuclées périphériques sanguines (PBMCs). Les diètes méditerranéennes ou l'ajout d'huile d'olive étaient fréquemment comparés à des diètes élevées en acides gras saturés ou encore à une diète de type Western. Une étude a également comparé l'effet de la diète méditerranéenne standard versus une diète méditerranéenne enrichie en antioxydants. Les études mesuraient pour la plupart l'expression génique par réaction en chaîne de la polymérase à transcription quantitative inversée (qRT-PCR) et quelques-unes avaient utilisé des micropuces sur le génome entier. Les études démontraient principalement une diminution dans l'expression

postprandiale des gènes impliqués dans les processus inflammatoires comparativement à une diète riche en gras saturés. Une autre étude publiée en 2013 a examiné auprès de 34 individus de la cohorte *Prevencion Con Dieta Mediterranea* (PREDIMED) que les diètes méditerranéennes enrichies en huile d'olive ou encore en noix modifiaient toutes deux les voies métaboliques reliées à l'athérosclérose et à l'hypertension comparativement à une diète faible en gras [84].

Ravasco et collaborateurs [85], ont étudié l'impact de l'alimentation habituelle sur les niveaux d'expression du gène *nuclear factor kappa B* (*NFKB*), un gène codant pour un facteur de transcription impliqué dans les processus inflammatoires. Ces derniers ont observé que les individus avec une consommation plus élevée de protéines animales, de glucides raffinés, de gras saturés, d'AGPI oméga-6 (n-6) et d'alcool avaient des niveaux d'expression de *NFKB* plus élevés tandis que l'opposé avait été observé pour les individus avec une consommation élevée d'AGPI n-3, de fibres alimentaires, de vitamine E, de flavonoïdes, d'isoflavones, de bêta-carotène et de sélénium [85].

D'autres études se sont plutôt penchées sur l'effet de la restriction calorique sur l'expression génique. Chez les souris, la restriction calorique affecterait principalement des gènes impliqués dans des voies métaboliques telles que le métabolisme du glutathion, la réponse immunitaire, le métabolisme des lipides et du cholestérol de même que la phosphorylation oxydative [86]. Chez les humains, des diètes faibles ou très faibles en calories ont mené à des changements dans l'expression de gènes reliés au métabolisme des glucides, de la synthèse des lipides, aux macrophages et aux adipocytes [87-89]. Par exemple, l'expression des gènes *ATP citrate lyase* (*ACLY*), *acetyl-CoA carboxylase alpha* (*ACACA*), *fatty acid synthase* (*FASN*) et *stearoyl-CoA desaturase* (*SCD*) impliqués dans la voie de la lipogénèse de novo, du gène *carnitine palmitoyltransferase 1B* (*CPT1B*) impliqué dans la bêta-oxydation des acides gras et des gènes *serpin peptidase inhibitor, clade F* (*SERPINF1*) et *secreted protein, acidic, cysteine-rich* (*SPARC*) impliqués dans la résistance à l'insuline, était diminuée suite à la diète très faible en calories [88]. Finalement, Mutch et collaborateurs [90] ont observé des différences entre des femmes qui perdaient du poids et le maintenaient suite à une diète faible en calories comparativement à celles qui

perdaient du poids et le reprenaient suite à une diète faible en calories, dans l'expression de gènes impliqués dans les voies métaboliques du métabolisme des lipides, du cycle de Krebs, de la phosphorylation oxydative et dans l'apoptose.

Les différents types de gras et leurs effets sur l'expression génique ont été également étudiés. La consommation, durant huit semaines, d'une diète élevée en gras saturés comparativement à une diète élevée en gras monoinsaturés induirait un profil d'expression génique davantage pro-inflammatoire [91]. Une étude de 2014 chez 18 adultes en santé qui a comparé l'effet d'une diète élevée en acides gras saturés de type palmitique versus une diète faible en acide palmitique et élevée en acide oléique (acide gras monoinsaturé), a observé une diminution de l'expression du gène *insulin induced gene 1* (*INSIG1*), un gène impliqué dans la régulation du métabolisme du cholestérol induit par l'insuline [92]. Bergouignan et collaborateurs [93] ont observé des différences dans l'expression des gènes *pyruvate dehydrogenase kinase* (*PDK4*) et *CD36 molecule (thrombospondin receptor)* (*CD36*) suite à la consommation d'une diète isocalorique élevée en gras après seulement deux jours de consommation chez 19 participants. Les auteurs concluaient que l'augmentation de l'énergie consommée provenant des lipides conduisait à une augmentation de l'oxydation des lipides dans les muscles squelettiques [93]. Une autre étude a observé qu'une diète élevée en gras diminuait l'expression dans les muscles squelettiques de gènes impliqués dans le système endocannabinoïde [94].

Quelques études se sont également penchées sur l'effet des AGPI n-3/huile de poisson sur l'expression génique [95-98]. La consommation habituelle d'AGPI n-3 a été associée à des différences dans les voies métaboliques du métabolisme des TG et la signalisation des lipides [96]. La prise d'AGPI n-3/huile de poisson affecte l'expression de gènes reliés à l'inflammation tels que *CD68 molecule* (*CD68*), *matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)* (*MMP9*) et *chemokine (C-X-C motif) ligand 8* (*CXCL8*) [95,97,98]. Selon une étude issue de notre groupe de recherche, la prise de 5g d'huile de poisson durant six semaines a pour effet de modifier l'expression génique de gènes impliqués dans les voies métaboliques reliées à *PPARA*, à *NFKB* et au stress oxydatif [98]. Des différences dans l'expression génique en fonction des diètes ne sont toutefois pas

toujours observées. Meneses et collaborateurs [99] ont testé l'impact de quatre diètes (élevée en gras saturés, élevée en gras monoinsaturés, élevée en AGPI et faible en gras/ élevée en glucides) consommées durant 12 semaines sur la réponse inflammatoire postprandiale dans le tissu adipeux d'individus ayant le syndrome métabolique. Aucune différence entre les diètes dans l'expression des gènes candidats reliés à l'inflammation sélectionnés n'a été observée. Bjermo et collaborateurs [100] n'ont pas non plus observé de différence dans l'expression de gènes impliqués dans le métabolisme des lipides et l'inflammation suite à la consommation de deux diètes (diète riche en AGPI n-6 versus diète riche en acides gras saturés provenant du beurre) durant 10 semaines.

1.1.4 Profils alimentaires et profils de métabolites

Les apports alimentaires et les profils de métabolites sont depuis les cinq dernières années de plus en plus étudiés [101]. Le métabolome est influencé par l'alimentation de deux façons, d'abord par le métabolome endogène et ensuite par le métabolome issu des aliments («*food metabolome*») [101]. Le métabolome endogène représente tous les métabolites d'un hôte. Ses variations sont un reflet de l'effet métabolique de l'alimentation sur la santé [101]. Le métabolome issu des aliments est plutôt la sommation de tous les métabolites qui sont directement issus de la digestion des aliments, leur absorption dans l'intestin et la biotransformation dans les tissus de l'hôte et du microbiote [101,102].

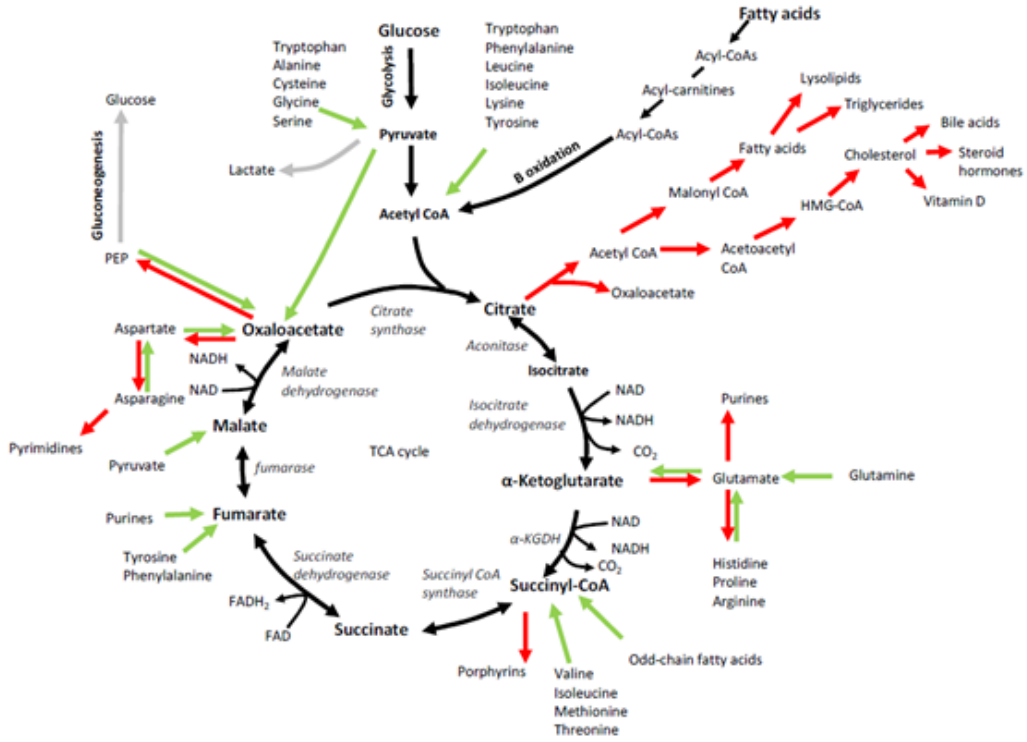
Impact de l'alimentation sur les profils de métabolites

Les profils de métabolites pourront éventuellement être utilisés comme biomarqueurs de l'impact métabolique de l'alimentation et des apports alimentaires [101]. Entre autres, Scalbert et collaborateurs [102] ont observé que des métabolites spécifiques issus du sérum sont des marqueurs des apports alimentaires en certains aliments tels que certains fruits (citron, pomme, banane, fraises, etc.), certains légumes, entre autres les légumes crucifères, les carottes et des légumes verts, les viandes rouges, le poulet et le poisson de même que d'autres aliments ou boissons tels que le chocolat, les croustilles et café. Guertin et collaborateurs [20] ont répliqué en 2014 certaines associations entre les apports en certains aliments et les métabolites issus du sérum tels que le citron et la stachydrine de même

qu'ils ont observé de nouvelles associations tels que la consommation d'arachides et le bétaïne tryptophane. O'Sullivan et collaborateurs [103], quant à eux ont observé que l'O-acetylcarnitine et le phenylacetylglutamine, issus de l'urine, étaient associés positivement avec les apports en viandes rouges et en légumes, respectivement. Des métabolites issus de l'urine permettent également de distinguer la consommation d'une diète élevée en composés phytochimiques issus des légumes et fruits d'une diète sans aucun légume et fruit [104]. Les métabolites les plus présents lors de la diète sans légume et fruit étaient la riboflavine, les acylcarnitines et les acides aminés [104]. Des différences dans les concentrations de métabolites issus du plasma ont aussi été observées entre la consommation de brocolis à teneur élevée en glucoraphanine comparativement à la consommation de brocolis standards ou encore de pois verts [105]. Les auteurs concluaient que les différences observées dans les concentrations de métabolites durant la consommation de la diète avec les brocolis à teneur élevée en glucoraphanine semblaient indiquer un rebalancement des réactions anaplérotiques (qui synthétisent les intermédiaires du cycle de Krebs) et cataplérotiques (qui catabolisent les intermédiaires du cycle de Krebs) de même qu'une meilleure intégration de la bêta-oxydation des acides gras et de l'activité du cycle de Krebs tel que présenté à la figure 1.2 [105].

Xu et collaboteurs [106] ont examiné l'effet de la diète ou du sexe sur les profils de métabolites issus de l'urine. Ces derniers ont déterminé que les profils alimentaires lactovégétarien versus omnivores affectaient davantage les concentrations de métabolites urinaires que le sexe [106]. La consommation de noix de Grenoble affecte certains métabolites issus de l'urine reliés au métabolisme des lipides, des composés dérivés de l'ellagitanin et de la voie métabolique du tryptophane et de la sérotonine [107].

Figure 1.2 Le cycle de Krebs et les réactions anaplérotiques en vert et cataplérotiques en rouge. [105]



La quantité d'acides gras saturés de la diète principalement l'acide palmitique semble avoir un impact sur les profils de métabolites. La consommation d'une diète élevée en acide palmitique durant trois semaines comparativement à la consommation d'une diète faible en acide palmitique et élevée en acide oléique, a produit une augmentation des concentrations d'acylcarnitines chez neuf femmes en santé [92]. Dans cette étude, une augmentation de l'oxydation des gras avec la diète élevée en acide palmitique a également été observée [92]. Conséquemment, les auteurs mentionnaient que l'augmentation des acylcarnitines avec la diète élevée en acide palmitique était possiblement due à une augmentation du catabolisme des gras afin de fournir de l'énergie [92]. Une récente étude a observé des associations entres des profils alimentaires dérivés par *reduced rank regression* et les profils de métabolites mesurés dans le sérum [108]. Dans cette étude, les auteurs ont observé que les profils alimentaires habituels des individus étaient associés avec les concentrations de métabolites circulant dans le sérum. Un profil alimentaire caractérisé par une consommation élevée en viandes rouges, poulet, beurre et autre matières grasses d'origine

végétale et une consommation faible de pains à grains entiers, de thé, de margarine, de soupe, de pâtes et de riz était associée à un profil de métabolites relié à un risque plus élevé de diabète de type 2. Ce profil de métabolites issus du sérum était composé entre autres d'acides aminés ramifiés, d'acides aminés aromatiques et de propionylcarnitine (C3) [109]. Un profil alimentaire caractérisé par des apports plus élevés en pommes de terre, produits laitiers et céréales à déjeuner de type cornflakes expliquait significativement les concentrations de méthionine et d'acides aminés ramifiées [108]. Une autre étude a observé chez 1 977 Afro-Américains qu'un profil alimentaire caractérisé par une consommation élevée d'aliments et de breuvages sucrés modulait les concentrations de certains métabolites (métabolites de la voie métabolique du 2-hydroxybutyrate, γ-glutamyl dipeptides et acides gras insaturés à longues chaînes) associés au stress oxydatif et au métabolisme des lipides [21]. En 2014, au sein d'une cohorte de 2 380 participants une association positive entre les concentrations d'acylcarnitines mesurées dans le sérum et l'obésité a été observée tandis qu'une association inverse a été observée entre les concentrations d'acylcarnitines et la consommation de pains à grains entiers [110].

Profils de métabolites et risque de maladies cardiovasculaires

D'autres études ont plutôt observé des associations entre les profils de métabolites et différents facteurs de risque des MCV tels que le diabète de type 2 ou encore l'obésité [111-113]. Tel que mentionné précédemment, les métabolites reflètent le métabolisme endogène. Des patients ayant subi une chirurgie bariatrique ont été étudiés afin de mieux comprendre les changements métaboliques survenant suite à ce type d'opération [111,114]. Des changements dans les concentrations plasmatiques d'acides aminés ramifiés, de méthionine, de phénylalanine et d'acides gras insaturés ont été observés suite à la chirurgie bariatrique [111,114,115]. L'observation des profils de métabolites pourrait contribuer à déterminer précocement la qualité du fonctionnement du métabolisme d'une personne, et cela, avant même l'apparition des modifications au niveau des facteurs risque traditionnels. Par exemple, Wang et collaborateurs [116] ont suivi durant 12 ans 2 422 individus ayant une glycémie normale. Sur ces 2 422 individus, 201 ont développé le diabète. Des concentrations élevées d'acides aminés ramifiés (leucine, isoleucine et valine) et d'acides aminés aromatiques (tyrosine et phénylalanine) étaient associées avec un risque augmenté

de développer le diabète [116]. Un risque augmenté de résistance à l'insuline a même été observé chez des enfants et des adolescents ayant des concentrations plasmatiques plus élevées d'acides aminés ramifiés indépendamment de l'IMC [117]. Plusieurs auteurs ont également observé des associations entre les acides aminés ramifiés, leurs dérivés (les acylcarnitines C3 et isovaleryl carnitine (C5)) et les acides aminés aromatiques de même que la méthionine et le risque de diabète de type 2 ou de résistance à l'insuline [112,117-119]. Une étude chez 1 872 participants a permis de déterminer qu'un profil de métabolites caractérisé par des concentrations plus élevées d'acides aminés ramifiés était, indépendamment de l'IMC, associé à un métabolisme altéré défini par la présence d'au moins deux des caractéristiques suivantes : (1) une glycémie à jeun élevée, (2) de l'hypertension, (3) des TG élevées, (4) des concentrations de C-HDL bas et (5) une résistance à l'insuline [120].

1.2 Les acides gras polyinsaturés oméga-3

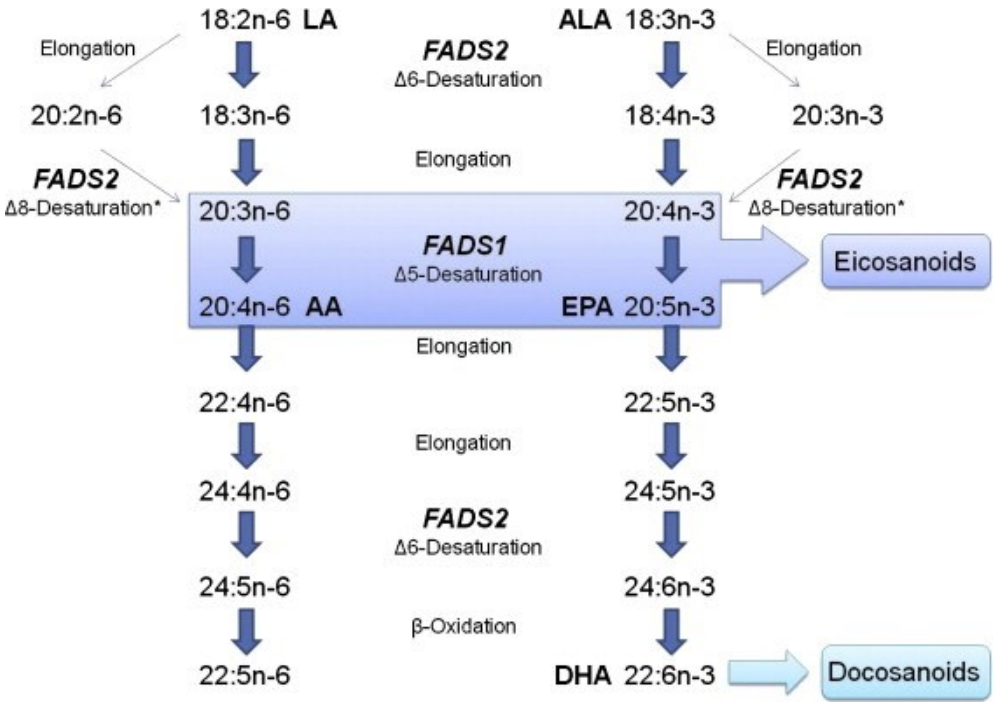
De nombreuses études se sont penchées sur les divers effets sur la santé des AGPI n-3. Dans cette section, les différents types d'AGPI n-3, leurs effets sur le risque de MCV, leurs effets hypotriglycéridémiant, la variabilité interindividuelle observée dans la réponse des concentrations de TG suite à la prise d'AGPI n-3 et leurs effets sur la glycémie et l'insulinémie seront présentés.

1.2.1 Les types d'acides gras polyinsaturés oméga-3

Il existe plusieurs types d'AGPI n-3 dont, l'acide α-linolénique, l'AEP, l'ADH et l'acide docosapentaénoïque (ADP). L'acide α-linolénique est le seul acide gras de type n-3 qui est considéré comme étant essentiel puisque le corps humain ne peut le synthétiser [121]. L'autre acide gras essentiel est l'acide linoléique qui fait partie de la famille des AGPI n-6. Le corps humain ne peut synthétiser ces deux acides gras puisqu'il ne possède pas les enzymes Δ^{12} et Δ^{15} désaturases qui incorporent des doubles liaisons à ces positions. L'AEP et l'ADH peuvent être synthétisés à partir de l'acide α-linolénique bien que les taux de conversion soient très faibles, c'est-à-dire des taux entre 1 et 5% [122]. Il existe également des différences entre les sexes pour la conversion de l'acide α-linolénique en AGPI à très longues chaînes. La conversion vers l'ADH est plus efficace chez les femmes que les hommes [123]. Cette différence pourrait être due à l'effet des œstrogènes [123]. L'acide α-linolénique est principalement retrouvé dans les aliments d'origine végétale tels que la graine de lin, la noix de Grenoble et la graine de chia, tandis que l'AEP et l'ADH sont surtout contenus dans les poissons gras tels que le hareng, le saumon, les anchois, le maquereau et la sardine [124]. L'apport nutritionnel recommandé pour l'acide α-linolénique est de 1,6 g/jour pour les hommes et de 1,1 g/jour pour les femmes [125]. Au Canada, il n'existe pas de recommandations formelles pour les apports en AEP et ADH. Toutefois, Santé Canada recommande la consommation de deux portions de poisson gras par semaine, une portion (75g poisson gras cuit) équivaut à environ 0,6g à 1,6g d'AEP+ADH [126]. Les recommandations de plusieurs pays suggèrent un apport en acides gras n-3 à très longues chaînes d'au moins 250mg/jour ce qui équivaut à environ deux portions de poissons gras par semaine [127].

Tel que présenté à la figure 1.3, l'acide α-linolénique (n-3) et l'acide linoléique (n-6) utilisent les mêmes enzymes afin d'être convertis en AGPI à chaînes plus longues. L'AEP et l'ADH sont les deux principaux constituant de l'huile de poisson suivis par l'ADP (22:5n-3) qui est également présent dans l'huile de poisson [128]. L'acide α-linolénique est converti vers l'AEP, l'ADP et l'ADH tandis que l'acide linoléique a pour produit terminal l'acide arachidonique (22:5n-6) [129].

Figure 1.3 Voies métaboliques pour la synthèse des AGPI à très longues chaînes à partir des acides gras essentiels. [130]



Conséquemment, le ratio n-6/n-3 détermine quels acides gras terminaux (AEP, ADH ou acide arachidonique) seront principalement synthétisés et incorporés dans les phospholipides membranaires [129]. L'AEP, l'ADH et l'acide arachidonique conduisent tous les trois à la formation d'eicosanoïdes. Les eicosanoïdes sont des médiateurs lipidiques qui modulent la réponse inflammatoire. Les eicosanoïdes dérivés de l'AEP et de l'ADH auraient des effets davantage anti-inflammatoire (prostaglandines et thromboxanes de séries

3 et leucotriènes de séries 5) et pro-résolution (résolvines, protectines et marésines) que les eicosanoïdes dérivés de l'acide arachidonique (prostaglandines et thromboxanes de séries 2 et leucotriènes de séries 4) [129,130].

1.2.2 Méthodes de mesures des acides gras polyinsaturés oméga-3

Afin de vérifier les apports alimentaires ou le statut en AGPI n-3, plusieurs méthodes peuvent être employées [131]. Par ailleurs, il n'existe pas à ce jour de biomarqueur officiellement accepté indiquant le statut en AGPI n-3 suite à sa consommation ou supplémentation [131]. Par contre, la mesure des AGPI n-3 dans les phospholipides du plasma et dans les membranes érythrocytaires sont deux mesures fréquemment utilisées [131]. La mesure de l'AEP et de l'ADH dans les phospholipides du plasma serait un bon biomarqueur des apports alimentaires en AEP et ADH sur une période à court terme, c'est-à-dire autour de quatre semaines, tandis que la mesure de l'AEP et de l'ADH dans les membranes érythrocytaires permettrait d'observer les apports alimentaires à plus long terme (quelques mois) étant donné leur plus longue demi-vie [132,133]. De plus, certains facteurs tels que les concentrations d'AGPI n-3 de départ, l'âge, le sexe et également la fréquence de consommation, influencent le degré d'incorporation des AGPI n-3 [134-136]. Par exemple, Walker et collaborateurs [134] ont observé que les femmes avaient augmenté davantage leurs concentrations d'AEP dans les TG que les hommes et que ces concentrations étaient également plus élevées chez les individus plus âgés suite à une supplémentation en AEP et ADH d'une durée de 12 mois. Quant à eux Browning et collaborateurs [135] ont observé que la consommation d'AEP et d'ADH quotidienne comparativement à la consommation intermittente, soit deux fois par semaine, était plus efficace afin d'enrichir les plaquettes et les cellules mononuclées du plasma en AEP et ADH.

1.2.3 Acides gras polyinsaturés oméga-3 et risque de maladies cardiovasculaires

Dès les années 60, les AGPI n-3 d'origine marine ont été reconnus comme potentiellement bénéfiques pour la santé cardiovasculaire. À cette époque, Bang et Dyerberg ont entrepris des expéditions vers le Groenland suite à des rapports anecdotiques révélant une faible

incidence de MCV chez les Inuits [122,137]. Ces derniers avaient remarqué chez les Inuits du Groenland une faible incidence d'infarctus du myocarde ainsi que des profils lipidiques favorables pour la santé cardiovasculaire [122,137]. Ces effets ont été attribués à la diète Inuit traditionnelle composée d'apports élevés en animaux marins dont les tissus sont riches en AGPI n-3 [137]. Depuis ce temps, plusieurs études épidémiologiques ont rapporté un risque plus faible de MCV chez les consommateurs d'AGPI n-3 d'origine marine [122,127,138]. Les AGPI n-3 d'origine marine ont plusieurs effets physiologiques cardioprotecteurs. Par exemple, ils ont un impact sur la morphologie et la stabilité de la plaque athérosclérotique, des effets antiarythmiques, antithrombotiques, antihypertenseurs et anti-inflammatoires, une influence sur la réactivité vasculaire de même qu'un impact sur les lipides sanguins et la distribution des sous-fractions de lipoprotéines [24,127]. L'effet le plus marqué sur les lipides sanguins suite à la prise d'AGPI n-3 d'origine marine est la diminution des concentrations de TG [139]. Malgré l'accumulation de nombreuses évidences démontrant que la prise de 0,85g à 1,8g par jour d'AGPI n-3 peut contribuer à réduire le risque de MCV, de récentes études ne sont pas parvenues à reproduire cet effet et concluaient que la prise d'AGPI n-3 ne diminuait pas les risques de MCV [138]. Par exemple, l'étude *Alpha Omega Trial* a étudié l'effet de la prise de 2g/jour d'acide α-linolénique et/ou 400mg d'AEP+ADH chez 4 837 individus ayant des antécédents d'infarctus du myocarde [140]. Les auteurs n'ont observé aucun effet protecteur sur le risque d'événements coronariens [140]. Par contre, la dose consommée d'AEP et ADH était relativement basse comparativement aux études *Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico* (GISSI) et *Prevenzione*, *Japan EPA Lipid Intervention Study* (JELIS)) ayant démontré des effets favorables [141,142]. De plus, les participants de cette étude consommaient à la base une quantité d'AGPI n-3 déjà plus élevée que la moyenne américaine [138]. Deux autres études issues de la même cohorte ont démontré des effets bénéfiques de la prise d'AGPI n-3, une chez des patients diabétiques et l'autre chez des patients ne prenant pas de statines [138,143,144]. Dans une autre étude, l'*Omega Trial*, aucun effet bénéfique de la prise de 840mg (AEP+ADH) n'a été observé en prévention secondaire chez 3 804 patients ayant subi récemment un infarctus du myocarde [145]. Toutefois, cette étude a fait l'objet de nombreuses critiques, entre autres, l'*Omega Trial* utilisait un supplément d'huile d'olive comme placebo qui aurait également des effets

bénéfiques sur la santé cardiovasculaire [146]. De plus, l'étude *Omega Trial* manquait de puissance (3 804 participants; $p<0,05$) comparativement à l'étude GISSI qui incluait plus de 18 000 patients [138,141]. Finalement, une autre étude, l'*Origin Trial*, qui elle avait suffisamment de puissance, n'a pas non plus détecté d'effets bénéfiques de la prise de 850mg/jour d'AEP+ADH chez 12 536 patients ayant une glycémie à jeun anormale, une intolérance au glucose ou étant diabétique [147]. Toutefois, dans cette étude, la prise habituelle avant l'étude des participants d'AEP+ADH était déjà de 210mg/jour ce qui est plus élevé que la moyenne américaine, tel que rapporté par Harris [138]. De plus, environ la moitié de ces patients n'avaient pas d'antécédents de maladies cardiaques ischémiques [138]. Globalement, les récentes études ayant démontré un effet neutre de la prise d'AEP+ADH sur le risque de MCV ont été critiquées [148]. D'abord, ces études ne tenaient pas compte de l'*Omega-3 Index* pré-intervention, c'est-à-dire le contenu en AEP+ADH présent dans les membranes érythrocytaires exprimé en pourcentage du contenu total en acides gras [148]. Les principaux déterminants de l'*Omega-3 Index* sont les apports alimentaires (20% de la variabilité), l'héritabilité (25% de la variabilité) et la présence de supplémentation en huile de poisson (15% de la variabilité) [148]. Ensuite, la biodisponibilité du supplément d'AEP+ADH consommé dans les études n'a pas été prise en considération [148]. La majorité des études ont utilisé un supplément sous la forme d'ester d'éthyle qui serait beaucoup mieux absorbé si ingéré durant un repas riche en lipides, ce qui n'a pas été considéré [148].

Certaines études ont également observé l'effet de l'acide α-linolénique sur le risque cardiovasculaire. Les résultats de ces études demeurent moins probants que ceux avec les AGPI n-3 d'origine marine. Toutefois, une récente revue de littérature de Rodriguez-Leyva et collaborateurs [149], présente plusieurs études ayant rapporté une association inverse entre la consommation d'acide α-linolénique et le risque d'un premier événement cardiovasculaire. Concernant l'effet de l'acide α-linolénique sur la réduction des concentrations de TG, les résultats sont moins probants, certaines études rapportent une diminution et d'autres aucun effet ou même une augmentation des concentrations de TG [127,149].

1.2.4 Effets hypotriglyceridémiant des acides gras polyinsaturés oméga-3

Tel que mentionné précédemment, le principal effet des AGPI n-3 sur les lipides plasmatiques est l'effet hypotriglycéridémiant. Les prochaines sections traiteront du métabolisme des TG, du lien entre les concentrations de TG et le risque de MCV, de l'effet hypotriglycéridémiant des AGPI n-3, des mécanismes potentiels pouvant expliquer cet effet et de la présence de variabilité interindividuelle dans la réponse des concentrations de TG suite à la prise d'AGPI n-3.

Métabolisme des triglycérides

Les TG dans le corps humain sont issus de deux principales sources, soit l'alimentation et le métabolisme endogène. Les TG représentent une proportion importante de l'énergie consommée. Les TG alimentaires sont d'abord hydrolysés puis incorporés dans des micelles pour être absorbés par la muqueuse intestinale et sécrétés dans la circulation lymphatique puis la veine cave sous la forme de chylomicrons [150,151]. Les TG des chylomicrons sont ensuite hydrolysés en acides gras libres et incorporés dans les tissus par la lipoprotéine lipase. Le foie est l'organe central du métabolisme endogène. Ce dernier synthétise et sécrète les VLDL qui sont des lipoprotéines riches en TG. Tel que pour les chylomicrons, les TG des VLDL sont hydrolysés par la lipoprotéine lipase et captés par les tissus ce qui conduit à la formation des lipoprotéines de densité intermédiaire (IDL) et éventuellement aux LDL [151]. La mesure des concentrations de TG est donc un reflet des TG présents principalement dans les chylomicrons et les VLDL.

Triglycérides et risques de maladies cardiovasculaires

Le rôle des TG en tant que facteur de risque indépendant ou de biomarqueur dans le risque de MCV demeure controversé [151]. Quelques études ont observé chez diverses populations que les concentrations de TG constituaient un facteur de risque indépendant des MCV [152-155]. Toutefois, d'autres études n'ont pas observé cette association. En 2009, une méta-analyse regroupant plus de 300 000 participants n'a pas observé d'association indépendante entre les concentrations de TG et le risque de MCV [156]. Les concentrations de TG sont étroitement liées aux concentrations des lipoprotéines riches en

TG de même qu'aux concentrations de C-HDL [151]. De plus, ils sont une composante importante du syndrome métabolique [151]. Selon la définition de la Fédération internationale du diabète, le syndrome métabolique est présent lorsqu'un individu présente de l'obésité abdominale (≥94cm hommes; ≥80cm femmes) et au moins deux des facteurs suivants : taux élevé de TG (≥1,7mmol/L), hypertension (≥130mmHg/≥85mmHg), taux de C-HDL bas (<1,03mmol/L; <1,29mmol/L femmes) et une glycémie à jeun élevée (≥5,6mmol/L) [157]. D'ailleurs l'*International Atherosclerosis Society* mentionnait dans sa dernière prise de position que le risque de MCV selon Framingham était sous-estimé par exemple chez les femmes post-ménopausées ayant le syndrome métabolique étant donné que ce dernier n'était pas entièrement considéré dans le modèle Framingham, par exemple les concentrations de TG ne sont pas considérées [158]. D'ailleurs, parmi les composantes du syndrome métabolique, les concentrations de TG seraient la composante la plus fortement associée à l'infarctus du myocarde et à l'accident vasculaire cérébral [159]. Des concentrations de TG élevées peuvent également être le reflet de désordres métaboliques associés à la lipotoxicité tels que la stéatose hépatique non alcoolique et le diabète de type 2 [151]. Toutefois, les mécanismes de même que les liens de cause à effet ne sont jusqu'à maintenant pas entièrement compris.

Différents mécanismes peuvent expliquer que les concentrations de TG s'élèvent. Entre autres, il est possible que l'absorption des TG soit augmentée, une augmentation de la formation de nouveaux TG ou encore une réduction du catabolisme des TG [151]. L'excès calorique et la résistance à l'insuline sont associés à l'hypertriglycéridémie, une augmentation du tissu adipeux intra-abdominal et une stéatose hépatique non alcoolique [151]. De plus, une consommation élevée d'aliments contenant des sucres simples, du fructose et/ou une consommation élevée d'alcool peut contribuer à augmenter les concentrations de TG [160]. Le fructose a un effet particulier étant donné que ce dernier n'est pas métabolisé via la phosphofructokinase, une étape limitante de la glycolyse. Cette enzyme est inhibée lorsque les concentrations d'ATP et de citrate augmentent ce qui régule le métabolisme hépatique du glucose. Conséquemment, même lorsque la phosphofructokinase est inhibée, le fructose peut continuer à être métabolisé et ainsi contribuer à augmenter la production hépatique TG et de VLDL [160].

Effet hypotriglycéridémiant des acides gras polyinsaturés oméga-3

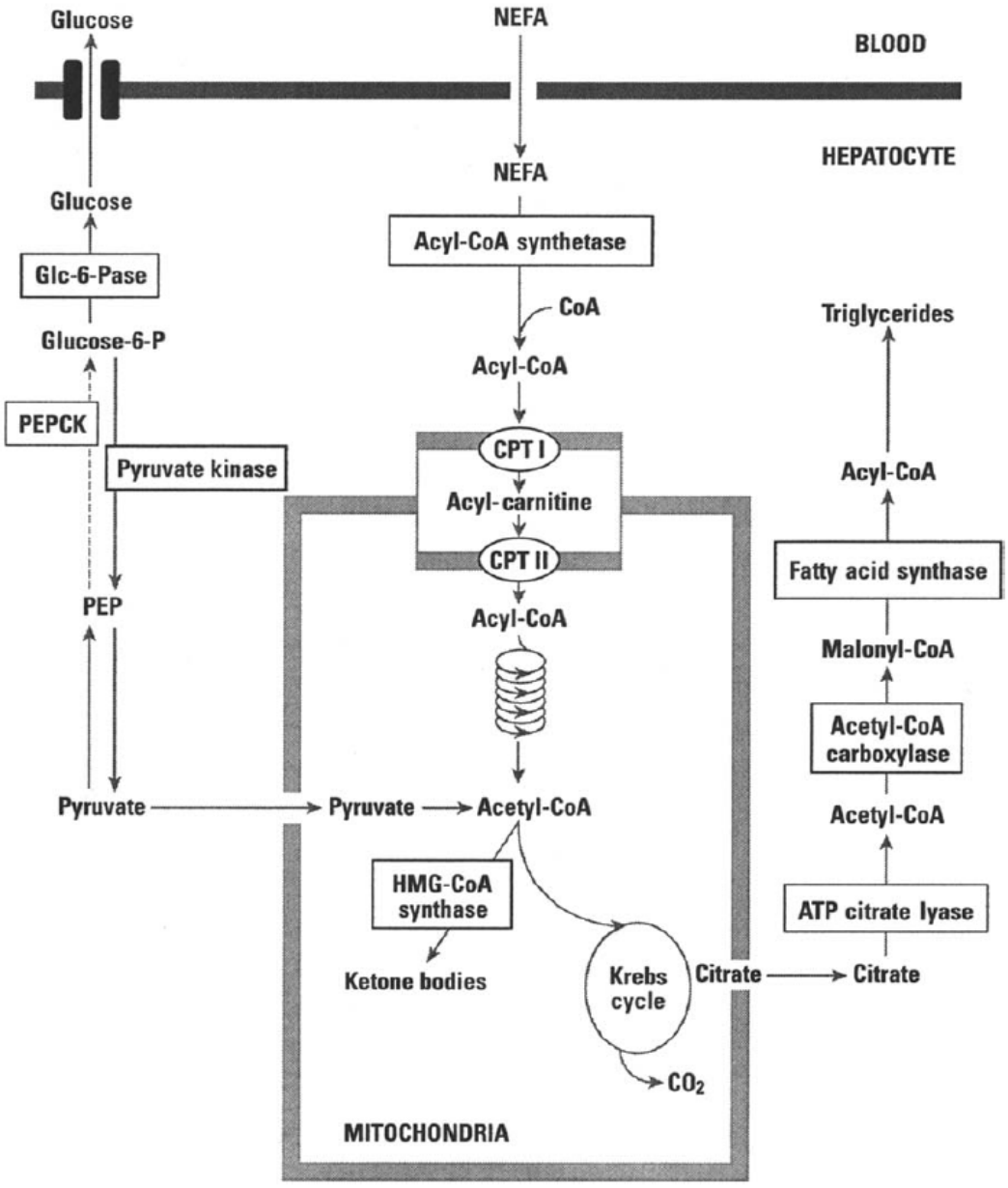
Tel que mentionné précédemment, l'effet le plus marqué de la prise d'AGPI n-3 d'origine marine ou d'huile de poisson sur les lipides sanguins est la réduction des concentrations de TG. Cet effet semble chez des individus hypertriglycéridémiques comparable à l'effet du gemfibrozil, un médicament utilisé pour diminuer les concentrations de TG [139]. L'*American Heart Association* recommande d'ailleurs la prise de 2 à 4 g d'AEP+ADH pour le traitement de l'hypertriglycéridémie [161]. La prise quotidienne de 3 à 4g d'AEP+ADH permet d'abaisser les concentrations de TG de 25 à 30% [139]. L'effet de la prise d'AEP+ADH est largement dose-dépendant, c'est-à-dire que plus les concentrations de TG de départ sont élevées et plus la réduction sera importante [139,162]. De plus, la prise d'AEP+ADH peut également augmenter les concentrations de cholestérol des lipoprotéine de basse densité (C-LDL) (5-10%) et de cholestérol des lipoprotéines de haute densité (C-HDL) (1-3%) [139]. Toutefois, il semblerait que la prise d'AGPI n-3 conduise à une augmentation de la taille des particules LDL [163]. Les particules LDL plus larges seraient moins athérogéniques que les particules LDL petites et denses [128,164]. Conséquemment, il est possible que l'effet d'augmentation des concentrations de C-LDL ne soit pas délétère pour la santé cardiovasculaire [128].

Mécanismes expliquant l'effet hypotriglycéridémiant des acides gras polyinsaturés oméga-3

L'effet réducteur des AGPI n-3 d'origine marine sur les concentrations de TG semble en partie causé par la diminution de la production hépatique des lipoprotéines de très basse densité (VLDL) qui sont des lipoprotéines riches en TG [127,165]. La synthèse des VLDL est influencée par la disponibilité des TG, qui est elle-même modulée par les afflux de sucres et d'acides gras libres. La figure 1.4, présente les origines des acides gras nécessaires pour la synthèse des TG. D'autre part, la prise d'AGPI n-3 augmente l'hydrolyse des TG via une augmentation de l'activité de la lipoprotéine lipase [167]. Certaines études ont également observé une augmentation de la clairance des particules VLDL, toutefois la

majorité des études observant l'effet des AGPI n-3 sur le métabolisme des TG n'ont pas rapporté ce résultat [165].

Figure 1.4 Origine des acides gras permettant la synthèse des TG. [166]



Une partie de l'effet hypotriglycéridémiant des AGPI n-3 à très longues chaînes est attribuable à la diminution de la synthèse des particules VLDL qui pourrait être due à des modifications dans l'expression de gènes impliqués dans les voies métaboliques reliées indirectement au métabolisme des TG tels que la lipogénèse de novo et la bêta-oxydation des acides gras. En effet, les AGPI n-3 sont des ligands de certains récepteurs nucléaires encodés par les gènes *liver X receptor* (*LXR*), *hepatocyte nuclear factor 4 alpha* (*HNF4A*), *nuclear receptor subfamily 1, group H, member 4* (*NR1H4*), *SREBF1*, *PPARA*, *PPARG* et *PPARD* [166,168]. Conséquemment, les AGPI n-3 vont se lier à ces protéines et en moduler leur action. Ensuite, ces récepteurs nucléaires affecteront l'expression d'autres gènes impliqués entre autres dans les métabolismes des lipides et des glucides [166]. Plus précisément, ces récepteurs nucléaires se lient aux éléments de réponse, qui sont des séquences spécifiques de l'ADN, de différents gènes cibles et en affectent ainsi l'expression [166]. Par exemple, LXR régule entre autres l'activité du gène *SREBF1* qui affecte ensuite le métabolisme de la lipogénèse de novo [166].

Les prochaines sections se pencheront sur les mécanismes pouvant affecter la disponibilité des TG et principalement sur les voies métaboliques de la lipogénèse de novo et de la bêta-oxydation des acides gras.

Lipogénèse de novo

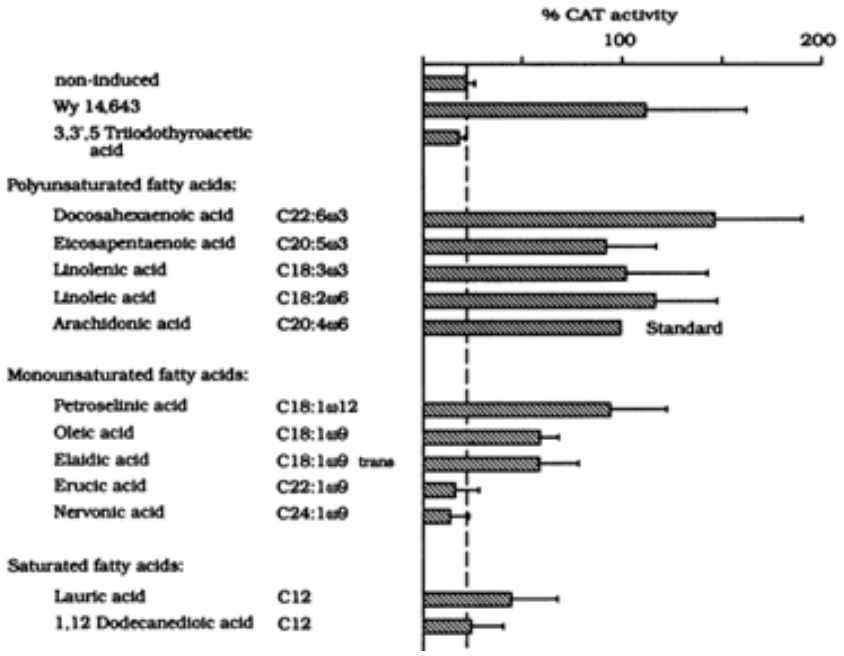
La synthèse de nouveaux acides gras à partir du citrate, un intermédiaire du cycle de Krebs, survient via la lipogénèse de novo qui est régulée par le facteur de transcription encodé par le gène *SREBF1* [127,165]. Tel que mentionné précédemment, une augmentation de la lipogénèse de novo augmente la disponibilité des acides gras pour la synthèse éventuelle des TG qui seront incorporés dans les VLDL [169,170]. Tanaka et collaborateurs [27] ont observé que la prise d'un supplément d'AEP chez des souris inhibait la maturation du *sterol regulatory element-binding protein 1* (SREBP1) via la diminution de l'expression des gènes *SREBP cleavage-activating protein* (*SCAP*) et *site-1 protease* (*S1P*). Il a également été observé que l'AEP permettait d'accélérer la dégradation de l'ARNm du gène *SREBF1* ce qui en diminuait son expression [171]. *SREBF1* régule non seulement

l'expression de différents gène encodant pour des enzymes impliquées dans la voie de la lipogenèse de novo tels que les gènes *ACACA* et *ACLY* mais également le gène *glucokinase* (*GCK*) impliqué dans la glycolyse [172]. La voie métabolique de la lipogénèse de novo est présentée à la figure 1.4 à partir de l'acétyl-CoA issu de la conversion du citrate par l'enzyme ACLY (ou ATP citrate lyase) provenant du cycle de Krebs vers la production de l'acyl-CoA.

Bêta-oxydation des acides gras

Tel que mentionné précédemment, les AGPI n-3 se lient également aux récepteurs nucléaires nommés PPARs. Jusqu'à maintenant, trois isotypes PPARs ont été identifiés : alpha, gamma et delta [173]. Le gène *PPARA* encode une protéine qui en se liant au PPRE de différents gènes cibles est un régulateur primordial du métabolisme des lipides [174]. La protéine PPARA est au cœur de l'activation des acides gras, de la bêta-oxydation mitochondriale et peroxisomale des acides gras, de la cétogénèse, du catabolisme des TG, de la gluconéogénèse de même que de la synthèse et la sécrétion de la bile [174]. *PPARA* exercerait son effet sur les concentrations de TG entre autres via une augmentation de la bêta-oxydation des acides gras dans les mitochondries et les peroxisomes ce qui diminuerait la disponibilité des acides gras pour la synthèse des TG [127,165,166]. Les études démontrent que la bêta-oxydation des acides gras est augmentée suite à une supplémentation en AGPI n-3 d'origine marine [165]. PPARA forme un hétérodimère avec la protéine *retinoid x receptor alpha* (RXRA). La formation de ce complexe est nécessaire à la liaison subséquente aux PPREs [175]. L'hétérodimère PPARA/RXRA régule l'activité de différents gènes impliqués dans la bêta-oxydation mitochondriale tels que *carnitine palmitoyltransferase 1a* (*CPT1A*), *acyl-coa dehydrogenase very long chain* (*ACADVL*) et *acetyl-coa acyltransferase 2* (*ACAA2*) et dans la bêta-oxydation peroxisomale tels que *ATP-binding cassette, sub-family D (ALD)* (*ABCD2*), *acyl-CoA oxidase 1, palmitoyl* (*ACOX1*) et *acetyl-CoA acyltransferase 1* (*ACAA1*) [172]. Tel que présenté à la figure 1.5 bien que les acides gras saturés puissent également se lier à PPARA, les AGPI à longues et très longues chaînes et principalement l'ADH, ont une affinité plus élevée avec ce récepteur nucléaire [80]. Les dérivés des AGPI n-3, les eicosanoïdes, se lient également à PPARA et sont des activateurs encore plus puissants que les AGPI n-3 [176].

Figure 1.5 L'activation de PPARA par les acides gras. [80]



1.2.5 Variabilité interindividuelle dans la réponse des triglycérides plasmatiques suite à la prise d'acides gras polyinsaturés oméga-3/huile de poisson

Malgré que les effets sur la santé cardiovasculaire des AGPI n-3 d'origine marine soient bien établis, une variabilité interindividuelle dans la réponse du profil lipidique suite à une supplémentation en AGPI n-3 d'origine marine ou d'huile de poisson a été observée [23,24]. Ces différences seraient dues à l'âge, au sexe, à l'état de santé, à l'utilisation de médicaments, aux habitudes alimentaires et d'activité physique ainsi qu'au profil lipidique de départ et à l'hérédité. Selon la FINGEN Study [23,24], 31% des individus ne diminuent pas leurs concentrations de TG suite à une supplémentation de 1,8 g/jour en AEP+ADH. Dans la cohorte du projet de recherche intitulé FAS qui est la cohorte principale étudiée dans le cadre de ce projet de doctorat, notre groupe de recherche a observé que 29% des individus n'avaient pas diminué leurs concentrations de TG suite à une supplémentation de 5g/jour d'huile de poisson dont 3g/jour d'AEP+ADH [177]. Dans la FINGEN Study [23], il

a également été observé que les hommes portant l'allèle *E4* du gène de l'*apolipoprotéine E* (*APOE*) avaient eu les effets hypotriglycéridémiants les plus importants.

Dans la littérature, d'autres gènes ayant potentiellement un effet sur l'action hypotriglycéridémique des AGPI n-3 ont été étudiés. Par exemple, il a été observé que les porteurs de l'allèle *Ala12* du polymorphisme *Pro12Ala* du gène *PPARG* répondaient davantage à une supplémentation de 2,4g/jour d'AEP+ADH que les autres individus [178]. La réponse des concentrations de TG suite à une supplémentation en AGPI n-3 d'origine marine semble également influencée par la présence de SNPs dans des gènes tels que *CD36*, *Complement component 3* (*C3*) et *PPARA* [24,179-181]. Toutefois, le pourcentage de variance expliquée par ces gènes demeure faible. Conséquemment, l'étude d'autres gènes candidats s'avère nécessaire afin de mieux comprendre les différences interindividuelles observées dans la réponse des concentrations de TG suite à une supplémentation en AGPI n-3.

1.2.6 Acides gras polyinsaturés oméga-3 et le métabolisme du glucose, de l'insuline et le diabète de type 2

Les AGPI n-3 affecteraient également les concentrations de glucose, d'insuline et le risque de diabète de type 2. Les prochaines sections traiteront des effets des AGPI n-3 sur les concentrations de glucose, d'insuline et le risque de diabète de type 2, des méthodes de mesure de la sensibilité à l'insuline et des possibles mécanismes d'action des AGPI n-3 sur les concentrations de glucose, d'insuline et la sensibilité à l'insuline.

Effets des acides gras polyinsaturés oméga-3 sur le glucose, l'insuline, la sensibilité à l'insuline et le risque de diabète de type 2

La prise d'AGPI n-3 à très longues chaînes pourrait affecter les concentrations de glucose, d'insuline ou encore la sensibilité à l'insuline. Une concentration de glucose à jeun élevée (≥5,6mmol/L) peut faire partie du syndrome métabolique tel que décrit précédemment [157]. La présence du syndrome métabolique, c'est-à-dire la présence de l'intolérance au

glucose, de l'hypertension artérielle, une dyslipidémie et de l'obésité, particulièrement l'obésité abdominale, est fortement associée aux risques de MCV et de diabète de type 2 [182]. La résistance à l'insuline survient lorsque la réponse à l'insuline des trois tissus principaux, soient les muscles squelettiques, le foie et le tissu adipeux, est diminuée ce qui entraîne un hyperinsulinisme et éventuellement une augmentation des concentrations de glucose [183,184].

Les études chez les animaux révèlent que les AGPI n-3 auraient des effets bénéfiques sur la glycémie, l'insulinémie et la sensibilité à l'insuline [183,185-188]. La plupart de ces résultats ont été observés chez des rongeurs et les résultats sont jusqu'à maintenant difficiles à observer chez l'humain. Récemment, une étude chez des macaques a observé que le développement de l'hypertriglycéridémie et de la résistance à l'insuline induit par une diète riche en fructose étaient prévenu par la consommation de 4g d'huile de poisson/jour (16% AEP et 11% ADH) [186].

Tel que mentionné précédemment, chez les humains, les résultats sont beaucoup moins concluants. D'abord, plusieurs études ont examiné l'effet des AGPI n-3 sur le risque ou l'incidence de diabète de type 2. Une récente méta-analyse étudiait l'effet des AGPI n-3 sur l'incidence de diabète de type 2 chez 540 184 personnes dont 25 670 cas de diabète de type 2 [189]. Dans cette étude la consommation d'AEP+ADH ou encore de poisson de même que les concentrations circulantes d'AGPI n-3 n'affectaient pas l'incidence de diabète de type 2. Toutefois, les auteurs rapportent une association inverse non significative entre la consommation d'acide α-linolénique de même que les concentrations circulantes d'acide α-linolénique et l'incidence de diabète de type 2. D'autres auteurs rapportent une grande hétérogénéité, en fonction des régions géographiques (États-Unis, Europe et Asie/Australie), de l'effet de la consommation d'AGPI n-3 ou encore de poisson sur le risque de diabète de type 2 [190]. Une autre méta-analyse n'a pas observé chez des individus diabétiques de type 2 d'effet des AGPI n-3 sur les concentrations de glucose ni d'insuline [191]. Une étude chez des diabétiques de type 2 a examiné l'effet de la prise de 4g d'AGPI n-3 par jour comparativement à un placebo durant dix semaines sur les concentrations d'acides gras non-estérifiés (acides gras libres), la sensibilité à l'insuline et

les concentrations de glucose [192]. Les auteurs rapportent une amélioration de la sensibilité à l'insuline pour le groupe prenant les AGPI n-3 et une diminution des concentrations d'acides gras non-estérifiés. Une autre étude chez des adultes en santé ayant un surplus de poids a observé une amélioration de la sensibilité à l'insuline suite à la prise durant 6 semaines de 0,3g d'AGPI n-3 à très longues chaînes (AEP+ADH) [193]. D'autres études ont démontré des effets bénéfiques des AGPI n-3 sur les concentrations de glucose, d'insuline et la sensibilité à l'insuline chez différentes populations telles que chez des femmes ayant le syndrome des ovaires polykystiques [194] et chez des enfants et adolescents obèses [195]. Dasarathy et collaborateurs [196], ont étudié l'effet de la prise de 3,6g d'AGPI n-3 (AEP+ADH) chez des patients diabétiques de type 2 ayant une stéatose hépatique non-alcoolique. Ces auteurs ont observé une détérioration des concentrations de glucose et de la résistance à l'insuline mesurée par l'indice *homeostasis model assessment* (HOMA). Une augmentation des concentrations de glucose et/ou d'insuline suite à la prise d'AGPI n-3 a également été rapportée par d'autres auteurs [197,198]. Toutefois, les méta-analyses observant l'effet de la prise d'AGPI n-3 ou d'huile de poisson sur le contrôle glycémique d'individus diabétiques n'ont pas observé de détérioration [191,199,200]. Abeywardena et collaborateurs [183] concluent que les bienfaits des AGPI-n3 sur les concentrations de glucose et d'insuline de même que sur la sensibilité à l'insuline et le risque de diabète de type 2 devraient être vus de manière systémique en considérant l'impact de l'incorporation dans les membranes cellulaires des AGPI n-3 et leur effet sur les concentrations de TG. Par exemple, une plus grande incorporation des AGPI n-3 dans les membranes cellulaires pourrait affecter les voies de signalisation de l'insuline [168,188].

Certaines études ont étudié les associations entre les concentrations d'AGPI n-3 dans les phospholipides ou encore dans les érythrocytes. Lou et collaborateurs [201] ont observé que les concentrations en AGPI n-3 dans les phospholipides du sérum étaient plus basses chez les individus diabétiques et ayant une stéatose hépatique non-alcoolique comparativement aux individus en santé. Mahendran et collaborateurs [202], n'ont pas observé d'association entre les concentrations érythrocytaires d'AGPI n-3 à très longues chaînes (AEP et ADH) et les niveaux d'intolérance au glucose. Toutefois, une association

inverse a été observée entre les concentrations érythrocytaires d'acide linoléique et les niveaux d'intolérance au glucose [202].

En résumé, toutes ces études démontrent bien que l'effet des AGPI n-3 sur les concentrations de glucose, d'insuline et la sensibilité à l'insuline chez l'humain est encore mal connu. Il est possible que plusieurs facteurs affectent la réponse de ces paramètres à la prise d'AGPI n-3, par exemple, il est possible que la variabilité des résultats observés chez l'humain soit attribuable aux différences dans les quantités d'AGPI n-3 fournies aux participants, la durée de la période d'exposition des participants aux AGPI n-3, l'état métabolique d'un individu ou encore certains facteurs génétiques [184].

Mesure de la sensibilité à l'insuline

Afin de mesurer les niveaux de sensibilité et/ou de résistance à l'insuline différentes méthodes directes et indirectes peuvent être utilisées [203]. Par exemple, les niveaux de sensibilité ou de résistance à l'insuline peuvent être mesurés directement par des clamps euglycémiques hyperinsulinémiques [203]. Afin de mesurer indirectement les niveaux de sensibilité ou de résistance à l'insuline, il est possible de réaliser le test oral de tolérance au glucose (OGTT) ou encore d'utiliser des indices. Par exemple, chez les individus en santé, une augmentation des concentrations d'insuline à jeun reflète une augmentation de la résistance à l'insuline, conséquemment l'indice 1/(insuline à jeun) est parfois utilisé [203]. Le ratio glucose/insuline est aussi parfois utilisé mais ce dernier ne reflète pas adéquatement les mécanismes sous-jacents à la sensibilité à l'insuline [203]. La méthode la plus fréquemment utilisée se nomme HOMA qui a été développée en 1985 [204]. Cette méthode tient compte des interactions entre le glucose et l'insuline afin d'estimer la résistance à l'insuline de même que la fonction des cellules bêta du pancréas. L'indice HOMA considère que les concentrations de glucose sont régulées par la production hépatique de glucose en fonction des concentrations d'insuline et que les concentrations d'insuline sont elles-mêmes dépendantes de la réponse des cellules bêta du pancréas aux concentrations de glucose [203]. La formule permettant de calculer l'indice HOMA-*insulin resistance* (IR) est la suivante :

$$\text{HOMA-IR} = (\text{insuline mU/L X glucose mmol/L})/22{,}5$$

Un autre indice mesurant la sensibilité à l'insuline se nomme *quantitative insulin sensitivity check index* (QUICKI). Cet indice est obtenu avec une transformation mathématique des concentrations de glucose et de l'insuline à jeun. L'indice QUICKI utilise les logarithmes du glucose et de l'insuline selon la formule suivante [203] :

$$\text{QUICKI} = 1/(\log(\text{insulin(mU/L)}) + \log(\text{glucose (mg/dL)}))$$

L'indice QUICKI est davantage corrélé aux résultats issus du clamp euglycémique hyperinsulinémique que l'indice HOMA. Toutefois, lorsque les valeurs obtenues avec l'indice HOMA sont transformées avec une transformation logarithmique le log (HOMA) et le QUICKI sont plutôt comparables [203]. Il a également été observé que l'indice QUICKI était davantage reproductible que l'indice HOMA avec des coefficients de variation de 3,9% versus 26,7%, respectivement [205].

Mécanismes d'action des acides gras polyinsaturés oméga-3 sur les concentrations de glucose, d'insuline et de la sensibilité à l'insuline

L'héritabilité de la glycémie serait d'environ 10% à 26% chez les Caucasiens [206,207]. Pour les concentrations d'insuline l'héritabilité serait d'environ 36% à 48% [208,209]. Conséquemment, une part de la variabilité observée dans les concentrations de glucose et d'insuline est due à des facteurs génétiques. Une multitude de gènes sont impliqués de près ou de loin à la voie de signalisation de l'insuline et au métabolisme du glucose. Les AGPI affectent l'activité des facteurs de transcriptions encodés par les gènes *PPARs* et *SREBF1* [184]. Clarke [210] proposait en 2000 un modèle où les AGPI n-3 affectaient la sensibilité à l'insuline à travers leurs effets sur les facteurs de transcription entre autres, *PPARA* et *SREBF1*, tel qu'illustré à la figure 1.6. L'activation de *PPARA* augmente la voie de la bêta-oxydation des acides gras tandis que l'inhibition de *SREBF1* diminue la voie de la

lipogénèse de novo ce qui aurait pour effet de diminuer la disponibilité des acides gras, améliorer la captation du glucose et diminuer la résistance à l'insuline.

Figure 1.6 Impact des AGPIs sur la régulation des facteurs de transcriptions (*PPARs* et *SREBF1*). [210]

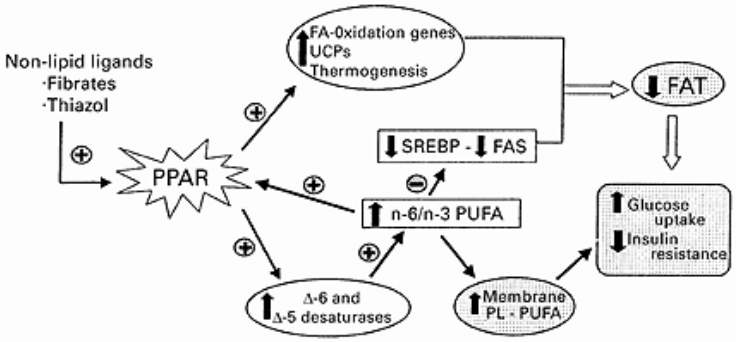


Fig. 1. Schematic mechanism of PUFA regulation of fuel partitioning. Thiazol, thiazolidinediones; PPAR, peroxisome proliferator activated receptor; UCPs, mitochondrial uncoupling proteins; SREBP, sterol response element-binding protein; FAS, fatty acid synthase; PUFA, polyunsaturated fatty acids; PL, phospholipids; +, activation or induction; –, suppression.

Chez les individus obèses, les adipocytes de plus grandes dimensions exhiberaient un stress augmenté au niveau du réticulum endoplasmique et des anormalités dans les mitochondries [184]. Ce stress pourrait également conduire à des défauts de régulation du gène *SREBF1* et favoriser la lipogénèse de novo de même qu'affecter la sensibilité à l'insuline [184]. Il a été observé que le DHA diminuait l'activation du gène *SREBF1* via le récepteur nucléaire LXRA [184]. De plus, la translocation nucléaire de SREBP1 serait modulée par les AGPI n-3 via l'activité de certaines protéines kinases telles que *protein kinase C* (PKC) et *extracellular-signal-regulated kinase* (ERK) [211]. Une consommation plus élevée d'AGPI n-3 modifie également la composition des membranes cellulaires ce qui pourrait contribuer à améliorer la captation du glucose et diminuer la résistance à l'insuline [210]. Il a été observé que les AGPI sont des constituants importants des phospholipides des parois cellulaires [212]. Des apports plus élevés en AGPI n-3 produisent un enrichissement des parois cellulaires entre autres des cellules reliées à l'immunité et à l'inflammation telles que les lymphocytes, les macrophages et les neutrophiles. Cette augmentation se fait au détriment du contenu en acide arachidonique dans les parois cellulaires [212]. Ces modifications pourraient permettre un fonctionnement optimal des protéines membranaires, améliorer la fluidité de la membrane cellulaire et affecter la formation des radeaux

lipidiques [212]. Tous ces changements pourraient affecter la signalisation cellulaire et ainsi moduler par exemple, la sensibilité à l'insuline.

1.3 L'application des connaissances

Étant donné que la génomique nutritionnelle n'est pas une science présentement utilisée d'emblée dans la pratique des professionnels de la santé, l'étude de cette science ne pourrait être complète sans un volet dédié à l'application des connaissances. La prochaine section décrit le troisième et dernier volet de cette thèse de doctorat et porte sur l'application des connaissances en lien avec la génomique nutritionnelle vers les professionnels de la santé. Il existe plusieurs définitions pour l'application des connaissances, également nommé transfert des connaissances [213]. Les Instituts de recherche en santé du Canada l'ont récemment décrit comme étant un «processus dynamique et itératif qui englobe la synthèse, la dissémination, l'échange et l'application conforme à l'éthique des connaissances dans le but d'améliorer la santé des Canadiens, d'offrir de meilleurs produits et services de santé et de renforcer le système de santé» [214]. L'application des connaissances est un champ de pratique et de recherche qui aurait le potentiel d'augmenter l'utilisation des connaissances à tous les niveaux et également dans la prise de décision [213]. Un des éléments clés de l'application des connaissances est le dialogue bidirectionnel entre les chercheurs et les utilisateurs des connaissances tels que les professionnels de la santé [215]. Les prochaines sections traitent de l'application des connaissances spécifique au domaine de la génomique nutritionnelle et de l'utilisation d'un cadre théorique chez les professionnels de la santé afin de mieux comprendre les déterminants de l'intention de réaliser un comportement ou du comportement lui-même.

1.3.1 L'application des connaissances en nutrigénomique

La génomique nutritionnelle est un domaine émergeant qui comprend la nutrigénomique, la nutrigénétique et l'épigénomique nutritionnelle [216]. La récente position de l'*Academy of Nutrition and Dietetics* mentionne que la génomique nutritionnelle donne un aperçu de la façon dont la diète et le génome interagissent afin de moduler le phénotype [216]. Les diététistes sont des professionnels de la santé spécialisés dans le domaine de la nutrition et seraient les mieux outillés afin d'intégrer des recommandations issues de la génomique nutritionnelle dans leur pratique professionnelle autant clinique que publique [217,218]. D'ailleurs, les diététistes travaillent déjà fréquemment auprès de patients et/ou clients qui

présentent des maladies issues de facteurs environnementaux et génétiques telles que le diabète de type 2, les MCV et le cancer. Toutefois l'*Academy of Nutrition and Dietetics* stipule que les diététistes ne devraient pas être responsables de la divulgation des résultats de tests génétiques à leurs patients ou clients mais plutôt travailler de concert avec un professionnel de la génétique ou un médecin [216]. De leur côté, les Diététistes du Canada n'ont pas émis d'avis concernant la pertinence de la génomique nutritionnelle dans la pratique professionnelle des diététistes.

Il existait déjà en 2008, environ une trentaine de compagnies offrant des tests de nutrigénétique disponibles directement aux consommateurs (*Direct-to-consumer*) [219]. Depuis les dix dernières années, ces tests sont devenus très présents étant donné la diminution des coûts de génotypage, l'amélioration des techniques de laboratoire et le manque de législation [216]. Les professionnels de la santé ne sont la plupart du temps pas impliqués par les compagnies offrant des tests de nutrigénétique [220]. Il a été observé que ces tests n'étaient pas appuyés par de solides évidences scientifiques. Les compagnies offrant ces tests prônent plusieurs bienfaits, toutefois l'efficacité réelle de ces tests sur l'optimisation de la santé n'a pas été validée [221-223]. De plus, ceux-ci n'utilisent que quelques SNPs pour prédire un risque plus élevé de maladie alors que chacun de ces SNPs n'expliquent qu'une faible partie de la variance observée dans la réponse à un nutriment, un aliment ou un type d'alimentation particulier [219]. Ce phénomène est très fréquent lorsque des maladies multifactorielles sont étudiées telles que les MCV [224,225]. Cela indique que potentiellement plusieurs SNPs pourraient avoir des effets synergiques, additifs ou encore antagonistes. Actuellement, l'utilité clinique de ces tests est discutable et serait plutôt faible [222,226,227]. D'ailleurs, Corella et collaborateurs [228] de même que Minihane et collaborateurs [229], ont révisé récemment les évidences dans le domaine et ont conclu que davantage d'études devront être réalisées avant de pouvoir appliquer concrètement ce savoir.

Le manque d'études en génomique nutritionnelle n'est pas le seul obstacle, les professionnels de la santé, dont les diététistes ne sont pas non plus adéquatement préparés à appliquer la génomique nutritionnelle dans leur pratique. Selon Weir et collaborateurs

[230], les professionnels de la santé sentent qu'ils manquent de compétences afin de transmettre ce savoir à leurs patients et/ou clients. De plus, ces derniers sont septiques envers l'applicabilité immédiate de la génomique nutritionnelle étant donné l'état des connaissances actuelles [230]. Toutefois, la plupart des professionnels de la santé étaient en accord avec le fait qu'éventuellement la génétique sera au cœur de la pratique clinique [230]. Une autre étude menée au Royaume-Uni ciblant exclusivement les diététistes, a observé qu'en moyenne le niveau de connaissances en génétique et en génomique nutritionnelle était faible et qu'un niveau de connaissances plus élevé était associé avec le degré d'éducation universitaire de même que la participation à des formations continues [30]. Cette observation a également été remarquée aux États-Unis, où 81% des diététistes pensaient manquer de connaissances en regard de la génomique nutritionnelle [31]. Rosen et collaborateurs [31], ont étudié les besoins en formation continue en génomique nutritionnelle de diététistes américains. Dans leur étude, la plupart des diététistes étaient d'avis que la génomique nutritionnelle aurait des effets positifs tels qu'une plus grande individualisation des recommandations alimentaires, une plus solide base à ces recommandations et qu'elles permettraient de prévenir certaines maladies plus efficacement. Les besoins en formation continue les plus importants formulés par les diététistes étaient d'acquérir davantage de connaissances de base en génomique nutritionnelle de même que la façon de communiquer à leurs patients et/ou clients les notions issues de cette science. Une limitation observée était le nombre de diététistes experts en génomique nutritionnelle en mesure d'offrir des conférences et des ateliers de formation continue [31]. La situation auprès des diététistes québécois n'est pas différente de celle mentionnée précédemment chez les diététistes du Royaume-Uni et des États-Unis. Un récent sondage mené auprès de 373 diététistes membres de l'OPDQ (Ordre professionnel des diététistes) a observé que 76% des diététistes travaillant en nutrition clinique dans le réseau de santé publique et 63% des diététistes travaillant en nutrition clinique dans le secteur privé considéraient ne pas avoir suffisamment de connaissances afin d'incorporer la nutrigénomique dans leur pratique professionnelle [29]. La plupart des diététistes (77%) connaissaient le terme nutrigénomique toutefois une plus faible proportion des diététistes était au courant que des tests de nutrigénétique étaient disponibles directement pour les consommateurs sur le réseau Internet, plus précisément, 49% chez les

diététistes avec moins de 5 ans d'expérience et seulement 12% chez les diététistes avec plus de 25 ans d'expérience [29].

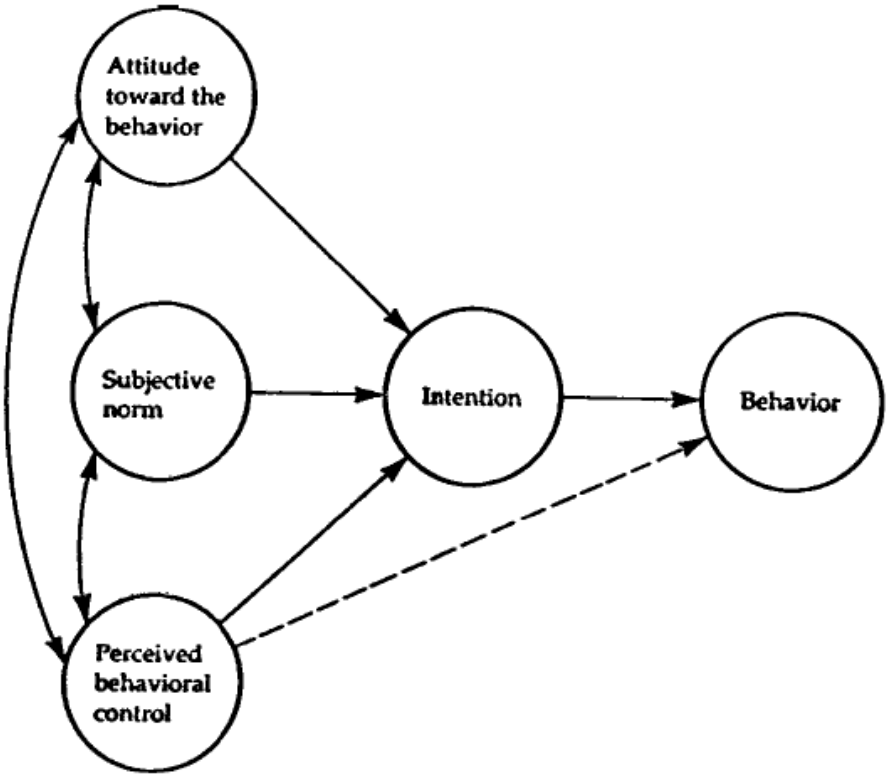
Malgré que plusieurs étapes restent à franchir avant l'application de la génomique nutritionnelle à la pratique professionnelle des diététistes, des tests de nutrigénétique disponibles directement au consommateur (DTC) existent déjà sur le marché et même certains tests disponibles via un diététiste tels que *Nutrigenomix* au Canada qui est déjà offert par presqu'une vingtaine de diététistes au Québec [231]. Conséquemment, le niveau de connaissances et d'aptitudes chez les diététistes doit être augmenté afin de maintenir à jour les connaissances de ces derniers en lien avec les avancées de la recherche en génomique nutritionnelle. Cela permettra éventuellement aux diététistes d'aborder de façon nuancée la génomique nutritionnelle avec leurs patients/clients ou encore les autres professionnels de la santé.

1.3.2 La théorie du comportement planifié et l'application des connaissances

Afin de promouvoir l'application des connaissances, de nombreuses stratégies peuvent être employées telles que des interventions visant à augmenter le niveau de connaissances, à amener un changement d'attitude ou encore un changement de pratique [232,233]. Avant de sélectionner une de ces stratégies, l'utilisation de cadre théorique afin de mieux comprendre les déterminants du comportement étudié s'avèrerait une étape importante afin d'élaborer des interventions efficaces [33,234]. Les théories sociales cognitives fournissent ces types de cadre théorique et considèrent les pensées et les cognitions comme des processus reliant un stimuli et la réponse d'une personne dans une situation précise [33]. Il a été observé que l'intention était un prédicteur valide de l'adoption d'un comportement chez les professionnels de la santé [235]. Environ 28% de la variance dans un comportement serait expliquée par l'intention [236]. Godin et collaborateurs [33] ont publié une revue de la littérature incluant 76 études utilisant un cadre théorique tel que l'action raisonnée/théorie du comportement planifié, la théorie des comportement interpersonnels et le modèle d'acceptation de technologie. Le pouvoir prédictif du comportement des études utilisant la théorie de l'action raisonnée ou encore son extension, la théorie du

comportement planifié, était plus élevé que les études utilisant d'autres théories [33]. Afin de prévoir l'intention, la théorie de l'action raisonnée/théorie du comportement planifié était la plus utilisée tandis que la théorie des comportements interpersonnels avait le pouvoir prédictif le plus élevé, suivi de la théorie de l'action raisonnée/théorie du comportement planifié [33]. La théorie du comportement planifié est la théorie sociale cognitive la plus utilisée dans le domaine des comportements relatifs à la santé [33]. Cette théorie a été élaborée par Ajzen et Fishbein afin d'améliorer le modèle de la théorie de l'action raisonnée pour les comportements qui sont sous contrôle partiellement volitif en ajoutant le concept de perception de contrôle au modèle théorique [237]. Tel qu'illustré à la figure 1.7, la théorie du comportement planifié comprend les concepts d'attitude envers le comportement, la norme subjective et la perception de contrôle qui peuvent prédire l'intention de même que l'intention et la perception de contrôle qui seraient prédicteur du comportement [237].

Figure 1.7 Diagramme de la théorie du comportement planifié. [237]



L'utilisation de la théorie du comportement planifié comme cadre théorique pourrait permettre dans le cadre de ce projet de doctorat d'identifier les déterminants de l'intention et du comportement des diététistes de discuter de nutrigénétique avec leurs patients/clients. La connaissance de ces déterminants permettra de cibler certains éléments clés à inclure dans un futur outil de formation pour les diététistes de l'OPDQ.

Chapitre 2 :
Problématique

L'étude de l'alimentation et de son impact sur la santé est complexe. Effectivement, chez l'humain, il est difficile d'isoler complètement l'effet d'un nutriment sur un paramètre de la santé étant donné que plusieurs autres facteurs tels que la variabilité génétique et les autres habitudes de vie vont également avoir une influence. L'impact de profils alimentaires globaux sur la santé est encore mal compris. Conséquemment, des études observant l'effet de l'alimentation globale sur non seulement les marqueurs sanguins mais également en amont dans le métabolisme du corps humain tel qu'au niveau de l'expression des gènes et des intermédiaires du métabolisme soit les métabolites, pourraient permettre de mieux comprendre l'effet systémique de l'alimentation sur le métabolisme et sur l'état de santé métabolique.

Étant donné les interactions complexes qui peuvent survenir entre les facteurs génétiques et alimentaires, il est également intéressant d'étudier l'effet isolé d'un seul nutriment afin de mieux comprendre la variabilité de la réponse observée. La prise d'huile de poisson est reconnue pour son effet hypotriglycéridémiant. Conséquemment, cela en fait un modèle idéal pour tenter de mieux comprendre ce qui affecte la variabilité interindividuelle observée dans la réponse des concentrations de TG. Dans le cadre de ce projet de doctorat, deux voies métaboliques en lien avec la réponse des concentrations de TG à une supplémentation en huile de poisson ont été étudiées, soit la lipogénèse de novo et la bêta-oxydation des acides gras. Les gènes candidats ont été sélectionnés pour leur implication dans l'une de ces voies métaboliques. De plus, les gènes candidats devaient être régulés par les facteurs de transcription encodés par les gènes *PPARA* et/ou *SREBF1*.

Non seulement, les connaissances et la recherche scientifique sont importantes mais également l'application des connaissances et ce projet de doctorat ne pourrait être complet sans un volet relié à ce domaine. De surcroit, selon la littérature, les diététistes ne détiennent pas, pour la plupart, les bases afin d'intégrer à leur pratique professionnelle certains aspects de la génomique nutritionnelle.

Conséquemment, l'objectif général de ce projet de doctorat était d'utiliser la génomique nutritionnelle afin de mieux comprendre l'impact de profils alimentaires sur la santé métabolique et la variabilité de la réponse des facteurs de risque de MCV à une supplémentation en huile de poisson.

Afin d'atteindre cet objectif, plusieurs objectifs spécifiques ont été déterminés et poursuivis à l'aide de trois différentes études. D'abord, l'étude nommée FAS au cours de laquelle j'ai participé autant au volet clinique de l'étude, qu'aux analyses de laboratoire et à l'analyse des données. Les résultats présentés aux chapitres 3, 4, 5, 6, 7 et 8 sont issus de l'étude FAS. Ensuite, l'étude nommée INFOGENE pour laquelle j'ai pu utiliser la base de données afin d'étudier et d'observer certaines associations qui avaient été détectées dans un premier temps durant l'étude FAS suite à une étude de GWAS. Les résultats issus de ces analyses sont présentés au chapitre 9. Finalement, l'étude effectuée auprès d'un échantillon de diététistes membres de l'OPDQ pour laquelle j'ai réalisé toutes les étapes allant de la conception à l'analyse des résultats de même qu'à l'écriture de l'article est présentée au chapitre 10.

Les objectifs spécifiques étaient les suivants :

1. Étudier les associations entre des profils alimentaires dérivés d'une analyse factorielle et l'expression génique sur le génome entier.

    Hypothèse

    1.1 Les profils alimentaires dérivés d'une analyse factorielle sont associés à des profils d'expression génique reliés aux maladies chroniques complexes.

2. Étudier les associations entre des profils alimentaires dérivés d'une analyse factorielle et les profils de métabolites, spécifiquement les acides aminés et les acylcarnitines.

    Hypothèse

    2.1 Les profils alimentaires dérivés d'une analyse factorielle sont associés aux profils de métabolites constitués d'acides aminés et d'acylcarnitine.

3. Étudier les associations entre des SNPs présents dans les gènes *SREBF1*, *ACLY*, *ACACA* et *GCK* encodant pour des enzymes impliquées ou reliées à la voie de la lipogénèse de novo et la réponse des concentrations de TG suite à la supplémentation en huile de poisson.

Hypothèse

3.1 La variabilité génétique des gènes *SREBF1*, *ACLY*, *ACACA* et *GCK* explique une partie de la variabilité interindividuelle observée dans la réponse des concentrations de TG suite à la supplémentation en huile de poisson.

4. Étudier les associations entre des SNPs présents dans les gènes *RXRA*, *CPT1A*, *ACADVL*, *ACAA2*, *ABCD2*, *ACOX1* et *ACAA1* encodant pour des enzymes impliquées dans la voie de la bêta-oxydation des acides gras et la réponse des concentrations de TG suite à la supplémentation en huile de poisson.

Hypothèse

4.1 La variabilité génétique des gènes *RXRA*, *CPT1A*, *ACADVL*, *ACAA2*, *ABCD2*, *ACOX1* et *ACAA1* explique une partie de la variabilité interindividuelle observée dans la réponse des TG suite à la supplémentation en huile de poisson.

5. Étudier les associations entre des SNPs présents dans le gène *SREBF1* et la réponse des concentrations d'insuline, de glucose et de la sensibilité à l'insuline suite à la supplémentation en huile de poisson.

Hypothèse

5.1 La présence de SNPs dans le gène *SREBF1* module la réponse du glucose, de l'insuline et de la sensibilité à l'insuline suite à la supplémentation en huile de poisson.

6. Étudier les associations entre des SNPs identifiés lors d'un précédent GWAS réalisé dans l'étude FAS et les concentrations de TG de même que les proportions d'acides gras dans les phospholipides du plasma dans la cohorte INFOGENE.

Hypothèse

6.1 Les SNPs qui ont été détectés au cours d'un précédant GWAS modulent les concentrations de TG et les proportions d'acides gras dans les phospholipides du plasma.

7. Étudier les facteurs influençant l'intention et le comportement des diététistes de discuter de nutrigénétique avec leur patient/client à l'aide d'un cadre théorique.

Hypothèse

7.1 Les variables de la théorie du comportement planifié sont associées à l'intention et au comportement de discuter de nutrigénétique avec leur patient/client.

Chapitre 3

Associations entre les profils alimentaires et les profils d'expression génique chez des hommes et des femmes en santé : une étude transversale

Annie Bouchard-Mercier, Ann-Marie Paradis, Iwona Rudkowska, Simone Lemieux, Patrick Couture et Marie-Claude Vohl

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Associations between dietary patterns and gene expression profiles of healthy men and women: a cross-sectional study

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This trial was registered at clinicaltrials.gov as NCT01343342.

Résumé

Introduction : L'alimentation régule l'expression génique par plusieurs mécanismes. L'objectif de cette étude était d'examiner l'expression génique en relation avec les profils alimentaires. **Méthodes** : Deux cent cinquante-quatre participants de la région métropolitaine de Québec ont été recrutés. Deux cent dix participants ont complété le protocole de l'étude. Les profils alimentaires ont été dérivés par analyse factorielle à partir de données issues de questionnaires de fréquence alimentaire (QFA). Pour trente participants à jeun, l'ARN a été extrait des cellules mononucléées périphériques sanguines (PBMCs) et les niveaux d'expression de 47 231 transcripts d'ARNm ont été mesurés par les puces *Illumina Human-6 v3 Expression BeadChips*®. Les données issues des micropuces ont été pré-traitées avec le logiciel *Flexarray* et analysées avec *Ingenuity Pathway Anaysis* (IPA). **Résultats** : Deux profils alimentaires ont été identifiés. Le profil alimentaire Prudent était caractérisé par une consommation élevée de légumes, fruits, produits céréaliers à grains entiers et une faible consommation de produits céréaliers raffinés tandis que le profils alimentaire Western était caractérisé par une consommation élevée de produits céréaliers raffinés, desserts, sucreries et viandes transformées. Lorsque les individus avec des scores élevés au profil alimentaire Prudent étaient comparés aux individus avec de faibles scores, 2 083 transcrits étaient différentiellement exprimés chez les hommes, 1 136 transcrits chez les femmes et 59 étaient différentiellement exprimés chez les hommes et les femmes. Pour le profil alimentaire Western, 1 021 transcrits étaient différentiellement exprimés chez les hommes ayant un score élevé comparativement à ceux ayant un score faible, 1 163 chez les femmes et 23 autant chez les hommes que les femmes. IPA indiquait que les gènes différentiellement exprimés pour les deux profils alimentaires étaient présents dans des voies métaboliques reliées au système immunitaire et/ou à la réponse inflammatoire, au cancer et aux maladies cardiovasculaires. **Conclusion** : Les profils d'expression étaient différents en fonction des scores aux profils alimentaires, ce qui pourrait moduler le risque de maladies chroniques.

Abstract

Background: Diet regulates gene expression profiles by several mechanisms. The objective of this study was to examine gene expression in relation with dietary patterns. **Methods:** Two hundred and fifty four participants from the greater Quebec City metropolitan area were recruited. Two hundred and ten participants completed the study protocol. Dietary patterns were derived from a food frequency questionnaire (FFQ) by factor analysis. For 30 participants (in fasting state), RNA was extracted from peripheral blood mononuclear cells (PBMCs) and expression levels of 47,231 mRNA transcripts were assessed using the Illumina Human-6 v3 Expression BeadChips®. Microarray data was pre-processed with Flexarray software and analysed with Ingenuity Pathway Analysis (IPA). **Results**: Two dietary patterns were identified. The Prudent dietary pattern was characterised by high intakes of vegetables, fruits, whole grain products and low intakes of refined grain products and the Western dietary pattern, by high intakes of refined grain products, desserts, sweets and processed meats. When individuals with high scores for the Prudent dietary pattern where compared to individuals with low scores, 2,083 transcripts were differentially expressed in men, 1,136 transcripts in women and 59 transcripts were overlapping in men and women. For the Western dietary pattern, 1,021 transcripts were differentially expressed in men with high versus low scores, 1,163 transcripts in women and 23 transcripts were overlapping in men and women. IPA reveals that genes differentially expressed for both patterns were present in networks related to the immune and/or inflammatory response, cancer and cardiovascular diseases. **Conclusion:** Gene expression profiles were different according to dietary patterns, which probably modulate the risk of chronic diseases.

Introduction

With the knowledge acquired by dietary patterns, dietitians can provide their patients or clients dietary recommendations that take into account not only one nutrient but the overall diet. Dietary patterns have been associated with several cardiovascular risk factors such as blood pressure, obesity, serum lipids, and inflammatory markers such as C-reactive protein (CRP) [1-4]. They have also been related to the risk of mortality from cardiovascular diseases and cancer [5]. In a recent systematic review, the Prudent dietary pattern was associated with a reduced risk of stroke and the Western pattern with an increased risk [6]. The Western dietary pattern was associated with an increased risk of colon cancer [7]. Additionally, Meyerhardt *et al*. [8] have shown an increase in colon cancer recurrence with the Western dietary pattern. Dietary patterns have also been associated with plasma proteomic biomarkers [9]. The Western dietary pattern was positively associated with a group of protein including proteins related to coagulation and lipid metabolism [9]. A few methods to generate dietary patterns, such as factor and cluster analyses, have been described in the literature [10]. The use of dietary patterns assessed by factor analysis has been proven to be a reproducible and valid method among different populations [11-13].

Microarray data can be used to study changes in gene expression for thousands of genes simultaneously. Gene expression studies have observed associations with diseases such as cancer and cardiovascular diseases [14,15]. Diet is an important regulator of gene expression [14,16]. Dietary patterns may impact gene expression through several mechanisms, for example certain dietary compounds bind to transcription factors and regulate their activity such as polyunsaturated fatty acids (PUFA) with *peroxisome proliferator-activated receptors* (*PPARs*) [17]. Studies regarding energy restricted diets and their effects on gene expression levels have observed down-regulation of genes involved in glycolytic and lipid synthesis pathways [40]. Distribution of macronutrients also seems to have an impact on gene expression regulation. Compared to a diet rich in monounsaturated fats, a diet rich in saturated fats resulted in a more proinflammatory gene expression profile [20]. The Mediterranean diet has been associated with a decreased in expression of genes involved in the inflammatory response [21].

To our knowledge, the effects of dietary patterns derived from factor analysis on gene expression profile have never been investigated. Thus, the objective of this study was to examine associations between dietary patterns derived from factor analysis and gene expression profiles.

Methods

Subjects and study design

Two hundred and fifty four participants were recruited between September 2009 and December 2011 from the greater Quebec City metropolitan area through advertisements in local news as well as by electronic messages sent to university students/employees. Women who were pregnant or breastfeeding were excluded. To be eligible, participants had to be between 18 to 50 years of age, non-smokers and free of any thyroid or metabolic disorders requiring treatment, such as diabetes, hypertension, severe dyslipidemia and coronary heart disease requiring treatment. The body mass index (BMI) of the participants was between 25 and 40 kg/m^2. Subjects drinking regularly more than 2 drinks per day, taking omega-3 PUFA (n-3 PUFA) supplements 6 months prior to the study and other medication or supplement affecting lipid and lipoprotein metabolism were excluded. A total of 210 participants completed the protocol which is described elsewhere [22] and were included in this cross-sectional study. Subjects all provided written consent to participate into the study, which was approved by the ethics committees of Laval University Hospital Research Center and Laval University. This trial was registered at clinicaltrials.gov as NCT01343342.

Anthropometric measurements

Body weight, height, and waist circumference were measured according to the procedures recommended by the Airlie Conference [23]. BMI was calculated as weight per meter squared (kg/m^2).

Biochemical parameters

The morning after a 12-hour overnight fast and 48-h alcohol abstinence, blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA. Blood samples were used to identify individuals with metabolic disorders, which were excluded. Plasma was separated by centrifugation (2500 x g for 10 minutes at 4°C), samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (TC) and triglyceride

concentrations were measured using enzymatic assays [24,25]. Infranatant (d >1.006 g/ml) with heparin-manganese chloride was used to precipitate very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) and then determine high-density lipoprotein (HDL) cholesterol concentrations (HDL-C) [26]. The equation of Friedewald was used to estimate LDL-cholesterol (LDL-C) [27]. Non-HDL-C was calculated by subtracting HDL-C from TC. CRP was measured by nephelometry (Prospec equipment Behring) using a sensitive assay, as described previously [28]. Plasma apolipoprotein B-100 (apoB) concentrations were measured by the rocket immunoelectrophoretic method of Laurell, as previously described [29]. Glucose concentrations were determined enzymatically [30] and plasma insulin was measured by radioimmunoassay with polyethylene glycol separation [31].

Blood pressure measurements

Resting blood pressure measurements (three readings) were performed after a 10-min rest in a sitting position, phases I and V of Korotkoff sounds being respectively used for systolic (SBP) and diastolic (DBP) blood pressures [32].

Dietary assessment and food grouping

Dietary intake of the past month was determined by a 91-items validated food frequency questionnaire (FFQ) [33] based on food habits of Quebecers, administered by a registered dietitian (RD). The RD asked participants how often they consumed each type of food: daily, weekly, monthly or none at all during the last month. To make sure each participant estimated correctly the portion eaten, examples of portion size were provided. Data obtained from FFQ were analysed using the Nutrition Data System for Research software version 2011, developed by the Nutrition Coordination Center (University of Minnesota, Minneapolis, MN). All the information was compiled and similar food items from the FFQ were grouped, as previously described [4]. Three criteria were used to form these groups: first, the similarity of nutrient profiles, second, the culinary usage of different types of food (similar to groups used in a previous study [11]) and third, the consideration of groups utilized in other studies to maintain consistency [34]. Some individual food items were

classified separately when their composition differed considerably from other foods (for example, pizza or eggs) or when they represented a different dietary habit (for example, liquor, wine, beer and French fries). On this basis, thirty-seven food groups were formed as described by Paradis *et al*. [4]. Food items from only thirty-five food groups were consumed by the participants in the present study. From these thirty-five food groups, eight were not normally distributed even after logarithmic transformation and were excluded as well. Consequently, twenty-seven foods groups were used for factor analyses to generate dietary patterns.

Food pattern derivation

Food patterns derivation methods have already been described in a previous study [4]. Briefly, variables with abnormal distribution where logarithmically transformed before further analyses. The FACTOR procedure from Statistical Analysis Software (SAS) was used to derive factors. To determine the number of factors to retain, components with eigenvalue > 1, values at Scree test and the interpretability were considered. Food groups with absolute factor loadings ≥ 0.30 were regarded as significant contributors to the pattern. The patterns (derived factors) were named according to the interpretation of the data and to previous literature [4]. Each participant was given a score for both dietary patterns. These scores were calculated from the sum of food groups multiplied by their respective factor loading with the SCORE procedure of SAS. These scores reflect the degree of each participant dietary intakes conforming to a dietary pattern. In order to form two groups for each dietary pattern, participants were divided according to their score. A score ≤ 0 was considered as «low» and a score > 0 was considered as «high».

Transcriptomics analyses

For transcriptomics analyses, the first 30 individuals who completed the study were included (13 men and 17 pre-menopausal women). In human nutrition studies 5 to 10 individuals in each group appear to be sufficient to detect differently expressed genes [35]. The following methods have been described by Rudkowska *et al*. [22]. Briefly, peripheral blood mononuclear cells (PBMCs) were collected into an 8-ml Cell Preparation Tube

(Becton Dickinson, Oakville, Ontario, Canada). Remarkable concordance (<80%) of gene expression profiles between PBMCs and different tissues including liver, kidney, stomach, spleen, prostate, lung, heart, colon and brain, has been reported [36]. Centrifugation at room temperature (1500g, 20 minutes) was executed to separate PBMCs. The RNeasy Plus Mini Kit (QIAGEN, Mississauga, Ontario, Canada) was used to extract total RNA according to the manufacturer's protocol. Microarray analyses were performed after spectrophotometric quantification and verification of the total RNA quality on the Agilent 2100 Bioanalyser (Agilent Technologies, Palo Alto, CA, United States). None of the samples had RNA integrity number (RIN) values less than 8 [37]. Consequently, all samples were included in the microarray analysis. However, as described previously [22], one outlier was excluded due to abnormal hybridization results. Thus, further analyses were conducted with 29 participants. The Illumina TotalPrep RNA Amplification kit (Ambion, Austin, TX, United States) was used to amplify and label 200ng of total RNA. The quality of complimentary RNA (cRNA) was evaluated by capillary electrophoresis on Agilent 2100 Bioanalyzer. 37,804 genes were analysed via expression levels of 48,803 mRNA transcripts with the Human-6 v3 Expression BeadChips® (Illumina, San Diego, CA, United States). The McGill University and Génome Québec Innovation Center (Montreal, Quebec, Canada) performed hybridization according to the manufacturer's instructions, as previously described [22]. Validation of the expression levels were assessed previously by polymerase chain reaction (PCR) [22].

Analysis of microarray data

Microarray data was analysed with Flexarray software [38]. The lumi Bioconductor package algorithm included in Flexarray software, was used to pre-process and normalize Illumina microarray data. Background correction was assessed using negative controls followed by $\log_2$ to stabilize variance and quantile normalization. According to Shi *et al*. [39] the use of control probes during background correction minimize false discovery rate (FDR). Fold changes obtained with this method also relate more to fold changes observed with PCR validation tests [39]. Participants where then stratified according to sex and scores for Prudent and Western dietary patterns. To assess differences (separately for men and for women) in gene expression levels between high versus low scores for Prudent and

Western dietary patterns, the Significant Analysis of Microarray (SAM) was performed. SAM is an adaptation of *t*-test for microarray data which assigns a score to each gene according to changes in gene expression relative to the standard deviation of repeated measurements [40]. SAM uses permutation of the repeated measurements to estimate FDR. Transcripts were declared differently expressed only when $P < 0.05$ and fold changes were either < 0.8 (down-regulated) or > 1.2 (up-regulated), as previously described [22].

Biological pathway analyses

Ingenuity Pathway Analysis (IPA) system (Ingenuity® System, www.ingenuity.com) was used to verify if differentially expressed genes were related more than expected by chance to networks, diseases and canonical pathways. IPA allows adding structure to the vast amount of data generated by microarrays. To begin, an input file containing fold changes and P values of all probe sets was uploaded into IPA system. From this file, dataset in Core Analysis was produced. General settings for IPA system as «Ingenuity® Knowledge Base (genes)» and «considered only molecules and/or relationships for humans» were used. IPA calculates a P value based on the right-tailed Fisher's exact test for each canonical pathway, which is a measure of the likelihood that the association of a data set with a pathway is due to random chance. A cutoff of 1.2 was set. Relevant pathways with P values smaller than 0.05, were taken into account. IPA suggests that canonical pathways with P values higher than 0.05 may also be biologically relevant. Furthermore, no direction is associated with pathways, in other words, they cannot be qualified as up- or down-regulated. Significantly relevant canonical pathways related to cancer, cardiovascular diseases, immune system and inflammation were considered. IPA also processes «Downstream Effects Analysis» which is based on expected causal effects between genes and functions. These causal effects are derived from literature and compiled in «Ingenuity® Knowledge Base». «Downstream Effects Analysis» compares the direction of the differently expressed genes with expectations based on the literature and predicts for each function a direction change using the «regulation z-score algorithm». In other words, if the observed direction change is mostly consistent with a particular activation state (increase or decrease) then IPA makes a directional prediction. The z-score algorithm is designed to reduce the chance that random data will generate significant predictions. Z-scores ≥ 2, indicate that the function is

significantly increased and z-scores ≤ -2, indicate that the function is significantly decreased. IPA also calculates a P value with the Fisher's Exact Test which represents the likelihood that the association between a set of genes from the experimental data set and a related function is due to random association.

Statistical analyses

Comparisons between individuals with high and low dietary pattern scores were performed through the General Linear Model (GLM) procedure and using the type 3 sum of squares (for unbalanced study design). Variables with abnormal distribution were logarithmically transformed. Age, BMI and energy intakes (for all dietary intakes, except energy intakes) were included as potential confounders. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed with SAS statistical package (version 9.2; SAS Institute, Inc., Cary, NC, USA).

Results

Two main dietary patterns were derived from factor analysis. Factor loadings for both dietary patterns are listed in Table 3.1. A factor loading ≥ 0.30 indicates a strong positive association with the dietary pattern whereas a factor loading ≤ -0.30 indicates a strong inverse association with the dietary pattern. The Prudent dietary pattern was positively associated with vegetables, fruits, whole grain products food groups and inversely associated with refined grain products food group. The Western dietary pattern was positively associated with refined grain products, desserts, sweets and processed meat food groups.

Descriptive characteristics of the 29 study participants are presented separately for men and women in Table 3.2 and Table 3.3 according to low (≤0) or high (>0) scores for both dietary patterns. Fasting insulin concentrations were significantly (P=0.03) lower in men with high scores for the Prudent dietary pattern, as compared to men with low scores for the Prudent dietary pattern. Also, men with a high score for the Western dietary pattern had a significantly higher systolic and diastolic blood pressure (P=0.0008 and P=0.01, respectively) than men with a low scores and a trend (P=0.05) was observed for higher fasting glucose concentrations. Women with a high score for the Western dietary pattern had higher fasting glucose concentrations (P=0.03) than those with a low score. When comparing cardiovascular risk factors for women with high scores versus low scores for the Prudent dietary pattern, only trends were observed. Women with high scores for the Prudent dietary pattern had a higher BMI (P=0.09) but lower plasma triglyceride concentrations (P=0.07). When comparing mean dietary intakes of men (n=12) between women (n=17), men had significantly higher total fat (33.85%±4.16% and 29.81%±3.62% respectively, P=0.01) and monounsaturated fat intakes (14.40%±1.94% versus 12.11%±1.79% respectively, P=0.004) than women, independently of age and BMI. Saturated fat, polyunsaturated fat and total fiber intakes were not significantly different when age and BMI were included as potential confounders. For the entire cohort (210 participants), only associations for the Prudent pattern were observed. Men (n=97) with high scores had lower fasting insulin (P=0.04) and glucose concentrations (P=0.003) as

compared to men with low scores (data not shown). Women (n=113) with high Prudent dietary pattern scores had lower apoB (P=0.04) and TC (P=0.04) concentrations (data not shown).

Intakes of the most associated food groups with the dietary patterns are presented in Table 3.4 and Table 3.5. Men with high scores for the Prudent dietary pattern had higher intakes of vegetables (P=0.03), fruits (P=0.02), whole grain products (P=0.004), fish (P=0.04), and nuts (P=0.02) than men with low scores. Women with high scores for the Prudent dietary pattern had higher intakes of non-hydrogenated fats (P=0.006), and lower intakes of sweets (P=0.05) than women with low scores. For the Western dietary pattern, both men and women with high scores had higher intakes of sweets than individuals with low scores (P=0.03 and P=0.007, respectively). Only for the women, the intake of desserts (P<0.0001) was significantly higher with a high Western dietary pattern score. The men with high scores for the Western dietary pattern had lower intakes of vegetables (P=0.03).

As previously described [22], approximately 55% of transcripts were detected in the PBMCs of study participants. As shown in Figure 3.1, when individuals with high scores for the Prudent dietary pattern where compared to individuals with low scores, 2,083 transcripts were differentially expressed in men, 1,136 transcripts in women and 59 transcripts were overlapping. In men comparing high scores to low scores for the Prudent dietary pattern, 1,045 transcripts were down-regulated (49%) and 1,097 were up-regulated (51%). For the women, 355 transcripts were down-regulated (30%) and 840 were up-regulated (70%). As shown in Figure 3.2, for the Western dietary pattern, 1,021 transcripts were differentially expressed in men with high versus low scores, 1,163 transcripts were differentially expressed in women and 23 transcripts were overlapping in men and women. In men comparing high scores to low scores for the Western dietary pattern, 410 transcripts were down-regulated (39%) and 634 were up-regulated (61%). For women comparing high scores to low scores for the Western dietary pattern, 440 transcripts were down-regulated (37%) and 746 were up-regulated (63%).

According to IPA, few canonical pathways were significantly modified in men and women when comparing high scores to low scores for both dietary patterns (Figure 3.3(A), 3.3(B), 3.4(A) and 3.4(B)).

Interestingly, IPA was able to predict an activation state for different functions when comparing high scores to low scores for the Prudent dietary pattern. In men, a decrease in colony formation (z-score -2.10; 40 molecules; P=0.009), decreased adhesion of prostate cancer cell lines (z-score -2.17; 6 molecules; P=0.01), increased cell death of connective tissue cells (z-score 2.69; 19 molecules; P=0.01) and smooth muscle cells (z-score 2.55; 6 molecules; P=0.03), an increase in the metabolism of phosphatidic acid (PA) (z-score 2.23; 14 molecules; P=0.04), of phospholipid (z-score 2.22; 17 molecules; P=0.04) and in apoptosis of connective tissue cells (z-score 2.77; 13 molecules; P=0.04) were predicted. For women, IPA predicted a decreased tumorigenesis (z-score -2.30; 258 molecules; P=0.0001), mitosis of tumor cell lines (z-score -2.39; 11 molecules; P=0.003), tyrosine phosphorylation (z-score -2.03; 13 molecules; P=0.003) and survival of tumor cell lines (z-score -2.11; 33 molecules; P=0.03). When comparing high scores to low scores for the Western dietary pattern in men, IPA predicted a decrease in apoptosis of tumor cell lines (z-score -3.42; 54 molecules; P=0.005), a decrease in cell death of tumor cell lines (z-score -2.73; 57 molecules; P=0.08), an increase in quantity of PA (z-score 2.11; 5 molecules; P=0.02) and of carbohydrate (z-score 2.31; 6 molecules; P=0.04). For women according to the Western dietary pattern, IPA was unable to make a prediction for the activation state of any function.

Discussion

The dietary patterns derived in this study resemble «Prudent» and «Western» dietary patterns from the literature [41]. The Prudent dietary pattern is usually associated with vegetables, fruits, whole grain products, fish and non-hydrogenated fats [4,42], whereas the Western dietary pattern is described as high in red meats, processed meats, refined grains, French fries and sweets/desserts [4,42]. Participants with high scores for the Prudent dietary pattern (~48%) approached dietary recommendations for vegetables and fruits consumption of Canada's Food Guide [43] (approximately 7 servings per day). They also ate more than half of their grain products as whole grains (approximately 4 servings of whole grain products and 2 servings of refined grain products per day). The intakes of meat and alternatives as well as milk and alternatives were around 3 servings per day (data not shown). Thus, high scores for the Prudent dietary pattern were clearly related with Canada's healthy eating guidelines [43].

It has been observed that high insulin concentrations often relate to insulin resistance [44]. This may indicate a higher risk of insulin resistance among individuals with low Prudent dietary pattern scores which had higher than normal fasting insulin concentrations [44]. Even though individuals with high Western dietary pattern scores had higher fasting glucose than individuals with low scores, these values remained within the normal range (<5.6 mmol/L [45]). Interestingly, individuals with high scores for the Western dietary pattern also had higher systolic and diastolic blood pressure. For individuals with high scores for the Prudent dietary pattern, blood pressure also seemed lower than for individuals with low scores. These associations with dietary patterns and blood pressure have also been observed in other populations [1,2]. For the entire cohort (n=210), high scores for the Prudent dietary pattern were associated with a more favorable blood lipid profile (data not shown).

Major differences in gene expression profiles were observed between men and women. These differences had been observed previously by our research group [22]. According to the scores for the Prudent dietary pattern, only the B Cell Receptor Signaling pathway was

significantly different both for men and women. Sex-specific differences in adipose tissue gene expression have been studied but gene expression differences according to sex in PBMCs are not as well documented [46]. Mechanisms involving sex hormones such as estrogen on transcription factors might partly explain these differences [47]. Moreover, Kawasaki *et al*. [48] reported fluctuations of the expression of certain genes related to immune and/or inflammatory response according to the menstrual cycle among women. In this study, women were pre-menopausal and the phase of the menstrual cycle was not taken into account, which might explain part of the differences observed.

For participants with high comparatively to low scores for the Prudent dietary pattern, IPA revealed 9 canonical pathways related to immune and/or inflammatory response and 6 to cancer whereas for the Western dietary pattern, 5 pathways related to cancer, 6 to immune and/or inflammatory response and 3 to cardiovascular signaling. Interestingly, predictions made by IPA were pointing towards directional changes in functions which may lead to a decreased risk of cancer among individuals with high scores for the Prudent dietary pattern which is considered a «healthier» pattern (observed among men and women) and changes in functions towards a potential increase of the risk of cancer for individuals with high scores for the Western diet (only observed among men). The PA metabolism also appeared to be modulated with both dietary patterns. PA is mainly formed by the hydrolysis of phosphatidylcholine by phospholipase D [49]. PA is important in heart function and has also been associated with cardiac hypertrophy [50]. For women with high scores for the Prudent dietary pattern, IPA predicted a decrease in tyrosine phosphorylation. Tyrosine phosphorylation may have a protective effect on cancer by reversing the effect of some protein kinase but it may also have a detrimental effect [51]. For example, an increase of phosphorylation in vascular endothelial cadherin tyrosine has been observed after the attachment of invasive breast cancer cells to endothelial cells [52].

Van Dijk *et al*. [20] have observed a more pro-inflammatory gene expression profile following a diet high in saturated fat compared to a diet high in monounsaturated fat. Saturated fats can modulate the expression of Toll-like receptors (TLRs) therefore increasing the expression of pro-inflammatory genes [53]. In women, according to the

Western pattern, the TLRs signaling pathway was different in participants with high versus low scores. Genes within the pathway appeared to be mostly up-regulated which could indicate an increase in the inflammatory response. Conversely, Bowens *et al*. [54], using shakes containing various amounts of saturated, polyunsaturated and monounsatured fats, observed an increased in the expression of genes involved in TLRs signaling following the high PUFA shake. However, these results were observed in the postprandial state which could explain discrepancies between studies. In addition, the B Cell Receptor Signaling pathway was different according to scores for the Prudent dietary pattern and this was also observed by Bowens *et al*. with the high PUFA shake [54]. In the present study, the intake of PUFA was higher among individuals with high scores for the Prudent dietary pattern. This pathway is important in humoral immune response and has also been related with chronic lymphocytic leukemia [55]. We hypothesise that the impact of the Prudent dietary pattern on B Cell Receptor Signaling pathway was beneficial due to the predictive results given by IPA and literature on the protective effect of a «healthy» dietary pattern on cancer [56]. When examining pathways common to both dietary patterns, only one was common to the Prudent and Western dietary patterns (among women), the Role of Nuclear Factor of Activated T cells (NFAT) in regulation of the immune response with only one gene overlapping both dietary patterns. The NFAT family of transcription factors induce gene transcription during the immune response. These transcription factors have been linked with cardiac hypertrophy which increases the risk of cardiovascular diseases and have a dual role in cancer acting as a tumor suppressors as well as oncogenes [77].

Results observed in this exploratory study support the scientific evidence regarding the beneficial effects of the consumption of a healthy diet and the deleterious impacts of a Western dietary pattern. These results also seem to indicate that gene expression profiles and expression of genes in pathways related to chronic disease are influenced by the presence of a few or more dietary characteristics according to a dietary pattern (high versus low scores). However, due to the small number of participants, these results should be interpreted with caution. In addition, many other factors associated with a healthy or unhealthy lifestyle may impact gene expression. For example, physical activity has an effect on gene expression profiles and was not taken into account in the analyses [59].

Conclusion

Data retrieved from this nutrigenomic study provide valuable information on biologically relevant pathways that might relate to chronic disease prevention or initiation. Transcriptomics analysis gives us further insights to understand the global effect of dietary patterns on health. In this study, both the Prudent and Western dietary patterns were related to biological pathways associated with cancer, immune and/or inflammatory response and cardiovascular signaling. It appears from these results that the Prudent dietary pattern has a protective effect on cancer initiation or development and the opposite is observed for the Western dietary pattern. However, these data reflect gene expression profile and statistical predictions and need to be confirmed by further research.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

SL, PC, IR, AMP and MCV designed research; AMP and ABM conducted research; SL, PC, IR, AMP and MCV provided essential reagents or provided essential materials; ABM analyzed data and performed statistical analysis; ABM wrote paper; ABM, SL, PC, IR, AMP and MCV had primary responsibility for final content; All authors read and approved the final manuscript.

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References

1. Sadakane A, Tsutsumi A, Gotoh T, Ishikawa S, Ojima T, Kario K *et al*.: Dietary patterns and levels of blood pressure and serum lipids in a Japanese population. *J Epidemiol* 2008, 18: 58-67.
2. Mikkila V, Rasanen L, Raitakari OT, Marniemi J, Pietinen P, Ronnemaa T *et al*.: Major dietary patterns and cardiovascular risk factors from childhood to adulthood. The Cardiovascular Risk in Young Finns Study. *Br J Nutr* 2007, 98: 218-225.
3. Meyer J, Doring A, Herder C, Roden M, Koenig W, Thorand B: Dietary patterns, subclinical inflammation, incident coronary heart disease and mortality in middle-aged men from the MONICA/KORA Augsburg cohort study. *Eur J Clin Nutr* 2011, 65: 800-807.
4. Paradis AM, Godin G, Perusse L, Vohl MC: Associations between dietary patterns and obesity phenotypes. *Int J Obes (Lond)* 2009, 33: 1419-1426.
5. Heidemann C, Schulze MB, Franco OH, van Dam RM, Mantzoros CS, Hu FB: Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. *Circulation* 2008, 118: 230-237.
6. Sherzai A, Heim LT, Boothby C, Sherzai AD: Stroke, food groups, and dietary patterns: a systematic review. *Nutr Rev* 2012, 70: 423-435.
7. Yusof AS, Isa ZM, Shah SA: Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies (2000-2011). *Asian Pac J Cancer Prev* 2012, 13: 4713-4717.
8. Meyerhardt JA, Niedzwiecki D, Hollis D, Saltz LB, Hu FB, Mayer RJ *et al*.: Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. *JAMA* 2007, 298: 754-764.
9. Garcia-Bailo B, Brenner DR, Nielsen D, Lee HJ, Domanski D, Kuzyk M *et al*.: Dietary patterns and ethnicity are associated with distinct plasma proteomic groups. *Am J Clin Nutr* 2012, 95: 352-361.
10. Hu FB: Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002, 13: 3-9.
11. Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A *et al*.: Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* 1999, 69: 243-249.
12. Khani BR, Ye W, Terry P, Wolk A: Reproducibility and validity of major dietary patterns among Swedish women assessed with a food-frequency questionnaire. *J Nutr* 2004, 134: 1541-1545.
13. Nanri A, Shimazu T, Ishihara J, Takachi R, Mizoue T, Inoue M *et al*.: Reproducibility and validity of dietary patterns assessed by a food frequency questionnaire used in the 5-year follow-up survey of the Japan Public Health Center-Based Prospective Study. *J Epidemiol* 2012, 22: 205-215.

14. Ross SA: Evidence for the relationship between diet and cancer. *Exp Oncol* 2010, 32: 137-142.

15. Schnabel RB, Baccarelli A, Lin H, Ellinor PT, Benjamin EJ: Next steps in cardiovascular disease genomic research--sequencing, epigenetics, and transcriptomics. *Clin Chem* 2012, 58: 113-126.

16. Barnes S: Nutritional genomics, polyphenols, diets, and their impact on dietetics. *J Am Diet Assoc* 2008, 108: 1888-1895.

17. Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W: Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc Natl Acad Sci U S A* 1993, 90: 2160-2164.

18. Ong KR, Sims AH, Harvie M, Chapman M, Dunn WB, Broadhurst D *et al.*: Biomarkers of dietary energy restriction in women at increased risk of breast cancer. *Cancer Prev Res (Phila)* 2009, 2: 720-731.

19. Franck N, Gummesson A, Jernas M, Glad C, Svensson PA, Guillot G *et al.*: Identification of adipocyte genes regulated by caloric intake. *J Clin Endocrinol Metab* 2011, 96: E413-E418.

20. van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG *et al.*: A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. *Am J Clin Nutr* 2009, 90: 1656-1664.

21. Yubero-Serrano EM, Gonzalez-Guardia L, Rangel-Zuniga O, Delgado-Lista J, Gutierrez-Mariscal FM, Perez-Martinez P *et al.*: Mediterranean diet supplemented with coenzyme Q10 modifies the expression of proinflammatory and endoplasmic reticulum stress-related genes in elderly men and women. *J Gerontol A Biol Sci Med Sci* 2012, 67: 3-10.

22. Rudkowska I, Paradis AM, Thifault E, Julien P, Tchernof A, Couture P *et al.*: Transcriptomic and metabolomic signatures of an n-3 polyunsaturated fatty acids supplementation in a normolipidemic/normocholesterolemic Caucasian population. *J Nutr Biochem* 2012.

23. Callaway, C.W.; Chumlea, W.C.; Bouchard, C.; Himes, J.H.; Lohman, T.G.; Martin, A.D.; Mitchell, C.D.; Mueller, W.H.; Roche, A.F.; Seefeldt, V.D. Standardization of Anthropometric Measurements. In The Airlie (VA) Consensus Conference; Lohman, T., Roche, A., Martorel, R., Eds.; Human Kinetics Publishers: Champaign, IR, USA, 1988; pp. 39-80.

24. McNamara JR, Schaefer EJ: Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin Chim Acta* 1987, 166: 1-8.

25. BURSTEIN M, SAMAILLE J: [On a rapid determination of the cholesterol bound to the serum alpha- and beta-lipoproteins]. *Clin Chim Acta* 1960, 5: 609.

26. Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L *et al*.: Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. *Clin Chem* 1978, 24: 853-856.

27. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972, 18: 499-502.

28. Pirro M, Bergeron J, Dagenais GR, Bernard PM, Cantin B, Despres JP *et al*.: Age and duration of follow-up as modulators of the risk for ischemic heart disease associated with high plasma C-reactive protein levels in men. *Arch Intern Med* 2001, 161: 2474-2480.

29. Laurell CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 1966, 15: 45-52.

30. Richterich R, Dauwalder H: [Determination of plasma glucose by hexokinase-glucose-6-phosphate dehydrogenase method]. *Schweiz Med Wochenschr* 1971, 101: 615-618.

31. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 1971, 33: 732-738.

32. Padwal RS, Hemmelgarn BR, Khan NA, Grover S, McKay DW, Wilson T *et al*.: The 2009 Canadian Hypertension Education Program recommendations for the management of hypertension: Part 1--blood pressure measurement, diagnosis and assessment of risk. *Can J Cardiol* 2009, 25: 279-286.

33. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S: Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. *Nutr J* 2004, 3: 13.

34. Newby PK, Tucker KL: Empirically derived eating patterns using factor or cluster analysis: a review. *Nutr Rev* 2004, 62: 177-203.

35. Fu WJ, Stromberg AJ, Viele K, Carroll RJ, Wu G: Statistics and bioinformatics in nutritional sciences: analysis of complex data in the era of systems biology. *J Nutr Biochem* 2010, 21: 561-572.

36. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA: The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J Lab Clin Med* 2006, 147: 126-132.

37. Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M *et al*.: The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Mol Biol* 2006, 7: 3.

38. Michal Blazejczyk, Mathieu Miron, Robert Nadon. *FlexArray*: Statistical Data Analysis Software for Gene Expression Microarrays, Made with Life Scientists in Mind. Canadian Bioinformatics Help Desk (CBHD) Newsletter 2007;69.

39. Shi W, Oshlack A, Smyth GK: Optimizing the noise versus bias trade-off for Illumina whole genome expression BeadChips. *Nucleic Acids Res* 2010, 38: e204.

40. Tusher VG, Tibshirani R, Chu G: Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A* 2001, 98: 5116-5121.

41. Bhupathiraju SN, Tucker KL: Coronary heart disease prevention: nutrients, foods, and dietary patterns. *Clin Chim Acta* 2011, 412: 1493-1514.

42. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willett WC: Prospective study of major dietary patterns and risk of coronary heart disease in men. *Am J Clin Nutr* 2000, 72: 912-921.

43. Health Canada. Eating well with Canada's Food Guide. Ottawa: Publications Health Canada, 2011.

44. Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y, Roth J: Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care* 2008, 31 Suppl 2: S262-S268.

45. Standards of medical care in diabetes--2012. *Diabetes Care* 2012, 35 Suppl 1: S11-S63.

46. Klaus S, Keijer J: Gene expression profiling of adipose tissue: individual, depot-dependent, and sex-dependent variabilities. *Nutrition* 2004, 20: 115-120.

47. Yoon M: PPARalpha in Obesity: Sex Difference and Estrogen Involvement. *PPAR Res* 2010, 2010.

48. Kawasaki M, Sekigawa I, Nozawa K, Kaneko H, Takasaki Y, Takamori K *et al.*: Changes in the gene expression of peripheral blood mononuclear cells during the menstrual cycle of females is associated with a gender bias in the incidence of systemic lupus erythematosus. *Clin Exp Rheumatol* 2009, 27: 260-266.

49. Tappia PS, Dent MR, Dhalla NS: Oxidative stress and redox regulation of phospholipase D in myocardial disease. *Free Radic Biol Med* 2006, 41: 349-361.

50. Dhalla NS, Xu YJ, Sheu SS, Tappia PS, Panagia V: Phosphatidic acid: a potential signal transducer for cardiac hypertrophy. *J Mol Cell Cardiol* 1997, 29: 2865-2871.

51. Laczmanska I, Sasiadek MM: Tyrosine phosphatases as a superfamily of tumor suppressors in colorectal cancer. *Acta Biochim Pol* 2011, 58: 467-470.

52. Haidari M, Zhang W, Caivano A, Chen Z, Ganjehei L, Mortazavi A *et al.*: Integrin alpha2-beta1 Mediates Tyrosine Phosphorylation of Vascular Endothelial Cadherin Induced by Invasive Breast Cancer Cells. *J Biol Chem* 2012.

53. Lee JY, Hwang DH: The modulation of inflammatory gene expression by lipids: mediation through Toll-like receptors. *Mol Cells* 2006, 21: 174-185.

54. Bouwens M, Grootte BM, Jansen J, Muller M, Afman LA: Postprandial dietary lipid-specific effects on human peripheral blood mononuclear cell gene expression profiles. *Am J Clin Nutr* 2010, 91: 208-217.

55. Burger JA: Inhibiting B-cell receptor signaling pathways in chronic lymphocytic leukemia. *Curr Hematol Malig Rep* 2012, 7: 26-33.

56. Kushi LH, Doyle C, McCullough M, Rock CL, Demark-Wahnefried W, Bandera EV *et al.*: American Cancer Society Guidelines on nutrition and physical activity for

cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. *CA Cancer J Clin* 2012, 62: 30-67.

57. Liu Q, Chen Y, Auger-Messier M, Molkentin JD: Interaction between NFkappaB and NFAT coordinates cardiac hypertrophy and pathological remodeling. *Circ Res* 2012, 110: 1077-1086.

58. Robbs BK, Cruz AL, Werneck MB, Mognol GP, Viola JP: Dual roles for NFAT transcription factor genes as oncogenes and tumor suppressors. *Mol Cell Biol* 2008, 28: 7168-7181.

59. Catoire M, Mensink M, Boekschoten MV, Hangelbroek R, Muller M, Schrauwen P *et al*.: Pronounced effects of acute endurance exercise on gene expression in resting and exercising human skeletal muscle. *PLoS One* 2012, 7: e51066.

Table 3.1 Factor loadings for Prudent and Western dietary patterns. (n=210)

Food groups (servings/day)	**Factor 1* Prudent**	**Factor 2* Western**
Vegetables	**0.71**	0.03
Fruits	**0.60**	-0.01
Whole grain products	**0.53**	0.21
Non-hydrogenated fat	**0.46**	0.02
Refined grain products	**-0.45**	**0.39**
Desserts	-0.01	**0.80**
Sweets	0.09	**0.77**
Beer	0.01	-0.03
Coffee	0.06	0.15
Poultry	-0.004	-0.06
Red meat	-0.11	0.11
Potatoes other than French fries	0.09	0.16
Processed meat	-0.10	**0.33**
Legumes	0.15	0.13
Tea	0.08	-0.02
High-fat dairy products	0.13	0.13
Low-fat dairy products	0.27	0.07
Eggs	0.27	-0.05
Cream soup	-0.11	0.12
Pizza	-0.23	-0.03
Fish and other sea food	0.28	-0.03
Fruit juices	-0.14	0.02
Nuts	0.26	0.06
Vegetable juices	0.12	0.05
Condiments	0.18	0.06
Snacks	-0.11	0.18
Saturated fat (butter and lard)	0.04	0.06
Variance explained (%)	12.96	10.62

*Principal component analysis using the FACTOR procedure.
Factor loading ≥ 0.30 or ≤ -0.30 are marked in bold.

Table 3.2 Descriptive characteristics of the study participants for men according to dietary pattern scores.

	Prudent dietary pattern (n=12)		*P*	Western dietary pattern (n=12)		*P*
	Low score (n=5)	High score (n=7)		Low score (n=3)	High score (n=9)	
Age (y)	34.40 ± 10.99	32.71 ± 5.88	0.74	31.00 ± 2.65	34.22 ± 9.09	0.57
BMI (kg/m^2)	28.86 ± 3.62	29.63 ± 5.56	0.88[1]	32.04 ± 8.04	28.40 ± 3.18	0.35[1]
Waist circumference (cm)	96.83 ± 9.24	95.53 ± 12.86	0.45[2]	102.87 ± 15.26	93.81 ± 9.27	0.68[2]
Systolic blood pressure (mmHg)	113.80 ± 5.89	107.86 ± 7.24	0.18[2]	101.67 ± 2.08	113.22 ± 5.56	0.0008[2]
Diastolic blood pressure (mmHg)	76.40 ± 2.70	68.86 ± 8.32	0.12[2]	63.33 ± 1.53	74.89 ± 6.17	0.01[2]
Fasting glucose (mmol/L)	5.20 ± 0.31	4.83 ± 0.63	0.32[2]	4.40 ± 0.46	5.18 ± 0.41	0.05[2]
Fasting insulin (pmol/L)	123.00 ± 3.49	73.29 ± 23.56	0.03[2]	73.67 ± 16.65	100.78 ± 55.29	0.14[2]
CRP (mg/L)	4.33 ± 5.81	2.02 ± 2.24	0.47[2]	2.88 ± 2.92	3.02 ± 4.52	0.92[2]
Total-C (mmol/L)	5.77 ± 0.85	5.08 ±1.04	0.33[2]	5.60 ± 0.98	5.29 ± 1.04	0.57[2]
LDL-C (mmol/L)	3.69 ± 0.55 (n=4)	3.18 ± 0.97	0.47[2]	3.74 ± 0.60	3.22 ± 0.92 (n=8)	0.33[2]
HDL-C (mmol/L)	1.09 ± 0.22	1.33 ± 0.39	0.23[2]	1.10 ± 0.35	1.27 ± 0.35	0.62[2]
Triglycerides (mmol/L)	2.48 ± 1.88	1.24 ± 0.54	0.20[2]	1.65 ± 0.29	1.79 ± 1.59	0.92[2]
ApoB (g/L)	1.14 ± 0.23	0.94 ± 0.27	0.26[2]	1.14 ± 0.11	0.98 ± 0.29	0.38[2]
Dietary intakes						
Total fat (%)	34.22 ± 2.23	33.58 ± 5.32	0.78[2]	32.52 ± 7.43	34.29 ± 3.02	0.40[2]
Saturated fat (%)	12.24 ± 1.47	9.77 ± 2.43	0.09[2]	8.57 ± 3.34	11.54 ± 1.57	0.03[2]
Monounsaturated fat (%)	14.34 ± 0.85	14.44 ± 2.53	0.97[2]	14.44 ± 3.06	14.38 ± 1.68	0.93[2]
Polyunsaturated fat (%)	4.98 ± 0.57	6.61 ± 1.50	0.08[2]	6.57 ± 0.91	5.72 ± 1.56	0.49[2]
Total fiber (g)	19.40 ± 8.31	31.50 ± 7.53	0.03[2]	26.04 ± 2.29	26.60 ± 11.36	0.82[2]

All values are means ± SDs. Tests for trends or differences were made by using generalized linear models.

[1]Adjusted for age.

[2]Adjusted for age and BMI.

Table 3.3 Descriptive characteristics of the study participants for women according to dietary pattern scores.

	Prudent dietary pattern (n=17)			Western dietary pattern (n=17)		
	Low score (n=10)	High score (n=7)	*P*	Low score (n=11)	High score (n=6)	*P*
Age (y)	34.80 ± 11.16	33.86 ± 9.51	0.86	35.45 ± 9.78	32.50 ± 11.64	0.59
BMI (kg/m^2)	28.09 ± 2.71	30.94 ± 3.58	0.09[1]	29.01 ± 2.73	29.73 ± 4.48	0.68[1]
Waist circumference (cm)	82.82 ± 8.07	91.14 ± 7.87	0.31[2]	86.05 ± 8.85	86.60 ± 9.58	0.88[2]
Systolic blood pressure (mmHg)	106.40 ± 8.42	103.57 ± 8.92	0.14[2]	103.45 ± 9.29	108.50 ± 6.12	0.11[2]
Diastolic blood pressure (mmHg)	67.70 ± 8.84	72.00 ± 8.79	0.91[2]	68.73 ± 9.23	70.83 ± 8.66	0.57[2]
Fasting glucose (mmol/L)	5.20 ± 1.08	4.66 ± 0.58	0.30[2]	4.74 ± 0.42	5.42 ± 1.42	0.03[2]
Fasting insulin (pmol/L)	83.10 ± 23.32	77.71 ± 31.39	0.15[2]	78.82 ± 27.99	84.67 ± 24.31	0.78[2]
CRP (mg/L)	7.03 ± 12.01	6.14 ± 9.90	0.51[2]	6.65 ± 11.32	6.69 ± 11.01	0.41[2]
Total-C (mmol/L)	5.37 ± 1.55	4.95 ± 1.23	0.85[2]	5.04 ± 1.35	5.48 ± 1.58	0.35[2]
LDL-C (mmol/L)	3.06 ± 1.12	2.76 ± 1.20	0.92[2]	2.90 ± 1.17	3.01 ± 1.15	0.59[2]
HDL-C (mmol/L)	1.64 ± 0.66	1.68 ± 0.39	0.49[2]	1.52 ± 0.47	1.91 ± 0.64	0.14[2]
Triglycerides (mmol/L)	1.48 ± 0.77	1.09 ± 0.52	0.07[2]	1.36 ± 0.72	1.25 ± 0.69	0.64[2]
ApoB (g/L)	0.98 ± 0.28	0.81 ± 0.29	0.37[2]	0.90 ± 0.28	0.94 ± 0.33	0.63[2]
Dietary intakes						
Total fat (%)	29.17 ± 4.02	30.73 ± 3.00	0.61[2]	28.55 ± 3.59	32.13 ± 2.50	0.08[2]
Saturated fat (%)	10.45 ± 1.54	9.45 ± 2.32	0.22[2]	9.32 ± 1.96	11.35 ± 0.81	0.05[2]
Monounsaturated fat (%)	11.45 ± 1.84	13.04 ± 1.33	0.13[2]	11.67 ± 1.91	12.90 ± 1.36	0.28[2]
Polyunsaturated fat (%)	4.81 ± 0.81	5.71 ± 0.69	0.05[2]	5.14 ± 0.98	5.26 ± 0.70	0.90[2]
Total fiber (g)	19.78 ± 3.88	28.14 ± 6.77	0.03[2]	24.14 ± 7.11	21.54 ± 5.77	0.36[2]

All values are means ± SDs. Tests for trends or differences were made by using generalized linear models.
[1]Adjusted for age.
[2]Adjusted for age and BMI.

Table 3.4 Food group intakes (number of servings) for men according to dietary pattern scores.

	Prudent dietary pattern (n=12)		*P*	Western dietary pattern (n=12)		*P*
	Low score (n=5)	High score (n=7)		Low score (n=3)	High score (n=9)	
Vegetables*	1.90 ± 0.65	3.93 ± 1.80	0.03[1]	4.47 ± 2.57	2.62 ± 1.23	0.03[1]
Fruits*	1.34 ± 1.18	3.20 ± 0.95	0.02[1]	2.98 ± 0.50	2.24 ± 1.55	0.28[1]
Whole grain products*	1.82 ± 1.43	4.37 ± 1.35	0.004[1]	3.33 ± 0.96	3.30 ± 2.12	0.35[1]
Non-hydrogenated fats*	4.02 ± 4.19	4.58 ± 3.02	0.75[1]	3.96 ± 2.58	4.47 ± 3.74	0.28[1]
Refined grain products*	3.26 ± 2.33	2.27 ± 1.30	0.23[1]	1.40 ± 1.25	3.10 ± 1.77	0.38[1]
Fish and other sea food*	0.61 ± 0.66	1.71 ± 0.88	0.04[1]	1.22 ± 0.87	1.26 ± 1.03	0.60[1]
Nuts*	0.33 ± 0.13	1.88 ± 1.88	0.02[1]	1.24 ± 0.68	1.23 ± 1.85	0.38[1]
Desserts*	1.03 ± 0.43	0.86 ± 0.85	0.75[1]	0.36 ± 0.52	1.12 ± 0.65	0.21[1]
Sweets*	4.86 ± 2.07	4.35 ± 2.60	0.60[1]	1.86 ± 1.17	5.47 ± 1.82	0.03[1]
Processed meats*	0.96 ± 0.91	0.72 ± 0.76	0.59[1]	0.12 ± 0.20	1.06 ± 0.78	0.16[1]

All values are means ± SDs. Tests for trends or differences were made by using generalized linear models.

[1]Adjusted for age, BMI and energy intakes.

*Vegetables, 1 serving = 125 ml of all vegetables; Fruits, 1 serving = 1 unit of fresh fruit, 125 ml of fruit compote (stewed), frozen and canned or 60 ml of dried fruit; Whole grain products, 1 serving = 1 unit of whole-wheat, whole-grain or other multigrain breads, ½ unit of whole-wheat, whole-grain and other multigrain bagels, tortillas and pitas, 125 ml of whole-wheat or whole-grain pasta, 125 ml of brown rice, 125 ml of oatmeal and wheat cream, 30 g of whole-grain cereal, 1 unit of whole wheat pancakes, 30 g of whole-wheat crackers; Non-hydrogenated fats, 1 serving = 5 ml of non-hydrogenated margarine, 5 ml of vegetable oil or 5 ml of salad dressing (all kinds); Refined grain products, 1 serving = 1 unit of white breads, ½ unit of white bagels, pitas and tortillas, 125 ml of white rice, white pasta and couscous, 1 unit of muffins (home-made), pancakes, waffles and granola bars, 30 g of refined cereal, 1 unit of rice cake or 30 g of salted crackers; Fish and other sea food, 1 serving = 30 g of fish and sea food (excluding breaded fish) or 1 unit of sushi; Nuts, 1 serving = 30 ml of all nuts and seeds or 30 ml of seed and nuts butter; Desserts, 1 serving = 2 units of cookies, 1/6 pies, 125 ml of pudding, 1/6 or 1 unit of cakes, doughnuts and pastries, 1 unit of croissants, muffins (commercial), date squares, banana breads and coated granola bars; Sweets, 1 serving = 5 ml of sugar, brown sugar, honey, maple taffy, jam, maple/corn syrup and nutella, 1 unit of candy, 5 g of chocolate bar or pieces; Processed meats, 1 serving = 5 slices of bacon, 1 unit of sausage, hotdogs and hotdogs on a stick, 30 g delicatessen, cretons, head cheese, liver pâté and terrine.

Table 3.5 Food group intakes (number of servings) for women according to dietary pattern scores.

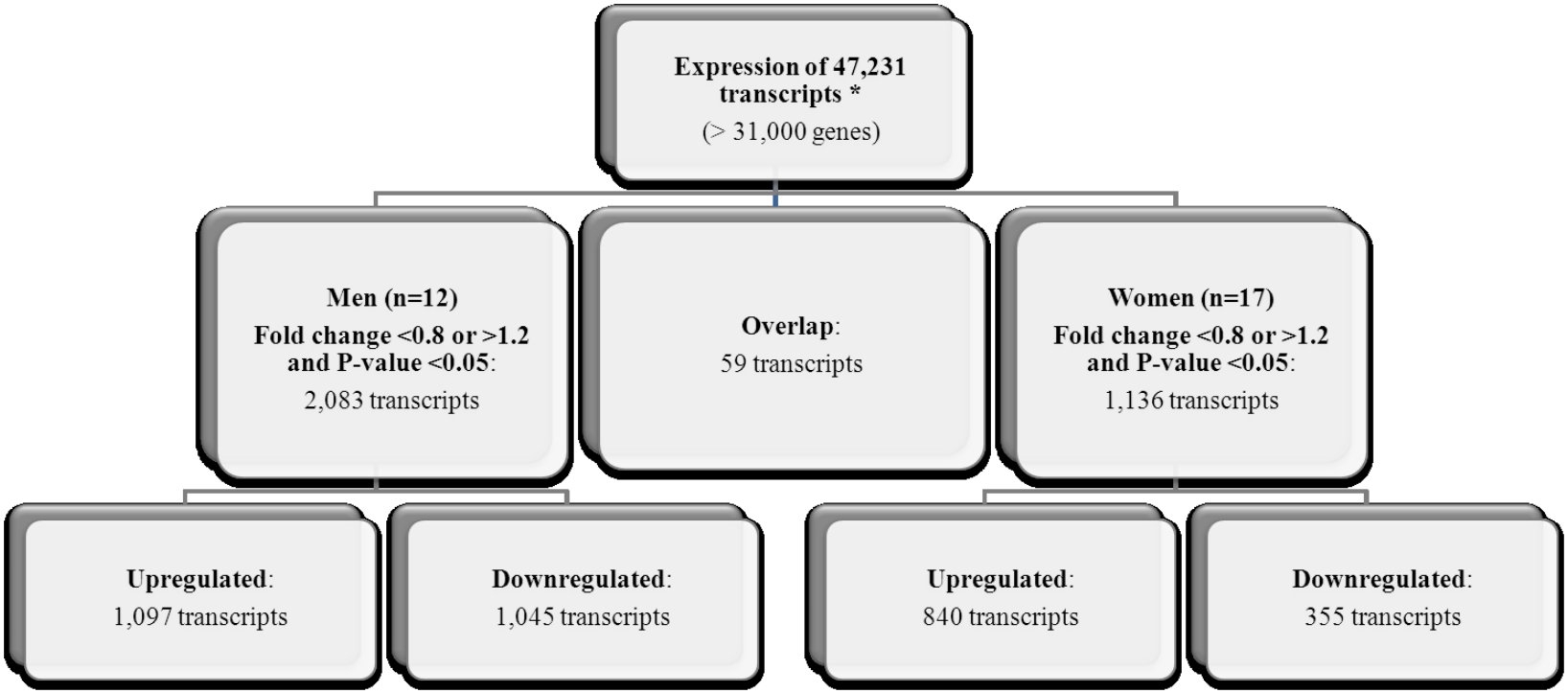
	Prudent dietary pattern (n=12)		*P*	Western dietary pattern (n=12)		*P*
	Low score (n=10)	High score (n=7)		Low score (n=3)	High score (n=9)	
Vegetables*	2.30 ± 1.55	4.38 ± 1.73	0.16[1]	3.09 ± 2.05	3.28 ± 1.73	0.77[1]
Fruits*	1.97 ± 1.14	3.34 ± 0.61	0.07[1]	2.56 ± 1.21	2.48 ± 1.18	0.62[1]
Whole grain products*	2.04 ± 1.33	4.02 ± 2.84	0.18[1]	3.25 ± 2.47	2.13 ± 1.70	0.13[1]
Non-hydrogenated fats*	1.52 ± 0.97	4.65 ± 3.09	0.006[1]	2.58 ± 2.66	3.24 ± 2.59	0.96[1]
Refined grain products*	3.14 ± 1.50	1.83 ± 0.61	0.06[1]	2.46 ± 1.31	2.86 ± 1.53	0.57[1]
Fish and other sea food*	1.01 ± 0.72	1.24 ± 0.88	0.35[1]	0.85 ±0.52	1.58 ± 0.96	0.11[1]
Nuts*	0.39 ± 0.39	0.59 ± 0.63	0.89[1]	0.56 ± 0.57	0.30 ± 0.30	0.29[1]
Desserts*	0.85 ± 0.75	0.62 ± 0.38	0.38[1]	0.38 ± 0.28	1.44 ± 0.42	<0.0001[1]
Sweets*	3.45 ± 1.69	2.93 ± 5.05	0.05[1]	1.90 ± 1.60	5.69 ± 4.43	0.007[1]
Processed meats*	0.38 ± 0.37	0.30 ± 0.17	0.13[1]	0.31 ± 0.28	0.43 ± 0.36	0.67[1]

All values are means ± SDs. Tests for trends or differences were made by using generalized linear models.

[1]Adjusted for age, BMI and energy intakes.

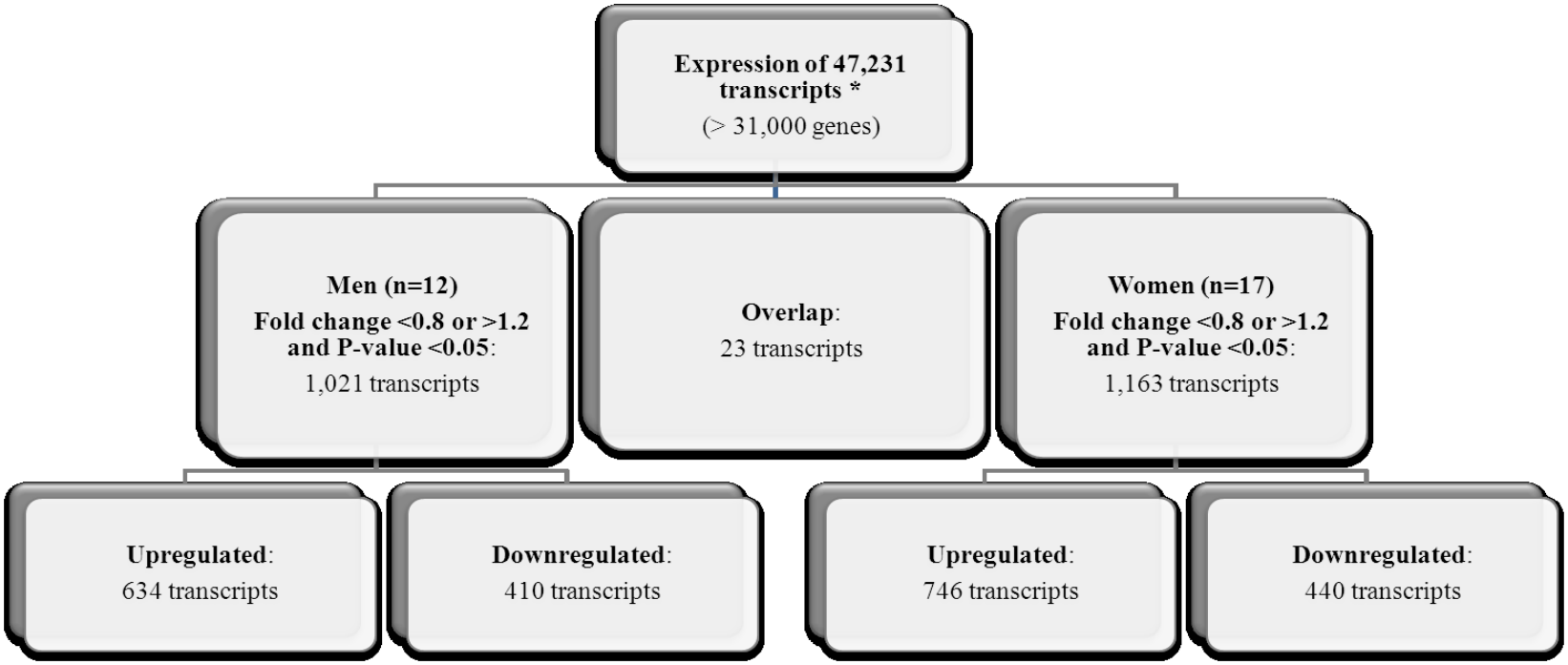
*Vegetables, 1 serving = 125 ml of all vegetables; Fruits, 1 serving = 1 unit of fresh fruit, 125 ml of fruit compote (stewed), frozen and canned or 60 ml of dried fruit; Whole grain products, 1 serving = 1 unit of whole-wheat, whole-grain or other multigrain breads, ½ unit of whole-wheat, whole-grain and other multigrain bagels, tortillas and pitas, 125 ml of whole-wheat or whole-grain pasta, 125 ml of brown rice, 125 ml of oatmeal and wheat cream, 30 g of whole-grain cereal, 1 unit of whole wheat pancakes, 30 g of whole-wheat crackers; Non-hydrogenated fats, 1 serving = 5 ml of non-hydrogenated margarine, 5 ml of vegetable oil or 5 ml of salad dressing (all kinds); Refined grain products, 1 serving = 1 unit of white breads, ½ unit of white bagels, pitas and tortillas, 125 ml of white rice, white pasta and couscous, 1 unit of muffins (home-made), pancakes, waffles and granola bars, 30 g of refined cereal, 1 unit of rice cake or 30 g of salted crackers; Fish and other sea food, 1 serving = 30 g of fish and sea food (excluding breaded fish) or 1 unit of sushi; Nuts, 1 serving = 30 ml of all nuts and seeds or 30 ml of seed and nuts butter; Desserts, 1 serving = 2 units of cookies, 1/6 pies, 125 ml of pudding, 1/6 or 1 unit of cakes, doughnuts and pastries, 1 unit of croissants, muffins (commercial), date squares, banana breads and coated granola bars; Sweets, 1 serving = 5 ml of sugar, brown sugar, honey, maple taffy, jam, maple/corn syrup and nutella, 1 unit of candy, 5 g of chocolate bar or pieces; Processed meats, 1 serving = 5 slices of bacon, 1 unit of sausage, hotdogs and hotdogs on a stick, 30 g delicatessen, cretons, head cheese, liver pâté and terrine.

Figure 3.1 Flowchart illustrating the significantly different transcripts according to scores for the Prudent dietary pattern.



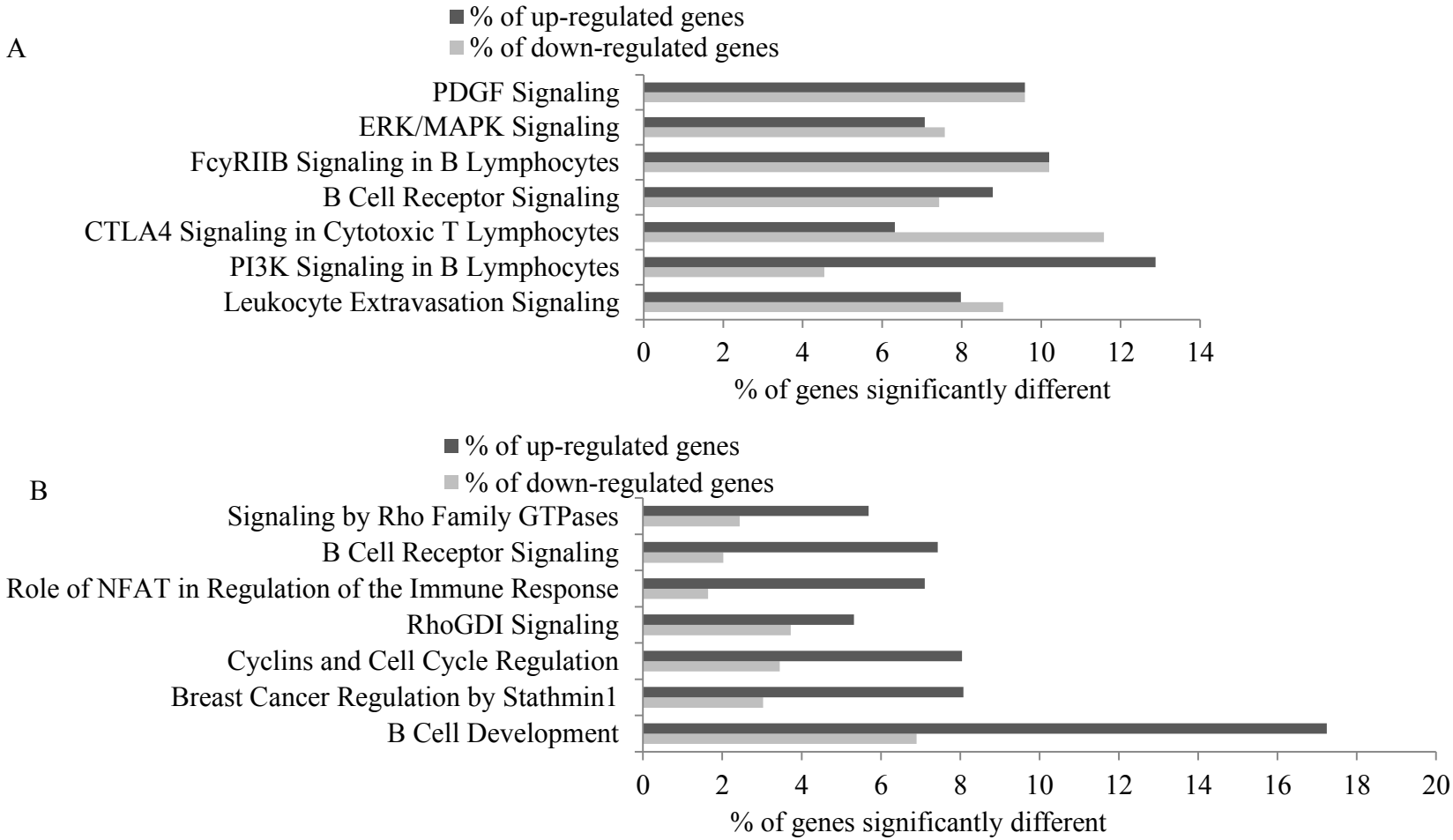
*HumanHT12v4 Expression BeadChips (Illumina)

Figure 3.2 Flowchart illustrating the significantly different transcripts according to scores for the Western dietary pattern.



*HumanHT12v4 Expression BeadChips (Illumina)

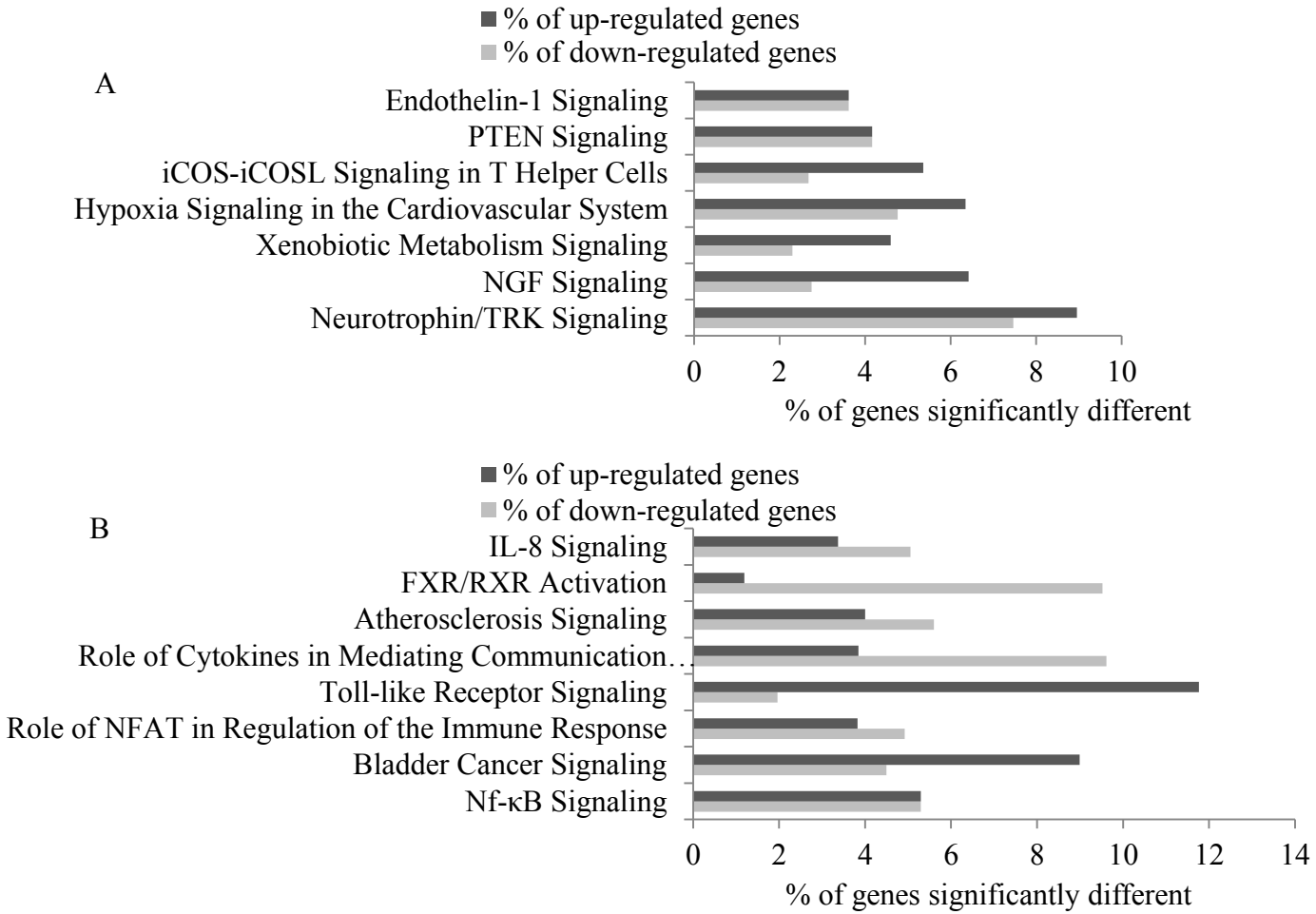
Figure 3.3 The modified canonical pathways according to scores for the Prudent dietary pattern.



Legend: Gene expression differences (≥ ± 1.2 fold change) in canonical pathways comparing high and low scores for the Prudent dietary pattern (A) For the men (B) For the women. P-values for Functional Analysis of dataset by IPA (Fisher's Exact Test) are presented. % of genes significantly up-regulated and down-regulated in each canonical pathway are presented (number of genes differently expressed/number of genes in the pathways*100). (A) PDGF Signaling: P=0.004, 14 genes significantly different; ERK/MAPK Signaling: P=0.004, 29 genes significantly different; FcyRIIB Signaling in B Lymphocytes: P=0.003, 10 genes significantly different; B Cell Receptor

Signaling: P=0.002, 24 genes significantly different; CTLA4 Signaling in Cytotoxic T Lymphocytes: P=0.003, 17 genes significantly different; PI3K Signaling in B Lymphocytes: P=0.0009, 23 genes significantly different; Leucocyte Extravasation Signaling: P=0.0002, 32 genes significantly different. (B) Signaling by Rho Family GTPase: P=0.02, 20 genes significantly different; B Cell Receptor Signaling: P=0.02, 14 genes significantly different; Role of NFAT in Regulation of the Immune Response: P=0.01, 16 genes significantly different; RhoGDI Signaling: P=0.009, 17 genes significantly different; Cyclins and Cell Cycle Regulation: P=0.007, 10 genes significantly different; Breast Cancer Regulation by Stathmin1: P=0.0005, 22 genes significantly different; B Cell Development: P=0.0004, 7 genes significantly different.

Figure 3.4 The modified canonical pathways according to scores for the Western dietary pattern.



Legend: Gene expression differences (≥ ± 1.2 fold change) in canonical pathways comparing high and low scores for the Western dietary pattern (A) For the men (B) For the women. P-values for Functional Analysis of dataset by IPA (Fisher's Exact Test) are presented. % of genes significantly up-regulated and down-regulated in each canonical pathway are presented (number of genes differently expressed/number of genes in the pathways*100). (A) Endothelin-1 Signaling: P=0.01, 12 genes significantly different; PTEN Signaling:

P=0.01, 10 genes significantly different; iCOS-iCOSL Signaling in T Helper Cells: P=0.008, 9 genes significantly different; Hypoxia Signaling in the Cardiovascular System: P=0.006, 7 genes significantly different; Xenobiotic Metabolism Signaling: P=0.005, 18 genes significantly different; NGF Signaling: P=0.004, 10 genes significantly different; Neurotrophin/TRK Signaling: P=0.00001, 11 genes significantly different. (B) IL-8 Signaling: P=0.04, 15 genes significantly different; FXR/RXR Activation: P=0.05, 9 genes significantly different; Atherosclerosis Signaling: P=0.02, 12 genes significantly different; Role of Cytokines in Mediating Communication between Immune Cells: P=0.02, 7 genes significantly different; Toll-like Receptor Signaling: P=0.01, 7 genes significantly different; Role of NFAT in Regulation of the Immune Response: P=0.01, 16 genes significantly different; Bladder Cancer Signaling: P=0.002, 12 genes significantly different; Nf-κB Signaling: P=0.002, 18 genes significantly different.

Chapitre 4
La signature métabolique associée avec le profil alimentaire Western : une étude transversale

Annie Bouchard-Mercier, Iwona Rudkowska, Simone Lemieux, Patrick Couture et Marie-Claude Vohl

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The metabolic signature associated with the Western dietary pattern: a cross-sectional study

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Trial registration: NCT01343342. http://clinicaltrials.gov/show/NCT01343342

Résumé

Introduction : Il a été observé que les profils de métabolites étaient associés au statut d'obésité et à la sensibilité à l'insuline. Les apports alimentaires influencent les voies métaboliques et conséquemment, différents profils alimentaires pourraient être reliés à des modifications dans les signatures métaboliques. L'objectif était de vérifier les associations entre les profils alimentaires et les profils de métabolites constitués d'acides aminés (AAs) et d'acylcarnitine (ACs). **Méthodes** : 210 participants ont été recrutés dans la région métropolitaine de Québec entre septembre 2009 et décembre 2011. Les profils alimentaires ont été décrits précédemment et ont été dérivés en utilisant une analyse en composante principale (ACP). Le profil alimentaire Prudent était caractérisé par des apports plus élevés en légumes, fruits, produits céréaliers à grains entiers, gras non-hydrogénés et plus bas en produits céréaliers raffinés, tandis que le profil alimentaire Western était associé à des apports plus élevés en produits céréaliers raffinés, desserts, sucreries et viandes transformées. Les métabolites ciblés (14 AAs et 41 ACs) ont été quantifiés chez 37 participants par spectrométrie de masse avec *Biocrates Absolute IDQ p150* (*Biocrates Life Sciences AG, Austria*). **Résultats** : L'analyse ACP avec les métabolites (AAs et ACs) a révélé deux composantes principales expliquant le plus de variance dans les données (13,8%). PC1 était composé surtout d'ACs à moyennes et à longues chaînes (C16:2, C14:2, C14:2-OH, C16, C14:1-OH, C14:1, C10:2, C5-DC/C6-OH, C12, C18:2, C10, C4:1-DC/C6, C8:1 et C2) tandis que PC2 incluait certains AAs et ACs à courtes chaînes (xLeu, Met, Arg, Phe, Pro, Orn, His, C0, C3, C4 et C5). Le profil alimentaire Western était négativement associé au PC1 et positivement au PC2 ($r = -0,34$, $p = 0,05$ et $r = 0,38$, $p = 0,03$, respectivement), indépendamment de l'âge, du sexe et de l'IMC. **Conclusion** : Ces résultats suggèrent que le profil alimentaire Western est associé avec une signature métabolique caractérisée par des concentrations plus élevées d'AAs incluant des acides aminés ramifiés et des ACs à courtes chaînes.

Abstract

Background: Metabolic profiles have been shown to be associated to obesity status and insulin sensitivity. Dietary intakes influence metabolic pathways and therefore, different dietary patterns may relate to modifications in metabolic signatures. The objective was to verify associations between dietary patterns and metabolic profiles composed of amino acids (AAs) and acylcarnitines (ACs). **Methods:** 210 participants were recruited in the greater Quebec City area between September 2009 and December 2011. Dietary patterns had been previously derived using principal component analysis (PCA). The Prudent dietary pattern was characterised by higher intakes of vegetables, fruits, whole grain products, non-hydrogenated fat and lower intakes of refined grain products, whereas the Western dietary pattern was associated with higher intakes of refined grain products, desserts, sweets and processed meats.Targeted metabolites were quantified in 37 participants with the Biocrates Absolute IDQ p150 (Biocrates Life Sciences AG, Austria) mass spectrometry method (including 14 amino acids and 41 acylcarnitines). **Results:** PCA analysis with metabolites including AAs and ACs revealed two main components explaining the most variance in overall data (13.8%). PC1 was composed mostly of medium- to long-chain ACs (C16:2, C14:2, C14:2-OH, C16, C14:1-OH, C14:1, C10:2, C5-DC/C6-OH, C12, C18:2, C10, C4:1-DC/C6, C8:1 and C2) whereas PC2 included certain AAs and short-chain ACs (xLeu, Met, Arg, Phe, Pro, Orn, His, C0, C3, C4 and C5). The Western dietary pattern correlated negatively with PC1 and positively with PC2 ($r = -0.34$, $p = 0.05$ and $r = 0.38$, $p = 0.03$, respectively), independently of age, sex and BMI. **Conclusion:** These findings suggest that the Western dietary pattern is associated with a specific metabolite signature characterized by increased levels of AAs including branched-chain AAs (BCAAs) and short-chain ACs.

Introduction

Single nutrients or single food components have been frequently studied in order to achieve a better understanding of their impact on health and on the development of chronic diseases. Many studies have also observed the effects of global diets or dietary patterns such as the Mediterranean diet on chronic diseases. Studying the effects of dietary patterns takes into account the interactions between nutrients [1]. Dietary patterns derived by principal component analysis (PCA) depict a portrait of the foods that are mainly consumed together within a population [1]. Dietary patterns, such as the Prudent (or Healthy pattern) and the Western dietary patterns have been associated positively or inversely with cardiovascular disease risk factors and mortality, as well as with certain types of cancer such as colorectal cancer [2-4]. Dietary patterns have also been associated with type 2 diabetes or related metabolic parameters. Schulze *et al*. [5] observed an association between the risk of type 2 diabetes and a dietary pattern high in foods such as sugar-sweetened soft drinks, refined grains as well as processed meats and low vegetable intake. In addition, Heidemann *et al*. [6] reported a decreased risk of type 2 diabetes with a dietary pattern characterised by high intakes of fruits and low intakes of foods such as high-caloric soft drinks and meats. These results have been confirmed by Esposito *et al*. [7] in a recent systematic review which observed that dietary patterns characterized by high intakes of fruit and vegetables, whole grains, fish, and poultry, and low intakes of red meat, processed foods, sugar-sweetened beverages, and starchy foods were associated with a reduced risk and a later development of type 2 diabetes.

Obesity pandemic represents a major health burden. In Canada, 26% of the adults were considered obese according to their body mass index (BMI) and when considering waist circumference, 37% were abdominally obese [8]. Obesity is closely related to insulin resistance [9]. However, the link between these two conditions is not well understood. Studying metabolites may help in further understanding the effects of diets, drugs and diseases at the cellular level and enhance our comprehension of the development of complex diseases such as type 2 diabetes (or insulin resistance) [10]. A few studies have investigated metabolic signatures in relation to insulin sensitivity or obesity [11-14]. For

example, Newgard *et al*. [12] have observed a metabolic signature including branched-chain amino acids (BCAA) and short-chain acylcarnitines (ACs) among obese and insulin resistant individuals. These observations have also been replicated in Chinese and Asian-Indian individuals where a metabolic signature characterised by higher concentrations of amino acids (AAs) such as leucine/isoleucine, phenylalanine, tyrosine and methionine was also associated with insulin resistance [13]. Other studies observed the effects of dietary variables on metabolic signatures including ACs. For example, the effects of a lactovegetarien diet versus an omnivorous diet, the intake of fruits and vegetables, coffee and garlic intakes and hypocaloric dieting were studied [15-17]. Dietary patterns derived from cluster analysis have also been associated with specific metabolites [18].

To our knowledge, the metabolic signatures associated with the Western and the Prudent dietary patterns have never been studied. Therefore, the objective of this study was to investigate the metabolic signatures, composed of AAs and ACs derived from PCA, associated with the Western and the Prudent dietary patterns in a sample of overweight men and women. Associations between the Western dietary pattern and principal components (PCs) were observed.

Methods

Subjects

A total of 254 subjects were recruited to participate in this clinical trial from the greater Quebec City metropolitan area 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, subjects had to be non-smokers and free of any thyroid or metabolic disorders requiring treatment such as diabetes, hypertension, severe dyslipidemia, and coronary heart disease. Participants had to be aged between 18 and 50 years with a BMI between 25 and 40 Kg/m^2. The subjects who had taken n-3 polyunsaturated fatty acid supplements during the six months preceding the study were excluded. A total of 210 subjects completed the study protocol which is described elsewhere [19] and were included in this cross-sectional study. All participants gave written informed consent and 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.

Dietary assessment and food grouping

Dietary assessment and food grouping has been previously described [20]. Briefly, dietary intake of the past month was determined by a 91-items validated food frequency questionnaire (FFQ) [21] based on food habits of Quebecers, administered by a registered dietitian (RD). All the information was compiled and similar food items from the FFQ were grouped, as previously described [22]. Three criteria were used to form these groups: first, the similarity of nutrient profiles, second, the culinary usage of different types of food (similar to groups used in a previous study [23]) and third, the consideration of groups utilized in other studies to maintain consistency [1]. Food items from only thirty-five food groups were consumed by the participants in the present study. From these thirty-five food groups, eight were not normally distributed even after logarithmic transformation and were excluded as well. Consequently, twenty-seven foods groups were used for PCA to generate dietary patterns as described previously [20]. Briefly, two main dietary patterns were derived from PCA analysis. The Prudent dietary pattern, characterized by higher intakes of

vegetables, fruits, whole grain products, non-hydrogenated fat and lower intakes of refined grain products, and the Western dietary pattern, associated with higher intakes of refined grain products, desserts, sweets and processed meats [20].

Metabolite profiling

The Biocrates Absolute IDQ p150 (Biocrates Life Sciences AG, Austria) mass spectrometry method was used to quantify 163 metabolites for the first 40 of the 254 participants. Three participants were excluded because of extreme values (standard out of range), resulting in 37 participants. For this study ACs and AAs were the main focus thus, 41 ACs (AC*x*:*y*, where *x* denotes the number of carbons in the side chain and *y* the number of double bonds) and 14 AAs (proteinogenic + ornithine) were studied. Assays used 10 µL of plasma from each subject. The metabolite profiling was carried out according to the manufacturer's instructions at CHENOMX (Edmonton, AL, Canada). For all analyzed metabolites the concentrations are reported in µM. Furthermore, metabolites with standard out of range and/or for which more than half of the values were below the limit of detection were excluded. Thus, 29 ACs and 13 AAs were included in the analyses.

Statistical analyses

Variables which were not normally distributed were logarithmically transformed. The distribution of glutaconyl-L-carnitine (C5_1_DC) was still not normally distributed after logarithmic transformation and thus was excluded from further analyses. The FACTOR procedure from Statistical Analysis Software (SAS) using PCA method was used to derive PCs describing metabolite signatures. Newgard *et al*. [11] described two main PCs when studying ACs and AAs which explained most of the variance in their data. In the present study, in order to determine the number of factors to retain, components with eigenvalue > 1, values at Scree test, variance explained (%) and the interpretability were considered. It was noticed that PC1 and PC2 had eigenvalues much higher (~8 and ~6, respectively) than the other PCs (<~3). Thus, the NFACTORS statement was added in the proc FACTOR procedure in order to retain only 2 main PCs and explain a maximum of variance. Metabolites with absolute factor loadings ≥ 0.50 were regarded as significant contributors

to the PC. Using the SCORE procedure of SAS, each participant was given a score for each PC. These scores are calculated from the sum of metabolic signature groups multiplied by their respective factor loading. These scores reflect the degree of each participant's metabolic signature conforming to PC1 and PC2.

Pearson correlations were used to detect associations between the Prudent and the Western dietary pattern scores with PC1 and PC2 scores. To further understand the relationships with PC1 and PC2 scores and dietary variables, partial correlations were performed with individual food groups (only the food groups which contributed to Prudent and Western dietary patterns) and macronutrients (expressed as energy percentages) adjusted for age, sex, BMI and energy intakes (only for the food groups). To facilitate interpretation, Prudent and Western dietary pattern scores as well as with food groups and macronutrients intakes were divided according to tertiles and associations with PC1 and PC2 were tested using the General Linear Model procedure implemented in SAS. A p-value <0.05 was considered significant. All statistical analyses were performed using SAS statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Descriptive characteristics and dietary patterns

Descriptive characteristics of the study participants are presented in Table 4.1. As described previously, there were two dietary patterns derived in this cohort, the Prudent and the Western dietary patterns [20]. The Prudent dietary pattern was characterised by high intakes of vegetables, fruits, whole grain products, non-hydrogenated fats and inversely associated with refined grain products food group and the Western dietary pattern by high intakes of refined grain products, desserts, sweets and processed meats [20]. In Table 4.2, dietary intakes, AC and AA concentrations according to dietary pattern score (low ≤ 0 or high > 0) are shown respectively for the Prudent and the Western dietary patterns. Individuals with high scores for the Prudent dietary pattern had lower saturated fat intakes than individuals with low scores. The opposite was observed for the Western dietary pattern scores. Regarding the associations between dietary patterns and cardiovascular disease risk factors, only a trend was observed for lower fasting insulin levels with higher scores for the Prudent dietary pattern ($r = -0.32$, $p = 0.07$). A positive association between fasting glucose levels and the Western dietary pattern was observed ($r = 0.38$, $p = 0.03$).

Principal component analysis of the metabolites

PC1 explained 7.97% of the variance in the data and PC2, 5.81%. As presented in Figure 4.1, PC1 was composed mostly of medium- to long-chain ACs (C16:2, C14:2, C14:2-OH, C16, C14:1-OH, C14:1, C10:2, C5-DC/C6-OH, C12, C18:2, C10, C4:1-DC/C6, C8:1 and C2) whereas PC2 was mainly composed of AAs and short-chain ACs (xLeu, Met, Arg, Phe, Pro, Orn, His, C0, C3, C4 and C5).

The Prudent dietary pattern was not correlated neither to PC1 nor to PC2 ($r = -0.19$, $p = 0.26$ and $r = -0.21$, $p = 0.21$, respectively). The Western dietary pattern, was correlated with PC2 ($r = 0.34$, $p = 0.04$). When further adjusted for the effects of age, sex and BMI, both PCs were associated with the Western dietary pattern. The Western dietary pattern correlated negatively with PC1 and positively with PC2 ($r = -0.34$, $p = 0.05$ and $r = 0.38$, p

= 0.03, respectively). To further explore the associations between the Western dietary pattern and PCs, subjects were divided into tertiles according to the Western dietary pattern score. The relationships between tertiles of the Western dietary pattern and PC1 and PC2 scores were not significant ($p = 0.10$ and $p = 0.15$, respectively).

In order to achieve a better understanding of the impact of dietary variables on the metabolic signature, the possible correlations between each dietary variable (food groups contributing either to Prudent or Western dietary pattern and macronutrient intake (expressed in energy percentages)) and the scores for each PC were tested (adjusted for age, sex, BMI and energy intakes (only for food groups)), as presented in Table 4.3. Briefly, PC1 was not correlated with any food groups. PC2 was negatively correlated with fruit intake and positively associated with dessert intake ($r = -0.38$, $p = 0.03$ and $r = 0.37$, $p = 0.04$, respectively) adjusted for age, sex, BMI and energy intakes. For the macronutrients, expressed as energy percentages, a positive association for PC2 with total fat and saturated fat intakes was observed ($r = 0.39$, $p = 0.02$ and $r = 0.50$, $p = 0.003$, respectively). Interestingly, when dietary total fat, saturated fat, fruit and dessert were divided into tertiles, only saturated fat intake tertiles were different according to PC2 scores (Figure 4.2) ($p = 0.01$). As shown in Figure 4.2, saturated fat intakes $\leq 11.30\%$ had negative PC2 scores, which indicate that their metabolic signature was not corresponding to PC2 characterised by higher concentrations of ACs and short-chain AAs. PC1 did not correlate with any macronutrients. In Table 4.4, partial correlations between dietary pattern scores and each AC and AA (only the metabolites which were associated with a PC) are shown. The Prudent dietary pattern score was positively associated with concentrations of C5-DC/C6-OH (glutaryl-L-carnitine) and C18:2 (octadecadienyl-L-carnitine). The Western dietary pattern score was positively associated with methionine and phenylalanine.

Dietary intakes and individual metabolites

To further explore the impact of dietary intakes on metabolites, partial correlations were tested for each metabolite. Briefly, intakes of vegetables and fruits were positively associated with C18:2 (octadecadienyl-L-carnitine) ($r = 0.49$, $p = 0.004$, for both) and

inversely associated with xleucine (r = −0.35, p = 0.05, for both). Fruit intakes were also inversely associated with methionine (r = −0.40, p = 0.02). The intakes of non-hydrogenated fats were positively associated with C14:1 (tetradecadienyl-L-carnitine) and C18:2 (octadecadienyl-L-carnitine) (r = 0.39, p = 0.02 and r = 0.46, p = 0.007, respectively) and inversely with histidine (r = −0.42, p = 0.01). The intakes of dessert were positively associated with three AAs, methionine, phenylalanine and xleucine (r = 0.49, p = 0.004, r = 0.40, p = 0.02 and r = 0.38, p = 0.03, respectively). The intake of sweets also was associated with methionine concentrations (r = 0.41, p = 0.02) and with C18:1-OH (hydroxyoctadecenoyl-L-carnitine) and C5:1-DC (glutaconyl-L-carnitine) (r = 0.37, p = 0.04 and r = 0.42, p = 0.01, respectively). When observing macronutrient intakes, saturated fat intakes expressed as energy percentages were positively associated with C5 (valeryl-L-carnitine) (r = 0.36, p = 0.04) and inversely with C18:2 (octadecadienyl-L-carnitine) (r = −0.46, p = 0.006). Monounsaturated fat intakes were positively associated with C8:1 (octenoyl-L-carnitine) (r = 0.50, p = 0.003) and inversely with C5-M-DC (methylglutaryl-L-carnitine) (r = −0.42, p = 0.01). Polyunsaturated fat intakes were also inversely associated with C5-M-DC (methylglutaryl-L-carnitine) (r = −0.35, p = 0.04) but as well with proline concentrations (r = −0.35, p = 0.04) and positively associated with C10:2 (decadienyl-L-carnitine) (r = 0.37, p = 0.03). Protein intakes expressed as energy percentages were associated with ornithine and histidine (r = 0.44, p = 0.009 and r = 0.42, p = 0.01, respectively). The opposite was observed for carbohydrate intakes which correlated inversely with ornithine concentrations (r = −0.55, p = 0.0008).

Discussion

The Prudent and the Western dietary patterns from this study had many similarities with the dietary patterns described in the literature. The Prudent dietary pattern is usually associated with high consumption of vegetables, fruits and whole grain products whereas the Western dietary pattern relates to higher intakes of red and processed meats, refined grain products and sweets [24]. In this study, an association with higher fasting glucose was observed among individuals with high scores for the Western dietary pattern. Associations between dietary patterns and type 2 diabetes have been frequently observed and have been reviewed recently by Alhazmi *et al*. [25].

PC1 was composed mainly of medium- to long-chain ACs whereas PC2 was composed of short-chain ACs and AAs including the BCAA xleucine and the aromatic AA phenylalanine. Recent studies have shown a link between plasma levels of certain AAs and the risk of insulin resistance. Newgard *et al*. [12] have observed a metabolic signature among obese individuals characterised by a combination of BCAA, methionine, aromatic AAs and short-chain ACs (C3 and C5) which was related to insulin resistance. It has also been observed that increased levels of BCAA and aromatic AAs were associated with the risk of developing future type 2 diabetes [26]. Laferrère *et al*. [11] have studied the impact on ACs and AAs of weight-loss induced by gastric bypass surgery or by a hypocaloric diet. In their study, the first PC (mostly medium- to long-chain ACs) was associated with improved insulin sensitivity and the second PC (mostly AAs and short-chain ACs) was associated with an increase in insulin resistance. The gastric bypass surgery was associated with a decrease in short-chain ACs: C3, C4-DC and C5 ACs and AAs: alanine, leucine/isoleucine, phenylalanine and tyrosine. The ACs C3 and C5 have been demonstrated to be, at least partly, the products of AAs catabolism, especially BCAA (leucine/isoleucine and valine), possibly indicating an increase in enzymes related to BCAA catabolism [12]. In this study, no correlations were observed between PCs and fasting insulin or glucose levels. This could be due to the fact that the individuals from this cohort were healthy as well as only slightly overweight. Thus, their metabolic profile was not deteriorated leading to too subtle differences to be detected according to PCs scores.

Interestingly, scores for the Western dietary pattern were inversely associated with PC1 (medium- to long-chain ACs) and positively with PC2 (short-chain ACs and AAs including the BCAA xleucine). Xu *et al*. [15] compared the metabolite profile between a lactovegetarian diet with an omnivorous diet. Among the most different metabolites, there was glycine which was higher among lactovegeterians and phenylalanine which was lower among lactovegeterians compared to omnivorous controls. The authors hypothesised that phenylalanine concentrations may have been higher among the omnivorous group due to the intakes of animal proteins which contain more phenylalanine than proteins from vegetal sources. In our study, higher AAs levels in PC2 was not associated to an increase in total protein, animal protein or vegetal protein intakes (data not shown). In addition, protein intakes correlated positively with the Prudent dietary pattern and negatively with the Western dietary pattern. Thus, changes may be due to a modification in rates of protein turnover or AA catabolism. Differences in the expression of the enzyme responsible for BCAA catabolism (branched-chain α-ketoacid dehydrogenase (BCKD) complex) have been reported among obese rats compared to their lean counterparts [27]. BCKD's activity was reduced among obese rats and also among diet induced obese mice, which were fed a diet containing from 45% to 60% energy from fat [27]. May *et al*. [16] have studied the effects on urine metabolomic profiles of a diet devoid of fruits and vegetables compared to a diet high in fruits and vegetables. They observed for the group deprived with fruits and vegetables, higher concentrations of short- to medium-chain ACs and higher concentrations of AAs and tricarboxylic cycle intermediates. They also hypothesised that these alterations could be due to a shift from glucose utilisation to fatty acid beta-oxidation. In our study, the Western dietary pattern was inversely associated with vegetable consumption (data not shown) and with a PC (PC2) characterised by higher concentrations of four short-chain ACs and seven AAs. In addition, an inverse association between fruit consumption and PC2 was observed in the present study. Thus, it seems that low fruit and vegetable intakes may be associated with a metabolic signature characterised by higher levels of shorter chain ACs and AAs.

When further examining the relationships between food groups and PCs, the most important correlation was observed between saturated fat intakes and PC2. Mechanisms behind these relations are unknown. Saturated fat intakes have been shown to be less potent activators than polyunsaturated fatty acids of an important transcription factor regulating fatty acid beta-oxidation, peroxisome proliferator-activated receptor alpha (*PPARA*) [28]. It has also been observed that oleate, compared to palmitate, the main saturated fat from the diet, increased mitochondrial fatty acid beta-oxidation [29]. However, other studies have reported the opposite. Stephenson *et al*. [30] have observed that among rats fed a «Western» diet (higher in fat, saturated fat and sucrose intakes) the activity of several mitochondrial enzymes involved in fatty acid beta-oxidation was increased. These discrepancies may be dependent of the overall effect of diet. Saturated fat alone may not have the same effect on fuel selection than when consumed in conjunction with higher intakes of sugary foods. In rat models, it has been observed that in long term, the rats fed a diet high in saturated fat and sucrose developed more severe symptoms of the metabolic syndrome than rats fed diets either high in saturated fat or high in sucrose alone [31].

Even though this cohort was generally healthy, differences in metabolic signatures have been observed and may be indicative of a higher or lower risk of future cardiometabolic diseases. A strength of this study is the analysis of dietary intakes grouped in dietary patterns from FFQ which represents real life intakes. Obviously, functional analyses are needed to understand underlying mechanisms behind these associations between dietary patterns and ACs and AAs concentrations in the plasma. One limitation of this study could come from the use of PCA. Results could be sample specific and strongly affected by subjective analytic decisions [1]. Nevertheless, to minimize subjectivity and allow data to be used in other studies, eigenvalue, Scree test and the literature were examined before selecting the number of PCs and for the dietary patterns, data from previous studies were also considered for food grouping.

Conclusion

In conclusion, the results of the present study indicate a relationship between the Western dietary pattern and saturated fat intakes with a metabolic signature characterised by higher levels of short-chain ACs and AAs including BCAA and an aromatic AA. Individuals eating according to the Western dietary pattern or with high saturated fat intakes may increase their long term risk of cardiometabolic diseases possibly via small metabolic alterations.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

IR, SL, PC, and MCV designed research; ABM conducted research with the research professionals; IR, SL, PC and MCV provided essential reagents or provided essential materials; ABM analyzed data and performed statistical analysis; ABM wrote paper; ABM, IR, SL, PC and MCV had primary responsibility for final content; All authors read and approved the final manuscript.

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References

1. Newby PK, Tucker KL: Empirically derived eating patterns using factor or cluster analysis: a review. *Nutr Rev* 2004, 62:177–203.
2. Bhupathiraju SN, Tucker KL: Coronary heart disease prevention: nutrients, foods, and dietary patterns. *Clin Chim Acta* 2011, 412:1493–1514.
3. Yusof AS, Isa ZM, Shah SA: Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies (2000–2011). *Asian Pac J Cancer Prev* 2012, 13:4713–4717.
4. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC: Dietary patterns, insulin resistance, and prevalence of the metabolic syndrome in women. *Am J Clin Nutr* 2007, 85:910–918.
5. Schulze MB, Hoffmann K, Manson JE, Willett WC, Meigs JB, Weikert C, *et al*: Dietary pattern, inflammation, and incidence of type 2 diabetes in women. *Am J Clin Nutr* 2005, 82:675–684.
6. Heidemann C, Hoffmann K, Spranger J, Klipstein-Grobusch K, Mohlig M, Pfeiffer AF, *et al*: A dietary pattern protective against type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam Study cohort. *Diabetologia* 2005, 48:1126–1134.
7. Esposito K, Kastorini CM, Panagiotakos DB, Giugliano D: Prevention of type 2 diabetes by dietary patterns: a systematic review of prospective studies and meta-analysis. *Metab Syndr Relat Disord* 2010, 8:471–476.
8. Janssen I: The public health burden of obesity in Canada. *Can J Diabetes* 2013, 37:90–96.
9. Abbasi F, Brown BW Jr, Lamendola C, McLaughlin T, Reaven GM: Relationship between obesity, insulin resistance, and coronary heart disease risk. *J Am Coll Cardiol* 2002, 40:937–943.
10. Ceglarek U, Leichtle A, Brugel M, Kortz L, Brauer R, Bresler K, *et al*: Challenges and developments in tandem mass spectrometry based clinical metabolomics. *Mol Cell Endocrinol* 2009, 301:266–271.
11. Laferrere B, Reilly D, Arias S, Swerdlow N, Gorroochurn P, Bawa B, *et al*: Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. *Sci Transl Med* 2011, 3:80re2.
12. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, *et al*: A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 2009, 9:311–326.
13. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, *et al*: Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia* 2010, 53:757–767.
14. Newgard CB: Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* 2012, 15:606–614.

15. Xu J, Yang S, Cai S, Dong J, Li X, Chen Z: Identification of biochemical changes in lactovegetarian urine using ^{1}H NMR spectroscopy and pattern recognition. *Anal Bioanal Chem* 2010, 396:1451–1463.

16. May DH, Navarro SL, Ruczinski I, Hogan J, Ogata Y, Schwarz Y, *et al*: Metabolomic profiling of urine: response to a randomised, controlled feeding study of select fruits and vegetables, and application to an observational study. *Br J Nutr* 2013, 110:1760–1770.

17. Menni C, Zhai G, Macgregor A, Prehn C, Romisch-Margl W, Suhre K, *et al*: Targeted metabolomics profiles are strongly correlated with nutritional patterns in women. *Metabolomics* 2013, 9:506–514.

18. O'Sullivan A, Gibney MJ, Brennan L: Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. *Am J Clin Nutr* 2011, 93:314–321.

19. Rudkowska I, Paradis AM, Thifault E, Julien P, Tchernof A, Couture P, *et al*: Transcriptomic and metabolomic signatures of an n-3 polyunsaturated fatty acids supplementation in a normolipidemic/normocholesterolemic Caucasian population. *J Nutr Biochem* 2012, 24:54-61.

20. Bouchard-Mercier A, Paradis AM, Rudkowska I, Lemieux S, Couture P, Vohl MC: Associations between dietary patterns and gene expression profiles of healthy men and women: a cross-sectional study. *Nutr J* 2013, 12:24.

21. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S: Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. *Nutr J* 2004, 3:13.

22. Paradis AM, Godin G, Perusse L, Vohl MC: Associations between dietary patterns and obesity phenotypes. *Int J Obes (Lond)* 2009, 33:1419–1426.

23. Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A, *et al*: Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* 1999, 69:243–249.

24. Sherzai A, Heim LT, Boothby C, Sherzai AD: Stroke, food groups, and dietary patterns: a systematic review. *Nutr Rev* 2012, 70:423–435.

25. Alhazmi A, Stojanovski E, McEvoy M, Garg ML: The association between dietary patterns and type 2 diabetes: a systematic review and meta-analysis of cohort studies. *J Hum Nutr Diet* 2013. [Epub ahead of print]

26. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, *et al*: Metabolite profiles and the risk of developing diabetes. *Nat Med* 2011, 17:448–453.

27. Lackey DE, Lynch CJ, Olson KC, Mostaedi R, Ali M, Smith WH, *et al*: Regulation of adipose branched-chain amino acid catabolism enzyme expression and cross-adipose amino acid flux in human obesity. *Am J Physiol Endocrinol Metab* 2013, 304:E1175–E1187.

28. Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, Brown PJ, *et al*: Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol Cell* 1999, 3:397–403.

29. Coll T, Eyre E, Rodriguez-Calvo R, Palomer X, Sanchez RM, Merlos M, *et al*: Oleate reverses palmitate-induced insulin resistance and inflammation in skeletal muscle cells. *J Biol Chem* 2008, 283:11107–11116.

30. Stephenson EJ, Camera DM, Jenkins TA, Kosari S, Lee JS, Hawley JA, *et al*: Skeletal muscle respiratory capacity is enhanced in rats consuming an obesogenic Western diet. *Am J Physiol Endocrinol Metab* 2012, 302:E1541–E1549.

31. Pranprawit A, Wolber FM, Heyes JA, Molan AL, Kruger MC: Short-term and long-term effects of excessive consumption of saturated fats and/or sucrose on metabolic variables in Sprague Dawley rats: a pilot study. *J Sci Food Agric* 2013, 93:3191-3197.

Table 4.1 Descriptive characteristics of the study participants.

Variables	All participants (n = 37)
Age (years)	34.59 ± 9.16
Sex (men/women)	16/21
BMI (Kg/m^2)	29.69 ± 4.17
Waist circumference (cm)	93.46 ± 11.89
Systolic blood pressure (mmHg)	108.03 ± 8.67
Diastolic blood pressure (mmHg)	71.89 ± 8.57
Fasting glucose (mmol/L)	5.01 ± 0.74
Fasting insulin (pmol/L)	84.57 ± 34.78
Total-C (mmol/L)	5.29 ± 1.35
LDL-C (mmol/L)	3.18 ± 1.13
HDL-C (mmol/L)	1.46 ± 0.48
Triglycerides (mmol/L)	1.43 ± 0.93
ApoB (g/L)	0.96 ± 0.30

Means ± SD.

Table 4.2 Dietary intakes and plasma AC and AA according to dietary pattern score.

	Low Prudent (≤0) (n = 20)	High Prudent (>0) (n = 17)	P-value[1]	Low Western (≤0) (n = 16)	High Western (>0) (n = 21)	P-value[1]
Dietary intakes						
Carbohydrate (%)	49.38 ± 5.99	50.48 ± 6.37	0.35	51.91 ± 7.21	48.35 ± 4.73	0.35
Protein (%)	17.14 ± 2.78	18.46 ± 2.26	0.20	18.20 ± 2.66	17.40 ± 2.58	0.43
Total fat (%)	32.57 ± 6.15	31.93 ± 4.19	0.56	30.52 ± 5.89	33.61 ± 4.45	0.29
Saturated fat (%)	11.41 ± 1.83	9.55 ± 2.11	**0.004**	9.11 ± 2.08	11.65 ± 1.47	**0.0006**
Monounsaturated fat (%)	13.24 ± 3.06	13.48 ± 2.04	0.92	12.83 ± 2.98	13.75 ± 2.28	0.81
Polyunsaturated fat (%)	5.30 ± 1.53	6.24 ± 1.30	0.07	5.97 ± 1.57	5.55 ± 1.44	0.17
Cholesterol (mg)	329.88 ± 167.61	357.86 ± 188.90	0.84	263.76 ± 167.15	402.92 ± 160.78	0.07
Total fiber (g)	19.38 ± 4.83	30.43 ± 7.03	**<0.0001**	25.71 ± 7.53	23.51 ± 8.54	0.31
Acylcarnitines (ACs)						
C0	25.16 ± 5.07	27.50 ± 7.09	0.24	23.78 ± 6.73	28.10 ± 4.97	0.24
C2	5.55 ± 1.36	5.20 ± 1.55	0.57	5.33 ± 1.48	5.44 ± 1.44	0.76
C3	0.36 ± 0.14	0.29 ± 0.10	**0.04**	0.30 ± 0.12	0.34 ± 0.13	0.95
C4	0.18 ± 0.06	0.16 ± 0.07	0.44	0.16 ± 0.08	0.18 ± 0.05	0.46
C4:1-DC/C6	0.05 ± 0.01	0.06 ± 0.02	0.08	0.05 ± 0.02	0.05 ± 0.02	0.39
C5	0.11 ± 0.04	0.11 ± 0.03	0.64	0.10 ± 0.03	0.12 ± 0.04	0.39
C5-DC/C6-OH	0.01 ± 0.00	0.02 ± 0.00	**0.01**	0.01 ± 0.01	0.01 ± 0.00	0.62
C8:1	0.14 ± 0.08	0.12 ± 0.06	0.63	0.12 ± 0.08	0.13 ± 0.07	0.83
C10	0.16 ± 0.07	0.22 ± 0.13	0.07	0.17 ± 0.09	0.20 ± 0.11	0.47
C10:2	0.02 ± 0.01	0.03 ± 0.01	0.11	0.02 ± 0.01	0.03 ± 0.01	0.12
C12	0.06 ± 0.03	0.08 ± 0.03	0.06	0.06 ± 0.03	0.08 ± 0.03	0.31
C14:1	0.17 ± 0.03	0.21 ± 0.09	**<0.05**	0.19 ± 0.08	0.19 ± 0.07	0.95
C14:1-OH	0.01 ± 0.00	0.01 ± 0.01	0.54	0.01 ± 0.01	0.01 ± 0.01	0.41
C14:2	0.03 ± 0.01	0.04 ± 0.02	0.02	0.03 ± 0.02	0.03 ± 0.02	0.89
C14:2-OH	0.01 ± 0.00	0.01 ± 0.01	0.19	0.01 ± 0.00	0.01 ± 0.00	0.63

C16	0.08 ± 0.02	0.08 ± 0.04	0.37	0.07 ± 0.03	0.08 ± 0.03	0.45
C16:2	0.01 ± 0.00	0.01 ± 0.01	0.19	0.01 ± 0.00	0.01 ± 0.01	0.65
C18:2	0.04 ± 0.01	0.05 ± 0.01	**0.002**	0.04 ± 0.01	0.04 ± 0.01	0.42
Amino acids						
xleucine	183.95 ± 46.81	161.29 ± 26.70	**0.03**	152.94 ± 21.48	189.24 ± 44.08	**0.03**
Methionine	28.37 ± 5.14	26.54 ± 4.32	0.05	24.25 ± 4.23	30.02 ± 3.60	**0.0004**
Arginine	99.91 ± 32.87	95.81 ± 23.14	0.64	93.54 ± 27.05	101.44 ± 29.76	0.79
Phenylalanine	48.18 ± 8.10	46.56 ± 4.91	0.31	43.86 ± 4.55	50.16 ± 7.02	**0.007**
Proline	167.32 ± 58.74	157.68 ± 39.76	0.47	154.47 ± 44.03	169.30 ± 55.06	0.89
Ornitine	49.06 ± 17.18	52.61 ± 15.80	0.48	45.44 ± 17.75	54.69 ± 14.51	0.33
Histidine	93.59 ± 20.35	93.79 ± 13.96	0.94	93.55 ± 16.26	93.78 ± 17.74	0.65

Means ± SD.

P-values in bold were considered significantly different.

[1]P-values of the GLM models are adjusted for age, sex and BMI.

Table 4.3 Partial correlations between metabolite PCs and dietary pattern scores, food groups and macronutrient intakes.

Dietary variables		**PC1**	**PC2**
Dietary patterns			
Prudent dietary pattern	r	−0.18	−0.25
	p[1]	0.31	0.15
Western dietary pattern	r	**−0.34**	**0.38**
	p[1]	**0.05**	**0.03**
Food groups			
Processed meats	r	−0.14	−0.12
	p[2]	0.45	0.52
Vegetables	r	−0.13	−0.27
	p[2]	0.46	0.13
Fruits	r	−0.10	**−0.38**
	p[2]	0.59	**0.03**
Whole grain products	r	−0.21	−0.24
	p[2]	0.23	0.18
Non-hydrogenated fats	r	−0.02	−0.17
	p[2]	0.93	0.34
Refined grain products	r	−0.14	0.04
	p[2]	0.43	0.85
Desserts	r	−0.09	**0.37**
	p[2]	0.60	**0.04**
Sweets	r	−0.18	0.25
	p[2]	0.32	0.16
Macronutrient intakes			
Total fat (%)	r	0.23	**0.39**
	p[1]	0.20	**0.02**
Saturated fat (%)	r	0.11	**0.50**
	p[1]	0.52	**0.003**
Monounsaturated fat (%)	r	0.28	0.28
	p[1]	0.10	0.10
Polyunsaturated fat (%)	r	0.06	0.07
	p[1]	0.74	0.70
Protein (%)	r	−0.00	−0.14
	p[1]	0.99	0.42
Carbohydrate (%)	r	−0.00	−0.29
	p[1]	0.99	0.10

P-values in bold were considered significantly different.

[1]P-values are adjusted for age, sex and BMI.

[2]P-values are adjusted for age, sex, BMI and energy intakes.

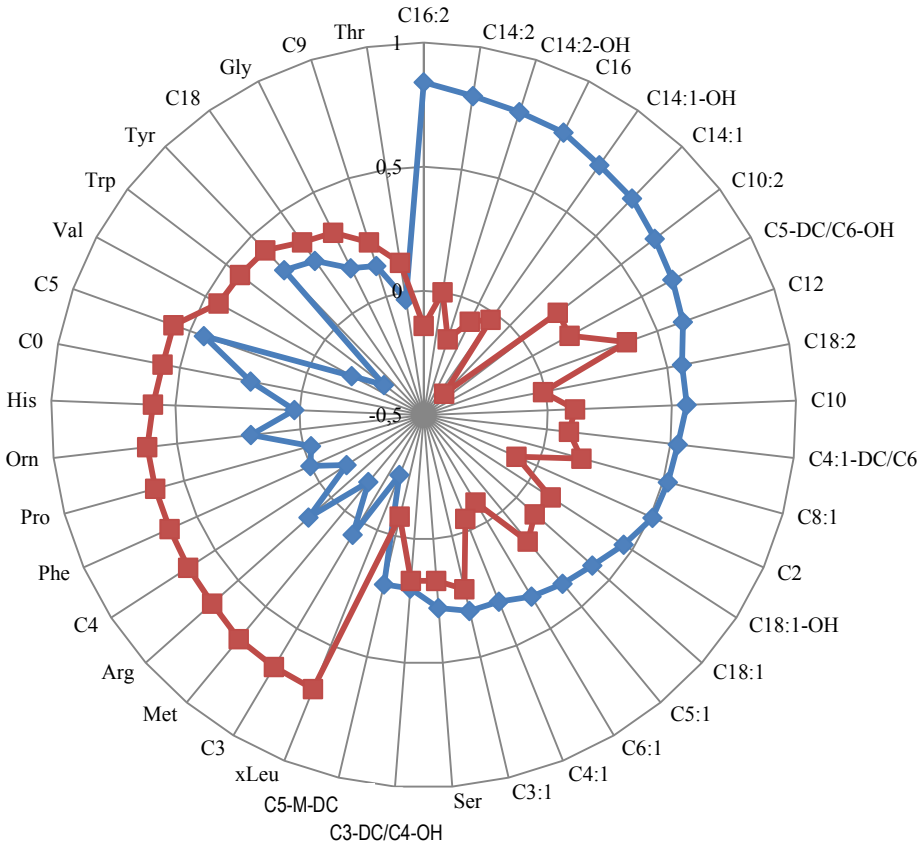
Table 4.4 Partial correlations between dietary pattern scores, ACs and AAs.

Acylcarnitines	**Prudent dietary pattern**		**Western dietary pattern**	
C0	r	0.09	r	0.18
	p	0.60	p	0.32
C2	r	−0.05	r	0.07
	p	0.76	p	0.68
C3	r	−0.23	r	−0.02
	p	0.20	p	0.92
C4	r	−0.15	r	0.15
	p	0.41	p	0.41
C4:1-DC/C6	r	0.26	r	0.13
	p	0.14	p	0.46
C5	r	−0.26	r	−0.02
	p	0.14	p	0.91
C5-DC/C6-OH	r	**0.35**	r	−0.06
	p	**0.04**	p	0.74
C8:1	r	−0.20	r	−0.10
	p	0.25	p	0.56
C10	r	0.16	r	0.22
	p	0.36	p	0.20
C10:2	r	0.19	r	0.30
	p	0.29	p	0.08
C12	r	0.18	r	0.32
	p	0.32	p	0.07
C14:1	r	0.27	r	0.06
	p	0.12	p	0.73
C14:1-OH	r	0.07	r	0.11
	p	0.69	p	0.52
C14:2	r	0.28	r	0.08
	p	0.11	p	0.67
C14:2-OH	r	0.20	r	0.05
	p	0.27	p	0.78
C16	r	0.18	r	0.13
	p	0.30	p	0.47
C16:2	r	0.15	r	0.09
	p	0.39	p	0.62
C18:2	r	**0.51**	r	−0.17
	p	**0.002**	p	0.34
Amino acids				
xleucine	r	−0.33	r	0.30
	p	0.06	p	0.08
Methionine	r	−0.30	r	**0.55**
	p	0.08	p	**0.0008**

Arginine	r	−0.22	r	0.22
	p	0.21	p	0.21
Phenylalanine	r	−0.13	r	**0.39**
	p	0.46	p	**0.02**
Proline	r	−0.26	r	0.03
	p	0.14	p	0.87
Ornitine	r	0.16	r	−0.02
	p	0.37	p	0.92
Histidine	r	−0.25	r	−0.17
	p	0.16	p	0.34

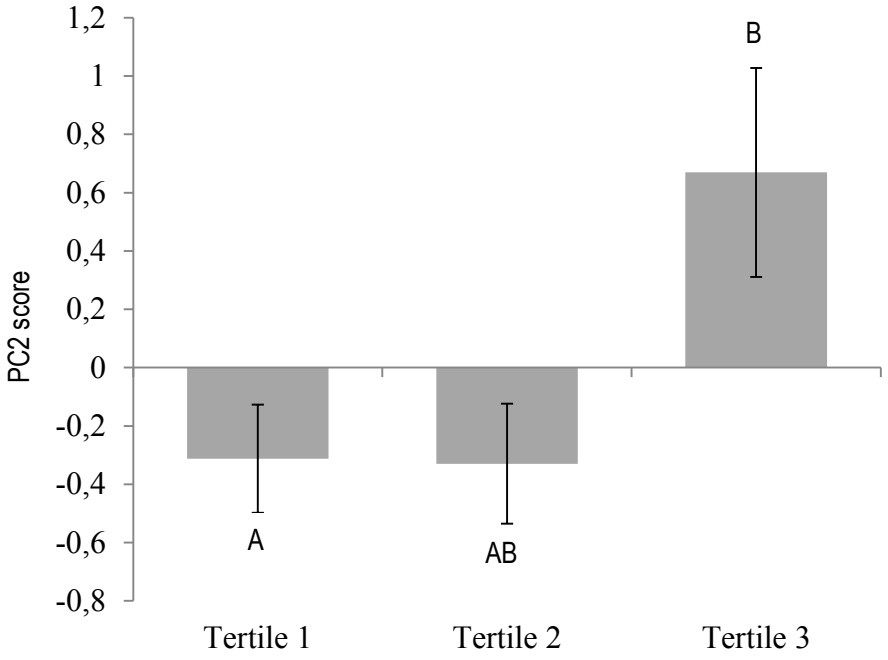
P-values in bold were considered significantly different.
P-values are all adjusted for age, sex and BMI.

Figure 4.1 ACs and AAs associated with PC1 and PC2.



Metabolites with absolute factor loadings ≥ 0.50 were regarded as significant contributors to the PC. The blue line and squares represent PC1 and the red line and squares represent PC2.

Figure 4.2 PC2 scores according to tertiles of saturated fat intake.



PC2 scores and tertile of saturated fat intake (means ± SE). Means with different letters are significantly different. Means of saturated fat intake according to tertiles: tertile 1 (4.72-10.13%, n = 12), tertile 2 (10.29-11.30%, n = 13) and tertile 3 (11.51-14.72%, n = 12). Tertile 1 versus tertile 3: p = 0.005. Tertile 1 versus tertile 2: p = 0.40. Tertile 2 versus tertile 3: p = 0.05.

Chapitre 5

Polymorphismes, lipogénèse de novo et réponse des triglycérides plasmatiques suite à une supplémentation en huile de poisson

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Polymorphisms, *de novo* lipogenesis and plasma triglyceride response following fish oil supplementation.

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Running head: SNPs and triglycerides after fish oil supplementation

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Résumé

Introduction : Une variabilité interindividuelle dans la réponse des concentrations de triglycérides plasmatiques (TG) suite à une supplémentation en huile de poisson a été observée. **Objectif** : Examiner les associations entre des polymorphismes nucléotidiques simples (SNPs) présents dans des gènes encodant pour des protéines impliquées dans la lipogénèse de novo et les changements relatifs dans les concentrations de TG suite à une supplémentation en huile de poisson. **Méthodes** : Deux cent huit participants ont été recrutés dans la région de Québec. Les participants ont complété une supplémentation en huile de poisson d'une durée de 6 semaines (5g d'huile de poisson/jour : 1,9-2,2g d'AEP et 1,1g d'ADH). Des SNPs présents dans les gènes *SREBF1*, *ACLY* et *ACACA* ont été génotypés en utilisant la méthodologie TAQMAN. **Résultats** : Après avoir corrigé le seuil de signification pour les comparaisons multiples, seuls deux SNPs, rs8071753 (*ACLY*) et rs1714987 (*ACACA*), étaient associés avec le changement relatif des concentrations de TG (p=0,004 et p=0,005, respectivement). Ces deux SNPs, rs8071753 et rs1714987, expliquaient 7,73% de la variance dans les changements relatifs des concentrations de TG suite à la supplémentation en huile de poisson. Les fréquences génotypiques pour le SNP rs8071753 en fonction de la réponse des concentrations de TG (répondeur versus non-répondeur) étaient différentes (p=0,02). **Conclusion** : La présence de certains SNPs des gènes *ACLY* et *ACACA*, encodant pour des protéines impliquées dans la lipogénèse de novo influence la réponse des concentrations de TG suite à la consommation d'huile de poisson.

Abstract

Background: Inter-individual variability in the response of plasma triglyceride concentrations (TG) following fish oil consumption has been observed. **Objective**: To examine the associations between single-nucleotide polymorphisms (SNPs) within genes encoding proteins involved in *de novo* lipogenesis and the relative change in plasma TG levels following a fish oil supplementation. **Design**: Two hundred and eight participants were recruited in the greater Quebec City area. The participants completed a 6-week fish oil supplementation (5g fish oil/day: 1.9-2.2g EPA and 1.1g DHA). SNPs within *SREBF1*, *ACLY* and *ACACA* genes were genotyped using TAQMAN methodology. **Results**: After, correction for multiple comparison, only two SNPs, rs8071753 (*ACLY*) and rs1714987 (*ACACA*), were associated with the relative change in plasma TG concentrations (p=0.004 and p=0.005, respectively). These two SNPs, rs8071753 and rs1714987, explained 7.73% of the variance in the plasma TG relative change following fish oil consumption. Genotype frequencies of rs8071753 according to the TG response groups (responders versus non-responders) were different (p=0.02). **Conclusion**: The presence of certain SNPs within genes such as *ACLY* and *ACACA*, encoding proteins involved in *de novo* lipogenesis seem to influence the plasma TG response following fish oil consumption.

Introduction

In Canada, cardiovascular disease (CVD) is the second leading cause of mortality after cancer (1). Many risk factors contribute to increase the risk of developing CVD. Among them, plasma triglyceride concentrations (TG) have been debated about whether they should be considered as an independent CVD risk factor. A review by Morisson *et al*. (2), indicated that plasma TG concentrations are an independent risk factor of coronary events in individuals without previous history of coronary heart disease (CHD). Plasma TG concentrations was the parameter of the metabolic syndrome the most strongly and independently associated with myocardial infarction and stroke (3). However, a recent meta-analysis has demonstrated no independent association between plasma TG concentrations and CVD risk (4). Still, the presence of high plasma TG concentrations is an important biomarker indicating alterations of lipid metabolism.

Omega-3 polyunsaturated fatty acids (n-3 PUFA) have been proven as an effective way to reduce plasma TG concentrations (5). However, a large inter-individual variability has been observed in plasma TG concentrations response following an n-3 PUFA supplementation (6-8). A decrease in *de novo* lipogenesis is one of a few pathways which could explain the n-3 PUFA plasma TG lowering effects. Lipogenesis is strongly regulated by the transcription factor *Sterol regulatory element binding transcription factor 1* (*SREBF1*) (9). N-3 PUFA decrease the expression of *SREBF1* (10, 11). *SREBF1* regulates the activity of several lipogenic genes such as *ATP citrate lyase* (*ACLY*) and *acetyl-CoA carboxylase alpha* (*ACACA*). ACLY is the primary enzyme responsible for the synthesis of acetyl-CoA in the cytosol (12). Acetyl-CoA may then be transformed by ACACA to malonyl-CoA which is the first product in fatty acid biosynthesis (13). Only a few polymorphisms within these genes have been studied. *SREBF1*, gly952gly (rs2297508), has been associated with obesity, type 2 diabetes and serum lipids (14-16) whereas a few SNPs within *ACACA* gene (rs1266175 and rs2229416) were associated with plasma TG concentrations after the intake of certain antipsychotic drugs (17). The effects of SNPs within these genes on plasma TG concentrations have never been studied in the context of an n-3 PUFA supplementation. Therefore, the aim of the present study was to examine the associations between

polymorphisms in genes involved in lipogenesis pathway and the plasma TG response following a marine n-3 PUFA supplementation.

Methods

Subjects

A total of 254 unrelated subjects were recruited to participate in this clinical trial from the greater Quebec City metropolitan area between September 2009 and December 2011 through advertisements in local newspapers as well as by electronic messages sent to university students/employees. It was determined that a group of 152 participants was sufficient to provide an 80% probability and a 5% significance level of detecting an anticipated difference of 0.25 mmol/L in plasma triglycerides concentrations after 6 weeks of fish oil supplementation with a genetic variation occurring in a relatively low frequency (5%) of the population. To be eligible, subjects had to be non-smokers and free of any thyroid or metabolic disorders requiring treatment such as diabetes, hypertension, severe dyslipidemia, and coronary heart disease. Participants had to be aged between 18 and 50 years old and with a body mass index (BMI) between 25 and 40 Kg/m^2. The subjects who had taken n-3 PUFA supplements during the six months preceding the study were excluded. A total of 210 subjects completed the n-3 PUFA supplementation period. However, plasma TG concentrations were available for 208 participants, thus the analyses were conducted on 208 participants. The experimental protocol was approved by the ethics committees of Laval University Hospital Research Center and Laval University. This clinical trial was registered at clinicaltrials.gov (NCT01343342). Informed written consent was obtained from all the study subjects.

Study design and diets

Subjects followed a run-in period of 2 weeks. Individual dietary instructions were given by a trained dietitian to achieve the recommendations from Canada's Food Guide. Subjects were asked to follow these dietary recommendations and maintain their body weight stable throughout the research protocol. Some specifications were given regarding the n-3 PUFA dietary intakes: not to exceed two fish or seafood servings per week, prefer white flesh fishes instead of fatty fishes (examples were given) and avoid enriched n-3 PUFA dietary products such as some milks, juices, breads and eggs. Subjects were also asked to limit their alcohol consumption during the protocol: two regular drinks per week were allowed.

In addition, subjects were not allowed to take n-3 PUFA supplements (such as flaxseed), vitamins or any natural health products during the protocol.

After the 2-week run-in period, each participant received a bottle containing n-3 PUFA capsules for the next 6 weeks. They were instructed to take five capsules (1 g of fish oil/capsule) per day (Ocean Nutrition, Nova Scotia, Canada), providing a total of 5 g of fish oil (1.9-2.2g EPA and 1.1 g DHA) per day. Capsules were provided in sufficient quantity for 6 weeks. Compliance was assessed from the return of bottles and by measuring plasma phospholipid fatty acid composition. Subjects were asked to report any deviation during the study protocol and to write down their alcohol and fish consumption as well as any side effects.

A validated food-frequency questionnaire (FFQ) was administrated to each participant before the run-in period by a trained registered dietitian (18). This FFQ is based on typical food items available in Quebec and contains 91 items with 27 items that have 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. Moreover, subjects completed two 3-d food records, before and after the n-3 PUFA supplementation period. Dietary data included both foods and beverages consumed at home and outside. A dietitian provided instructions on how to complete the food record with some examples and a written copy of these examples. All foods and beverages consumed on 2 representative weekdays and 1 weekend day were weighed or estimated and recorded in food diaries. 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 (19) and were taken before the run-in period, as

well as before and after the n-3 PUFA supplementation. BMI was calculated as weight per meter squared (Kg/m^2).

Biochemical parameters

The morning after a 12-hour overnight fast and 48-h alcohol abstinence, blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA. Blood samples were used to identify individuals with metabolic disorders, which were excluded. Plasma was separated by centrifugation (2500 x g for 10 minutes at 4°C), samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (total-C) and plasma TG concentrations were measured using enzymatic assays (20, 21). Infranatant (d >1.006 g/ml) with heparin-manganese chloride was used to precipitate VLDL and LDL and then determine HDL cholesterol concentrations (HDL-C) (22). The equation of Friedewald was used to estimate LDL-cholesterol concentrations (LDL-C) (23). Non-HDL-C was calculated by subtracting HDL-C from total-C. Plasma apoB-100 concentrations were measured by the rocket immunoelectrophoretic method of Laurell, as previously described (24).

Fatty acid composition of plasma phospholipids

Briefly, plasma lipids were extracted with chloroform:methanol (2:1, by volume) according to a modified Folch method (25). Total PL were separated by thin layer chromatography using a combination of isopropyl ether and acetic acid and fatty acids of isolated PL were then methylated. Capillary gas chromatography was then used to obtain FA profiles. The technique used for plasma analyses has been previously validated (26).

SNPs selection and genotyping

SNPs were selected with the International HapMap Project SNP database (HapMap Data Rel 28 Phase II+III, August 10, on National Center for Biotechnology Information (NCBI) B36 assembly, dbSNP b126). Tag SNPs (tSNPs) were determined with the tagger procedure in HaploView software version 4.2 with minor allele frequency (MAF) of >0.05

and pairwise tagging $R^2 \geq 0.80$. For each gene a minimum of 85% of the most common SNPs had to be captured by tSNPs. Additionally, tSNPs were prioritized according to the following criteria: (1) known SNPs from the literature, (2) SNPs within coding regions (exon), (3) SNPs within the promoter region (2500 bp before the start codon), (4) SNPs within 3' untranslated region (UTR) (500 bp after the stop codon) and (5) SNPs within 100 bp before an exon-intron splicing boundaries. Afterwards, as shown in Figure 5.1, linkage disequilibrium (LD) plot were generated with Haploview software version 4.2. All tSNPs were genotyped within INAF laboratories with the TAQMAN methodology (27), as described previously (8). Briefly, genotypes were determined using ABI Prism SDS version 2.0.5 (Applied Biosystem, Foster City, CA, USA). All SNPs were successfully genotyped. In an attempt to further understand the potential effects of the associated tSNPs on splice consensus sites or on intronic enhancer regions, NNSPLICE (28), Splice Site Finder (29, 30) and FASTSNP (31) web based programs were used.

Statistical analyses

Hardy-Weinberg equilibrium was tested with the ALLELE procedure of SAS version 9.3 using Fisher's exact test ($P<0.01$). When the genotype frequency for homozygotes individuals of the minor allele was <5%, carriers (heterozygotes and homozygotes individuals) of the minor allele were grouped.

Variables non-normally distributed were logarithmically transformed. To examine the difference between responders and non-responders for the plasma TG response, two groups were created. The responders group included individuals who decreased their plasma TG concentrations after the supplementation period (relative change in plasma TG < 0%). The non-responders group included individuals who did not change their plasma TG concentrations or even increased them (relative change in plasma TG ≥ 0%). Pre-supplementation and the relative change in the descriptive characteristics of the participants were compared between the responders and non-responders with an ANOVA including age, sex and BMI (except for BMI and waist circumference variables).

In order to verify the SNPs which were associated with the relative change in plasma TG concentrations, the GLM procedure of SAS was used and age, sex and BMI were included as confounding variables. To take into account the effects of multiple testing the simpleM procedure described by Gao *et al*. (32) was utilised. This method takes into consideration the impacts of LD between SNPs and has been demonstrated as efficient and accurate compared to permutation-based corrections (32). First, the composite LD correlation matrix was derived from the data set. Then, eigenvalues were calculated using the SAS PRINCOMP procedure and the number of effective independent tests was inferred so that the corresponding eigenvalues explain 99.5% of the variation in SNP data, as proposed by Gao *et al*. (32). The final step applies the Bonferroni correction formula to calculate the adjusted point-wise significance level, which was defined as α_G=0.05/9 (effective independent tests). Thus, p-values lower than 5.56×10^{-3} were considered significant.

To identify the variables the most tightly associated with the relative change in plasma TG concentrations, a regression model with stepwise selection, including and retaining only variables and tSNPs with $p<0.05$ was created. This regression model included age, sex, BMI and the tSNPs. Fisher's exact test was performed to verify the differences in the frequencies between responders and non-responders according to individual SNPs. All statistical analyses were performed using SAS statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Descriptive characteristics of the study population

Descriptive characteristics of the study participants are presented in Table 5.1. Briefly, mean BMI was slightly over 25 Kg/m^2 and blood lipids were around normal values (33). Descriptive characteristics of the study participants according to plasma TG response (responders vs non-responders), are shown in Table 5.2. Before the supplementation, non-responders had higher HDL-C concentrations and lower plasma TG concentrations than responders. After the supplementation, non-responders increased slightly more their BMI than responders. Non-responders increased their total-C and plasma TG concentrations and decreased their HDL-C while the opposite was observed for responders. These results are concordant with our previous results for a subset of 12 selected participants from this cohort (34).

TSNPs and baseline characteristics according to genotypes

The compliance rate for the fish oil supplementation was 94.41%±8.33%. The responders had a compliance rate of 93.98%±8.85% (n=148) and for the non-responders the compliance rate was 95.48%±6.71% (n=60) (p=0.17). No differences were observed in fatty acid incorporation into plasma phospholipids (total n-3 PUFA, EPA and DHA) between responders and non-responders (data not shown). The selected SNPs are presented in Table 5.3. All SNPs were in Hardy-Weinberg equilibrium. In Figure 5.1 (A to C), LD plot are presented. Briefly, three tSNPs covered 100% of the known genetic variability within *SREBF1* gene, three tSNPs covered 88% for *ACLY* and eight tSNPs covered 85% for *ACACA*.

Age, sex and baseline BMI did not differ by genotypes except for rs4925118 (*SREBF1*) for age, and rs12953299 (*SREBF1*) and rs1266175 (*ACACA*) for sex (p=0.01, p=0.01 and p=0.02, respectively). C/C homozygotes of rs4925118 (*SREBF1*) were slightly older than carriers of the rare allele (data not shown). A greater proportion of men were A/A homozygotes for rs12953299 (*SREBF1*) and G/G homozygotes for rs1266175 (63% for

both). Genotype frequencies according to the plasma TG response group seemed to differ only for rs8071753 (*ACLY*) (p=0.02) and trends were observed for three tSNPs within *ACACA* gene (rs2921368, rs1714987 and rs3815059) (Table 5.4).

Associations between tSNPs and baseline plasma TG levels

The SNP rs1714987 (*ACACA*) seemed associated with pre-supplementation plasma TG concentrations (p=0.006) (adjusted for age, sex and BMI). Carriers of the G allele (n=69) seemed to have higher baseline plasma TG concentrations than C/C homozygotes (n=139) (1.32±0.58 mmol/L compared to 1.15±0.65 mmol/L).

Association between tSNPs and the plasma TG relative change

As presented in Table 5.5, two SNPs (rs8071753: *ACLY* and rs1714987: *ACACA*) were associated with the relative change in plasma TG concentrations (adjusted for age, sex and BMI). Both associated tSNPs (rs8071753 and rs1714987) where located within intronic regions. Thus, to further examine the putative effects of these tSNPs on splice site, NNSPLICE, Splice Site Finder and FASTSNP were used. According to Splice Site Finder, A allele of rs8071753 seems to slightly decrease a putative donor site (72.61 to 74.59) compared to G allele. C allele of rs1714987 also slightly decreased a putative donor site compared to G allele (81.66 to 83.42). None of these predictions were confirmed by NNSPLICE program. According to FASTSNP program, rs8071753 and rs1714987 were not predicted to be in any transcription factor binding site or splicing site. Hence, it is unlikely that these SNPs may be responsible for possible alternative splice events or have a functional impact.

In an attempt to identify the variable the most tightly associated with the relative change in plasma TG concentrations, a regression model with stepwise selection including age, sex, BMI and the tSNPs was computed. Only the tSNPs rs8071753 (partial R^2=3.73%, p=0.005) and rs1714987 (partial R^2=4.01%, p=0.003) contributed to the model. In sum, the two SNPs allowed explaining 7.73% of the variance in the relative change of plasma TG concentrations. Age, sex and BMI were not contributors to the regression model, indicating

that they were not associated with the relative change in plasma TG concentrations following the n-3 PUFA supplementation.

The mean plasma TG relative change of individuals with both genotypes (A/A or A/G for rs8071753 and C/C for rs1714987) associated with a lower plasma TG response was -0.63%±30.54% (n=52) compared to a mean of -15.64%±23.09% (n=156) for the individuals with zero or one genotype (p=0.0001).

Discussion

According to the present results, certain SNPs within genes involved in *de novo* lipogenesis may explain part of the differences observed in plasma TG concentrations following an n-3 PUFA supplementation. The impact of n-3 PUFA on plasma TG concentrations may be modified by SNPs within genes involved in *de novo* lipogenesis pathway. SNPs may lead to an increase or a decrease in the activity of the enzymes or the affinity to certain transcription factors affecting the fatty acid metabolic pathway. In this study, two SNPs (rs8071753 and rs1714987) within genes involved in *de novo* lipogenesis were associated with the plasma TG response following an n-3 PUFA supplementation. This biological pathway contributes directly for only small amounts (<5%) to the NEFA pool utilized for the assembly of VLDL-TG among healthy individuals (35). However, among individuals with non-alcoholic fatty liver disease or with hypertriglyceridemia, it may contribute more importantly (around 15%) to the fatty acids used for VLDL-TG production (35-37). Thus, it is possible that among individuals with a more deteriorated metabolic profile, SNPs within genes related to *de novo* lipogenesis may have been more strongly associated with plasma TG response. It has been observed that NEFA derived from *de novo* lipogenesis are preferentially incorporated into the hepatic intracellular storage pool as TG (38). Intracellular TG can then be mobilized in the cytosol by lipolysis followed by re-esterification through a delayed pathway also contributing to VLDL-TG production (38, 39).

As described earlier, n-3 PUFA consumption induces a decrease in *de novo* lipogenesis mediated by *SREBF1* gene (9-11, 40). In the literature, two SNPs within *SREBF1* gene (rs2297508 and rs1889018) have been associated with a modest increase of type 2 diabetes risk, body weight (BMI or obesity), total-C, LDL-C and plasma TG concentrations (14, 16, 41, 42). *SREBF1* regulates the activity of both *ACLY* and *ACACA* genes. *ACLY* gene encodes for the enzyme converting citrate from the Krebs cycle to acetyl-CoA which enters the first step of *de novo* lipogenesis. The enzyme ACACA then produces malonyl-CoA from acetyl-CoA (12, 13). We hypothesized that SNPs within these genes may modify the affinity of sterol regulatory element binding protein 1 (SREBP1) with the sterol regulatory

element (SRE) within the promoter region or their function (43). This study is the first to report an association between rs8071753 within *ACLY* gene and plasma TG response following an n-3 PUFA supplementation. This SNP is located within an intronic region of *ACLY* gene and does not seem to have any regulatory impact on *ACLY* gene (44). In *ACACA* gene, the SNP rs1714987 was also associated the plasma TG response. Interestingly, carriers of the minor G allele seemed to have higher baseline plasma TG concentrations and decreased their plasma TG concentrations by almost twice as much as C/C homozygotes following the n-3 PUFA supplementation. Thus, rs1714987 seem to have a beneficial impact on the plasma TG response following the intake of n-3 PUFA. Diaz *et al*. (17), have observed associations between SNPs within *ACACA* gene (rs1266175 and rs2229416) and plasma TG concentrations following the intake of certain antipsychotic drugs that increase the risk of hyperlipidemia, globally having the opposite effect of n-3 PUFA on lipid metabolism by increasing lipogenesis and decreasing fatty acid oxidation (45, 46).

When the two SNPs associated with the relative change in plasma TG concentrations were grouped, results demonstrate that individuals having both «at risk» genotypes have hardly diminished their plasma TG concentration despite the intake of fish oil. It is likely that individuals who carry different combinations of SNPs may be at an increased risk of no change or even an increase of their plasma TG concentrations following an n-3 PUFA supplementation. However, these results need to be replicated and regression models including more SNPs are required in order to eventually generate a proper model leading to a clear identification of individuals who will have a beneficial plasma TG response following an n-3 PUFA supplementation. In this study, baseline plasma TG concentrations were within normal values, it is possible that with higher baseline values, there would have been fewer individuals considered as «non-responders» following the intake of fish oil.

Overall, this study shows that SNPs within genes involved in *de novo* lipogenesis may have an impact on the plasma TG response following the intake of fish oil. These SNPs may affect gene regulation by unknown mechanisms or are potentially in LD with other causal SNPs. More SNPs within genes involved in *de novo* lipogenesis but also in other metabolic

pathways such as fatty acid beta-oxidation, should be studied in order to further understand the genetic basis behind the observed variability in plasma TG response after fish oil consumption. The determination of SNPs associated with the plasma TG response after the intake of fish oil could help in the future to use fish oil more efficiently among hypertriglyceridemic individuals.

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Competing interests

The authors declare no conflict of interest.

Authors' contributions

IR, SL, PC, and MCV designed research; ABM conducted research with the research professionals; IR, SL, PC and MCV provided essential reagents or provided essential materials; ABM analyzed data and performed statistical analysis; ABM wrote paper; ABM, IR, SL, PC and MCV had primary responsibility for final content; All authors read and approved the final manuscript.

References

1. Statistics Canada. Leading Causes of Death in Canada. 2009. Internet: http://www.statcan.gc.ca/pub/84-215-x/2012001/hl-fs-eng.htm (accessed 23 November 2012).
2. Morrison, A., and J. E. Hokanson. 2009. The independent relationship between triglycerides and coronary heart disease. Vasc. Health Risk Manag. 5: 89-95.
3. Ninomiya, J. K., G. L'Italien, M. H.Criqui, J. L. Whyte., A. Gamst, and R. S.Chen. 2004. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. Circulation. 109: 42-46.
4. Di, A. E., N. Sarwar, P. Perry, S. Kaptoge, K. K. Ray, A. Thompson, A. M. Wood, S. Lewington, N. Sattar, C. J. Packard,R.Collins, S. G. Thompson, and J. Danesh. 2009. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 302: 1993-2000.
5. Singh, A., A. Schwartzbard, E. Gianos, J. S.Berger, and H. Weintraub. 2012. What should we do about Hypertriglyceridemia in Coronary Artery Disease Patients? Curr Treat Options Cardiovasc Med. 15: 104-117.
6. Madden, J., C. M. Williams, P. C.Calder, G. Lietz, E. A. Miles, H.Cordell, J. C. Mathers, and A. M. Minihane. 2011. The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. Annu Rev Nutr. 31: 203-234.
7. Caslake, M. J., E. A. Miles, B. M. Kofler, G. Lietz, P. Curtis, C. K. Armah, A. C. Kimber, J. P. Grew, L. Farrell, J. Stannard, F. L. Napper, A. Sala-Vila, A. L.West, J. C. Mathers, C. Packard, C. M. Williams, P. C.Calder, and A. M. Minihane. 2008. Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr. 88: 618-629.
8. Cormier, H., I. Rudkowska, A. M. Paradis, E. Thifault, V. Garneau, S. Lemieux, P. Couture, and M. C. Vohl. 2012. Association between Polymorphisms in the Fatty Acid Desaturase Gene Cluster and the Plasma Triacylglycerol Response to an n-3 PUFA Supplementation. Nutrients. 4: 1026-1041.
9. Eberle, D., B. Hegarty, P.Bossard, P. Ferre, and F. Foufelle. 2004. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 86: 839-848.
10. Xu, J., M. Teran-Garcia, J. H. Park, M. T. Nakamura, and S. D.Clarke. 2001. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. J Biol Chem. 276: 9800-9807.
11. Tanaka, N., X. Zhang, E. Sugiyama, H. Kono, A. Horiuchi, T. Nakajima, H. Kanbe, E. Tanaka, F. J. Gonzalez, and T. Aoyama. 2010. Eicosapentaenoic acid improves hepatic steatosis independent of PPARalpha activation through inhibition of SREBP-1 maturation in mice. Biochem Pharmacol. 80: 1601-1612.
12. Chypre, M., N. Zaidi, and K. Smans. 2012. ATP-citrate lyase: a mini-review. Biochem Biophys Res Commun. 422: 1-4.
13. Wakil, S. J., J. K. Stoops, and V. C.Joshi. 1983. Fatty acid synthesis and its regulation. Annu Rev Biochem. 52: 537-579.
14. Eberle, D., K.Clement, D. Meyre, M. Sahbatou, M. Vaxillaire, G. A. Le, P. Ferre, A.Basdevant, P. Froguel, and F. Foufelle. 2004. SREBF-1 gene polymorphisms are

associated with obesity and type 2 diabetes in French obese and diabetic cohorts. Diabetes 53: 2153-2157.
15. Zhang, Z., R. R. Gong, J. Du, L. Y. Xiao, W. Duan, X. D. Zhou, and D. Z. Fang. 2011. Associations of the SREBP-1c gene polymorphism with gender-specific changes in serum lipids induced by a high-carbohydrate diet in healthy Chinese youth. Appl Physiol Nutr Metab 36: 226-232.
16. Grarup, N., K. L. Stender-Petersen, E. A. Andersson, T.Jorgensen, K. BorchJohnsen, A. Sandbaek, T. Lauritzen, O. Schmitz, T. Hansen, and O. Pedersen. 2008. Association of variants in the sterol regulatory element-binding factor 1 (SREBF1) gene with type 2 diabetes, glycemia, and insulin resistance: a study of 15,734 Danish subjects. Diabetes 57: 1136-1142.
17. Diaz, F. J., A. Meary, M. J. Arranz, G.Ruano, A. Windemuth, and L. J. de. 2009. Acetyl-coenzyme A carboxylase alpha gene variations may be associated with the direct effects of some antipsychotics on triglyceride levels. SchizophrRes. 115: 136-140.
18. Goulet, J., G. Nadeau, A. Lapointe, B. Lamarche, and S. Lemieux. 2004. Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. NutrJ. 3: 13-22.
19. Callaway, C.W., W.C. Chumlea, C. Bouchard,J.H. Himes, T.G. Lohman, A.D. Martin, C.D. Mitchell, W.H. Mueller, A.F. Roche, V.D. Seefeldt. 1988. Standardization of Anthropometric Measurements, In: T. Lohman, A. Roche, R. Martorel. eds, The Airlie (VA) Consensus Conference, Human Kinetics Publishers, Champaign, IL. 39-80.
20. McNamara, J. R., and E. J. Schaefer. 1987. Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. Clin Chim Acta. 166: 1-8.
21. Burstein, M., and J. Samaille. 1960. On a rapid determination of the cholesterol bound to the serum alpha- and beta-lipoproteins. Clin Chim Acta. 5: 609.
22. Albers, J. J., G. R. Warnick, D. Wiebe, P. King, P. Steiner, L. Smith, C. Breckenridge, A.Chow, K. Kuba, S. Weidman, H. Arnett, P. Wood, and A. Shlagenhaft. 1978. Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. Clin Chem. 24: 853-856.
23. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. ClinChem. 18: 499-502.
24. Laurell, C. B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem. 15: 45-52.
25. Shaikh, N. A. and Downar, E. 1981. Time course of changes in porcine myocardial phospholipid levels during ischemia. A reassessment of the lysolipid hypothesis. Circ. Res. **49**: 316-325.
26. Kroger, E., Verreault, R., Carmichael, P. H., Lindsay, J., Julien, P., Dewailly, E., Ayotte, P., and Laurin, D. 2009. Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. Am. J. Clin. Nutr. **90**: 184-192.
27. Livak, K. J. 1999. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal. 14: 143-149.
28. Reese, M. G., F. H. Eeckman, D. Kulp, and D. Haussler. 1997. Improved splice site detection in Genie. J Comput Biol. 4: 311-323.

29. Shapiro, M. B., and P. Senapathy. 1987. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res. 15: 7155-7174
30. Desmet, F. O., D. Hamroun, M. Lalande, G. Collod-Beroud, M.Claustres, and C. Beroud. 2009. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 37: e67.
31. Yuan, H. Y., J. J.Chiou, W. H. Tseng, C. H. Liu, C. K. Liu, Y. J. Lin, H. H. Wang,A. Yao, Y. T.Chen, and C. N. Hsu. 2006. FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. Nucleic Acids Res. 34: W635-W641
32. Gao, X., J. Starmer, and E. R. Martin. 2008. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet. Epidemiol. 32: 361-369.
33. National Cholesterol Education Program (NCEP) Expert Panel. 2002. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 106: 3143-3421
34. Rudkowska, I., A. M. Paradis, E. Thifault, P.Julien, O.Barbier, P.Couture, S. Lemieux, and M. C. Vohl. 2012. Differences in metabolomic and transcriptomic profiles between responders and non-responders to an n-3 polyunsaturated fatty acids (PUFAs) supplementation. Genes Nutr. (Epub ahead of print)
35. Fabbrini, E., B. S. Mohammed, F. Magkos, K. M. Korenblat, B. W. Patterson, and S. Klein. 2008. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology. 134: 424-431
36. Diraison, F., P. Moulin, and M.Beylot. 2003. Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. Diabetes Metab. 29: 478-485.
37. Vedala, A., W. Wang, R. A. Neese, M. P. Christiansen, and M. K. Hellerstein.2006. Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and de novo lipogenesis in humans. J. Lipid Res. 47: 2562-2574.
38. Gibbons, G. F., S. M.Bartlett,C. E. Sparks, and J. D. Sparks. 1992. Extracellular fatty acids are not utilized directly for the synthesis of very-low-density lipoprotein in primary cultures of rat hepatocytes. Biochem J. 287 (Pt 3): 749-753.
39. Gibbons, G. F., D. Wiggins, A. M.Brown, and A. M. Hebbachi. 2004. Synthesis and function of hepatic very-low-density lipoprotein. Biochem Soc Trans. 32: 59-64.
40. Jump, D. B. 2008. N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. Curr Opin Lipidol. 19: 242-247.
41. Laudes, M., I. Barroso, J. Luan, M. A. Soos, G. Yeo, A. Meirhaeghe, L. Logie, A. Vidal-Puig, A. J. Schafer, N. J. Wareham, and S. O'Rahilly. 2004. Genetic variants in human sterol regulatory element binding protein-1c in syndromes of severe insulin resistance and type 2 diabetes. Diabetes. 53: 842-846.
42. Liu, J. X., J. Liu, P. Q. Li, X. D. Xie, Q. Guo, L. M. Tian, X. Q. Ma, J. P. Zhang, J. Liu, and J. Y. Gao. 2008. Association of sterol regulatory element-binding protein-1c gene polymorphism with type 2 diabetes mellitus, insulin resistance and blood lipid levels in Chinese population. Diabetes Res Clin Pract. 82: 42-47.

43. H. Q. Yin, M. Kim, J. H. Kim, G. Kong, K. S. Kang, H. L. Kim, B. I. Yoon, M. O. Lee, and B. H. Lee. 2007. Differential gene expression and lipid metabolism in fatty liver induced by acute ethanol treatment in mice. Toxicol Appl Pharmacol. 223: 225-233.
44. NCBI. dbSNP Short Genetic Variations. 2012. Internet: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1293329 (accessed 11 December 2012).
45. de, L. J., J. C.Correa, G.Ruano, A. Windemuth, M. J. Arranz, and F. J. Diaz. 2008. Exploring genetic variations that may be associated with the direct effects of some antipsychotics on lipid levels. Schizophr Res. 98: 40-46.
46. Harris, W. S., and D.Bulchandani. 2006. Why do omega-3 fatty acids lower serum triglycerides? Curr Opin Lipidol. 17: 387-393.

Table 5.1 Descriptive characteristics of the study participants (n=208).

Variables	Means ± SD
Age (years)	30.82±8.66
Sex (men/women)	96/112
BMI (Kg/m^2)	27.84±3.73
Waist circumference (cm)	Men: 94.85±10.98 Women: 91.99±10.44
Systolic blood pressure (mmHg)	112.03±13.64 (n=207)
Diastolic blood pressure (mmHg)	69.54±9.19 (n=207)
Fasting glucose (mmol/L)	4.95±0.52
Fasting insulin (pmol/L)	82.51±35.61 (n=206)
Total-C (mmol/L)	4.82±1.01
LDL-C (mmol/L)	2.79±0.87 (n=207)
HDL-C (mmol/L)	1.46±0.39
Triglycerides (mmol/L)	1.23±0.64
ApoB (g/L)	0.86±0.25 (n=207)

Means ± SD

Table 5.2 Descriptive characteristics of the study participants according to plasma TG response (pre- and post-supplementation).

Variables	Pre-supplementation			Post-supplementation		
	Responders (n=148)	**Non-responders (n=60)**	**P-value[a]**	**Responders (n=148)**	**Non-responders (n=60)**	**P-value[b]**
BMI (Kg/m^2)	27.81±3.68	27.79±3.91	0.98	Δ0.17±1.53% 27.86±3.79	Δ0.66±1.46% 27.98±4.01	0.03
Waist circumference (cm)	Men (n=66): 93.98±10.04 Women (n=81): 91.74±9.74	Men (n=30): 96.70±12.29 Women (n=30): 92.52±11.32	Men: 0.23 Women: 0.75	Men (n=66): Δ0.37±2.17% 94.31±10.08 Women (n=81): Δ-0.09±2.86% 91.63±10.19	Men (n=30): Δ0.35±1.81% 97.00±12.06 Women (n=30): Δ-0.13±2.21% 92.40±11.41	0.94 0.98
Total-C (mmol/L)	4.74±0.86	4.77±1.01	0.92	Δ-1.22±10.66% 4.66±0.88	Δ2.51±10.81% 4.87±1.07	0.02
LDL-C (mmol/L)	2.74±0.77	2.79±0.91	0.83	Δ1.61±16.40% 2.76±0.80	Δ2.27±16.76% 2.84±0.97	0.82
HDL-C (mmol/L)	1.41±0.34	1.50±0.39	0.03	Δ4.14±12.76% 1.47±0.40	Δ-1.26±10.28% 1.48±0.41	0.005
Triglycerides (mmol/L)	1.28±0.67	1.03±0.48	0.002	Δ-24.68±14.98% 0.95±0.50	Δ19.65±19.36% 1.20±0.54	<0.0001
ApoB (g/L)	0.84±0.23 (n=147)	0.83±0.26 (n=59)	0.66	Δ3.85±17.31% 0.86±0.23 (n=147)	Δ7.94±15.61% 0.88±0.25 (n=58)	0.12

[a]ANOVA including age, sex and BMI (except for BMI and waist circumference).
[b]ANOVA assessing the relative change in each variable including age, sex and BMI (except for BMI and waist circumference).
Means ± SD

Table 5.3 The selected single-nucleotide polymorphisms within *SREBF1*, *ACLY* and *ACACA* genes.

Genes	dbSNP No.[a]	Sequence[b]	Position	MAF	Genotype frequency		
SREBF1	rs4925115	GGTGGGC[A/G]GGGCAGA	Intron	0.425	G/G (n=66) 0.317	A/G (n=107) 0.514	A/A (n=35) 0.168
	rs4925118	GTCGGTT[C/T]GCGTCCT	Intron	0.185	C/C (n=140) 0.673	C/T (n=59) 0.284	T/T (n=9) 0.043
	rs12953299	GCAGGGG[A/G]CACTAAT	Intron	0.481	G/G (n=56) 0.269	A/G (n=104) 0.500	A/A (n=48) 0.231
ACLY	rs8071753	ACTACCA[A/G]TCCAAGT	Intron	0.214	G/G (n=127) 0.611	A/G (n=73) 0.351	A/A (n=8) 0.039
	rs8065502	CCTCCGG[A/G]TGCTTCC	Exon (synonymous [His] → [His])	0.075	G/G (n=178) 0.856	A/G (n=29) 0.139	A/A (n=1) 0.005
	rs2304497	TCTTGTC[G/T]TCAGGGG	Exon (missense [Glu] → [Asp])	0.091	T/T (n=174) 0.837	G/T (n=30) 0.144	G/G (n=4) 0.019
ACACA	rs2017571	CCTTCTC[C/T]TCCTCTT	Intron	0.202	T/T (n=133) 0.639	C/T (n=66) 0.317	C/C (n=9) 0.043
	rs2921368	TTACAGA[C/G]CTACTGG	Intron	0.202	C/C (n=131) 0.630	C/G (n=70) 0.337	G/G (n=7) 0.034
	rs9906044	CAGAATA[A/T]CTACTGC	Intron	0.349	A/A (n=87) 0.418	A/T (n=97) 0.466	T/T (n=24) 0.115
	rs2229416	GCTTTCA[A/C/G]ATGAACA	Exon (synonymous	0.137	C/C (n=155)	C/T (n=49)	T/T (n=4)

		[Gln] → [Gln])		0.745	0.236	0.019
rs1714987	CCCACCA[C/G]TGCCCCT	Intron	0.180	C/C (n=139) 0.668	C/G (n=63) 0.303	G/G (n=6) 0.029
rs1266175	GAACACC[A/G]CCTGGGT	Intron	0.389	A/A (n=78) 0.375	A/G (n=98) 0.471	G/G (n=32) 0.154
rs3815059	GAAATCA[A/T]GAAATTT	Intron	0.178	A/A (n=140) 0.673	A/T (n=62) 0.298	T/T (n=6) 0.029
rs829165	AATTTGG[C/T]GATTGTT	Intron near 5'UTR region	0.123	C/C (n=162) 0.779	C/T (n=41) 0.197	T/T (n=5) 0.024

[a]SNP reference id from dbSNP Short Genetic Variations NCBI Reference Assembly.
[b]Gene sequence from dbSNP Short Genetic Variations NCBI Reference Assembly.

Table 5.4 Frequencies of the genotypes according to the plasma TG response group.

Genes	SNPS	Responders	Non-responders	P-value[a]
ACLY	rs8071753	A/A + A/G n=50 (34%) G/G n=98 (66%)	A/A + A/G n=31 (52%) G/G n=29 (48%)	0.02
ACACA	rs2921368	C/G + G/G n=61 (41%) C/C n=87 (59%)	C/G + G/G n=16 (27%) C/C n=44 (73%)	0.06
	rs1714987	C/G + G/G n=55 (37%) C/C n=93 (63%)	C/G + G/G n=14 (23%) C/C n=46 (77%)	0.07
	rs3815059	A/T + T/T n=54 (36%) A/A n=94 (64%)	A/T + T/T n=14 (23%) A/A n=46 (77%)	0.07

[a]P-values from Fisher's Exact Test.

Table 5.5 The impact of SNPs on the plasma TG response after the intake of fish oil.

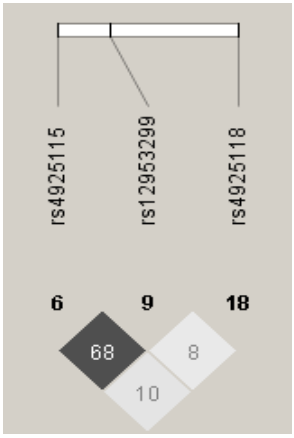
Genes	SNPs	Genotypes	% change in TG	P-value[a]
ACLY	rs8071753	A/A+A/G (n=81)	-5.64±28.85	0.004
		G/G (n=127)	-15.87±23.10	
ACACA	rs1714987	G/G+C/G (n=69)	-18.91±21.38	0.005
		C/C (n=139)	-8.40±27.29	
ACLY	rs8065502	A/A+A/G (n=30)	-2.27±32.10	0.02
		G/G (n=178)	-13.51±24.46	
ACACA	rs2017571	C/C+C/T (n=75)	-7.23±27.12	0.04
		T/T (n=133)	-14.52±24.92	
ACACA	rs3815059	T/T+A/T (n=68)	-16.31±23.36	0.08
		A/A (n=140)	-9.74±26.88	
SREBF1	rs4925118	T/T+C/T (n=68)	-7.25±27.02	0.08
		C/C (n=140)	-14.14±25.15	
ACACA	rs2229416	T/T+C/T (n=53)	-7.73±28.14	0.20
		C/C (n=155)	-13.21±25.04	
ACACA	rs2921368	G/G+C/G (n=77)	-14.86±22.31	0.23
		C/C (n=131)	-10.14±27.74	
ACLY	rs2304497	G/G+G/T (n=34)	-8.12±25.44	0.36
		T/T (n=174)	-12.63±26.01	
SREBF1	rs12953299	A/A (n=48)	-8.09±27.30	0.40
		A/G (n=104)	-14.42±25.32	
		G/G (n=56)	-10.44±25.75	
ACACA	rs829165	T/T+C/T (n=46)	-14.53±29.17	0.47
		C/C (n=162)	-11.14±24.96	
SREBF1	rs4925115	A/A (n=35)	-7.24±25.18	0.57
		A/G	-12.84±26.47	

		(n=107) G/G (n=66)	-12.82±25.49	
ACACA	rs9906044	T/T (n=24)	-10.10±26.38	0.82
		A/T (n=97)	-13.28±23.54	
		A/A (n=87)	-10.83±28.41	
ACACA	rs1266175	G/G (n=32)	-9.61±26.75	0.90
		A/G (n=98)	-12.52±25.30	
		A/A (n=78)	-12.04±26.61	

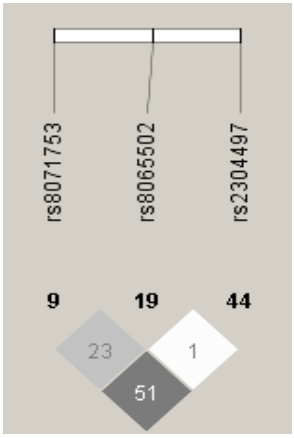
[a]P-values of the GLM models are adjusted for age, sex and BMI. P-values in bold were considered as significant ($<5.56 \times 10^{-3}$).
Means ± SD

Figure 5.1 Linkage disequilibrium (LD) plots of tSNPs in genes involved in the *de novo* lipogenesis pathway.

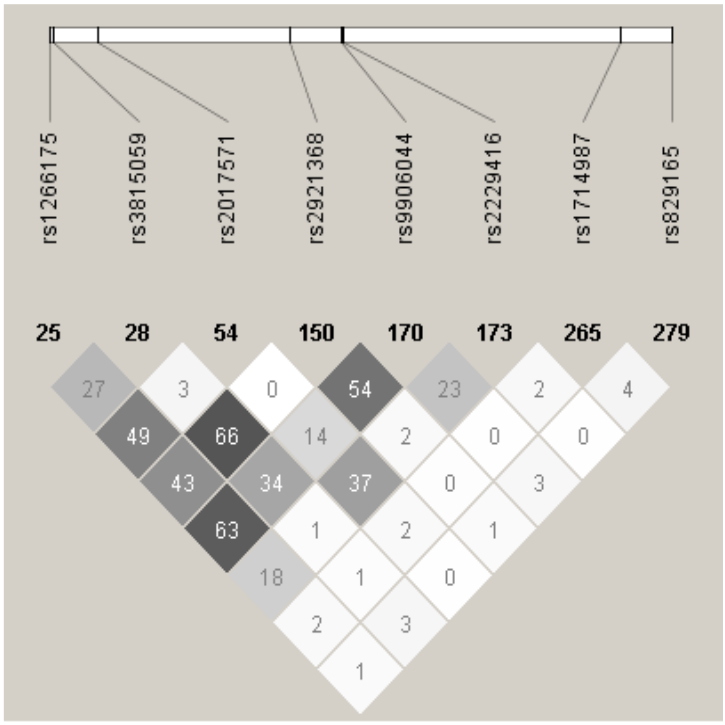
a)



b)



c)



Linkage disequilibrium (LD) plots of tSNPs in genes involved in the *de novo* lipogenesis pathway. A) *SREBF1* gene B) *ACLY* gene and C) *ACACA* gene. LD plots were generated by HaploView software version 4.2 using R^2 LD values.

Chapitre 6

Des polymorphismes présents dans des gènes impliqués dans la voie de la bêta-oxydation des acides gras interagissent avec les apports alimentaires en gras et modulent la réponse des triglycérides plasmatiques suite à une supplémentation en huile de poisson

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Polymorphisms in Genes Involved in Fatty Acid β-Oxidation Interact with Dietary Fat Intakes to Modulate the Plasma TG Response to a Fish Oil Supplementation

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Résumé

Introduction : Une importante variabilité interindividuelle dans la réponse des triglycerides plasmatiques (TG) suite à une supplémentation en acides gras polyinsaturés oméga-3 (AGPI n-3) a été observée. L'objectif était d'examiner les effets d'interaction gène-diète sur la réponse des concentrations de TG suite à une supplémentation en huile de poisson entre des polymorphismes nucléotidiques simples (SNPs) présents dans des gènes impliqués dans la β-oxydation des acides gras et les apports alimentaires en gras. **Méthodes** : Deux cent huit (208) participants ont été recrutés dans la région de Québec. Les participants ont complété une supplémentation de six semaines en huile de poisson (5g d'huile de poisson/jour : 1,9-2,2g d'AEP et 1,1g d'ADH). Les apports alimentaires en gras ont été mesurés avec des journaux alimentaires de trois jours. Des SNPs présents dans les gènes *RXRA*, *CPT1A*, *ACADVL*, *ACAA2*, *ABCD2*, *ACOX1* and *ACAA1* ont été génotypés en utilisant la méthodologie TAQMAN. **Résultats** : Des effets d'interaction gène-diète sur la réponse des TG plasmatiques ont été observés pour certains SNPs des gènes *RXRA* (rs11185660, rs10881576 et rs12339187) et *ACOX1* (rs17583163). Pour rs11185660, les changements dans les niveaux d'expression du gène *RXRA* étaient différents en fonction des apports alimentaires en gras saturés pour les homozygotes T/T. **Conclusion** : Les effets d'interaction gène-diète entre des SNPs présents dans des gènes impliqués dans la β-oxydation des acides gras et les apports alimentaires en gras pourraient expliquer une partie de la variabilité interindividuelle présente dans les concentrations de TG et dans la réponse des concentrations de TG suite à une supplémentation en huile de poisson.

Abstract

Introduction: A large inter-individual variability in the plasma triglyceride (TG) response to an omega-3 polyunsaturated fatty acid (*n*-3 PUFA) supplementation has been observed. The objective was to examine gene-diet interaction effects on the plasma TG response after a fish oil supplementation, between single-nucleotide polymorphisms (SNPs) within genes involved in fatty acid β-oxidation and dietary fat intakes. **Methods**: Two hundred and eight (208) participants were recruited in the greater Quebec City area. The participants completed a six-week fish oil supplementation (5 g fish oil/day: 1.9–2.2 g EPA and 1.1 g DHA). Dietary fat intakes were measured using three-day food records. SNPs within *RXRA*, *CPT1A*, *ACADVL*, *ACAA2*, *ABCD2*, *ACOX1* and *ACAA1* genes were genotyped using TAQMAN methodology. **Results**: Gene-diet interaction effects on the plasma TG response were observed for SNPs within *RXRA* (rs11185660, rs10881576 and rs12339187) and *ACOX1* (rs17583163) genes. For rs11185660, fold changes in *RXRA* gene expression levels were different depending on SFA intakes for homozygotes T/T. **Conclusion**: Gene-diet interaction effects of SNPs within genes involved in fatty acid β-oxidation and dietary fat intakes may be important in understanding the inter-individual variability in plasma TG levels and in the plasma TG response to a fish oil supplementation.

Introduction

Plasma triglyceride (TG) level is an important risk factor for cardiovascular disease [1]. Twin studies have revealed that plasma TG levels are highly heritable (19%–72%) with additive genetic effects accounting for around 40% of the variability observed [2,3]. The environment also contributes for an important part of the variability observed. Proportions of macronutrient intake have an impact on plasma TG levels. For example, high-fat/low-carbohydrate isocaloric diets lead to a decrease in plasma TG levels compared to low-fat/high-carbohydrate diets [4]. Polyunsaturated fats (PUFA), especially *n*-3 PUFA, have been reported to have a beneficial impact on plasma TG levels [4,5]. At the opposite, saturated fat (SFA) intakes seem to increase intrahepatic TG levels and plasma TG levels [6,7]. Fabbrini *et al*. [8] have observed that very-low-density lipoprotein (VLDL) TG secretion was almost doubled among obese individuals with high intrahepatic TG levels.

Following the intake of *n*-3 PUFA supplements, an important inter-individual variability has been observed in the plasma TG response. Approximately 30% of the individuals do not lower their plasma TG levels [9–11]. It has been observed that fish oil intake reduces VLDL production with or without a concomitant increase in VLDL clearance [12]. An increase in fatty acid β-oxidation via an increase in *peroxisome proliferator-activated receptor alpha* (*PPARA*) gene expression induced by fish oil, may decrease fatty acid availability for VLDL production [12,13]. *PPARA* forms a heterodimer with *retinoid X receptor alpha* (*RXRA*) and regulates the activity of several genes involved in the fatty acid metabolism [14]. In mitochondrial fatty acid β-oxidation, *PPARA* regulates genes such as *carnitine palmitoyltransferase 1A* (*CPT1A*), *acyl-CoA dehydrogenase* (*ACADVL*) and *acetyl-CoA acyltransferase 2* (*ACAA2*) [14]. *PPARA* also regulates enzymes involved in peroxisomal β-oxidation such as *ATP-binding cassette, sub-family (ALD)*, *member 2* (*ABCD2*), *acyl-CoA oxidase 1* (*ACOX1*) and *acetyl-CoA acyltransferase 1* (*ACAA1*) [14–16]. Single-nucleotide polymorphisms (SNPs) within these genes may have an impact on the plasma TG lowering effects of fish oil. A few studies have observed associations with plasma TG levels, coronary heart disease risk and metabolic syndrome with SNPs located within *RXRA* gene [17–19]. A SNP within the *CPT1A* gene (rs80356779) was associated

with high-density lipoprotein cholesterol (HDL-C) among Eskimos [20]. Interestingly, a gene-diet interaction effect on BMI was observed with the polymorphism pA275T of the *CPT1A* gene [21].

It is possible that gene-diet interaction effects modulate the plasma TG response to fish oil. The objective of this study is to examine the effects of SNPs within *RXRA*, *CPT1A*, *ACADVL*, *ACAA2*, *ABCD2*, *ACOX1* and *ACAA1* genes, dietary fat intakes and gene-diet interaction effects on the plasma TG response to fish oil. Gene-diet interaction effects with *RXRA* and *ACOX1* genes were observed on the plasma TG response to fish oil intake.

Methods

Participants

A total of 254 subjects were recruited between September 2009 and December 2011 from the greater Quebec City metropolitan area through advertisements in local newspapers as well as by electronic messages sent to university students/employees. To be eligible, subjects had to be non-smokers and free of any thyroid or metabolic disorders requiring treatment such as diabetes, hypertension, severe dyslipidemia, and coronary heart disease. Participants had to be aged between 18 and 50 years with a BMI between 25 and 40 kg/m^2. Subjects were excluded if they had taken *n*-3 PUFA supplements within 6 months prior to the study. A total of 210 subjects completed the *n*-3 PUFA supplementation period. However, TG levels were available for 208 participants, thus the analyses were conducted on 208 participants. 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

Subjects followed a run-in period of two weeks. Individual dietary instructions were given by a trained dietitian to achieve the recommendation from Canada's Food Guide. Subjects were asked to follow these dietary recommendations and stably maintain their body weight throughout the protocol. Some specifications were given regarding the *n*-3 PUFA dietary intakes: not to exceed two fish or seafood servings per week, prefer white flesh fishes instead of fatty fishes (examples were given) and avoid enriched *n*-3 PUFA dietary products such as some milks, juices, breads and eggs. Subjects were also told to limit their alcohol consumption during the protocol; two regular drinks per week were allowed. In addition, subjects were not allowed to take *n*-3 PUFA supplements (such as flaxseed), vitamins or natural health products during the protocol.

After the 2-week run-in, each participant received a bottle containing the *n*-3 PUFA capsules for the next six weeks. They were instructed to take five (1 g oil each) capsules per

day (Ocean Nutrition, Dartmouth, NS, Canada), providing a total of 5 g of fish oil (1.9–2.2 g EPA and 1.1 g DHA) per day. Capsules were provided in sufficient quantity for six weeks. Compliance was assessed from the return of bottles. Subjects were asked to report any deviation during the protocol and to write down their alcohol and fish consumption as well as any side effects. Before each phase, subjects received detailed written and oral instructions on their diet.

Subjects completed two 3-day food records (pre- and post- *n*-3 PUFA supplementation period). Dietary data included both foods and beverages consumed at home and outside. A dietitian provided instructions on how to complete the food record with some examples and a written copy of these examples. All foods and beverages consumed on two representative weekdays and one weekend day were weighed or estimated and recorded in food diaries. 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, USA.

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- fish oil supplementation. BMI was calculated as weight per meter squared (kg/m^2).

Biochemical Parameters

The morning after a 12-h overnight fast and 48-h alcohol abstinence, blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA. Blood samples were used to identify individuals with metabolic disorders, which were excluded. Plasma was separated by centrifugation (2500× *g* for 10 min at 4 °C), samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (TC) and TG were measured using enzymatic assays [23,24]. Infranatant (d > 1.006 g/mL) with heparin-manganese chloride was used to precipitate VLDL and low-density lipoprotein (LDL) and then determine HDL-

C [25]. The equation of Friedewald was used to estimate LDL-cholesterol (LDL-C) levels [26]. Non-HDL-C was calculated by subtracting HDL-C from TC. Plasma apolipoprotein B-100 (apoB) concentrations were measured by the rocket immunoelectrophoretic method of Laurell, as previously described [27].

SNPs Selection and Genotyping

Genetic analyses were performed on genomic DNA isolated from human leukocytes. DNA was extracted from 200 µL of buffy coat using the GenElute™ Blood Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA). Spectrophotometric quantification was realised with NanoDrop 2000C UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA). SNPs were selected with the International HapMap Project SNP database (HapMap Data Rel 28 Phase II + III, August 10, on National Center for Biotechnology Information (NCBI) B36 assembly, dbSNP b126). Tag SNPs (tSNPs) were determined with the tagger procedure in HaploView software version 4.2 with minor allele frequency (MAF) of >0.05 and pairwise tagging $R^2 \geq 0.80$. For each gene a minimum of 85% of the most common SNPs had to be captured by tSNPs. Additionally, tSNPs were prioritized according to the following criteria: (1) known SNPs from the literature; (2) SNPs within coding regions (exon); (3) SNPs within the promoter region (2500 bp before the start codon); (4) SNPs within 3' UTR region (500 bp after the stop codon) and (5) SNPs within 100 bp before an exon-intron splicing boundaries. Afterwards, as shown in Figures 6.1 and 6.2 and Supplementary Figures 6.S1–6.S4, linkage disequilibrium (LD) plots were generated with Haploview software version 4.2. All tSNPs within *RXRA*, *CPT1A*, *ACADVL*, *ACAA2*, *ABCD2*, *ACOX1* and *ACAA1* genes were genotyped with the TAQMAN methodology [28], as described previously [11].

Gene Expression Assessment

Blood samples (pre- and post- supplementation) were collected into an 8-mL Cell Preparation Tube (CPT) (Becton Dickinson, Oakville, ON, Canada). Peripheral blood mononuclear cells (PBMCs) were separated by centrifugation (1500× *g*, 20 min, at room temperature) and washed according to the manufacturer's instructions. Total RNA was

extracted with RNeasy Plus Mini Kit (QIAGEN, Mississauga, ON, Canada) according to manufacturer's protocol. Spectrophotometric quantification was realised with NanoDrop 2000C UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and cDNA was generated using 400 ng of total RNA with the High Capacity cDNA Reverse Transcription Kit (Life Technologies™, Carlsbad, CA, USA). cDNA was mixed with TaqMan OpenArray® Real-Time PCR Master Mix (#4462164, Life Technologies™, Carlsbad, CA, USA). The assays used were as follows: Hs01067636_m1 (NM_002957.4) (*RXRA*), Hs00912671_m1 (NM_001031847.2, NM_001876.3) (*CPT1A*), Hs00825606_g1 (NM_000018.3, NM_001033859.2, NM_001270447.1, NM_001270448.1) (*ACADVL*), Hs01557254_m1 (NM_006111.2) (*ACAA2*), Hs00193054_m1 (NM_005164.3) (*ABCD2*), Hs01074241_m1 (NM_001185039.1, NM_004035.6, NM_007292.5) (*ACOX1*) and Hs01576070_m1 (NM_001607.3, NR_024024.1) (*ACAA1*), and *GAPDH* Hs99999905_m1 as the housekeeping gene. All assays used the same fluorescent reporter probe (FAM dye labeled). All samples were run in triplicate on a QuantStudio™ 12K Flex Real-Time PCR (RT-PCR) System (Life Technologies™, Carlsbad, CA, USA) using 48-well plates TaqMan® OpenArray® RT PCR Inventoried Format 18 (Life Technologies™, Carlsbad, CA, USA). The RT-PCR results were analysed with ExpressionSuite software v1.0.1 (Life Technologies™, Carlsbad, CA, USA).

Statistical Analyses

Hardy-Weinberg equilibrium was tested with the ALLELE procedure of SAS [29] version 9.3 using Fisher's exact test ($p < 0.01$). When the genotype frequency for homozygotes of the minor allele was <5%, carriers (heterozygotes and homozygotes) of the minor allele were grouped in order to have appropriate statistical power.

Variables abnormally distributed were logarithmically transformed. Paired *t*-test were computed to detect differences in macronutrient intake (expressed as a proportion of energy intake) between pre- and post-supplementation values. To test the impact of dietary fat intake on the response of plasma TG to fish oil supplementation (relative difference: (post-supplementation plasma TG minus pre-supplementation plasma TG/pre-supplementation

plasma TG) × 100), subjects were divided on the basis of fat intake (%), including the fish oil supplement using the median value as a cut-off point. Subjects having an intake lower or equal to the median value were considered as having "low" intakes and subjects having an intake higher than the median value were considered as having "high" intakes of dietary fat.

Differences in the plasma TG response were tested using analyses of variance with the GLM procedure in SAS and the type 3 sum of squares for unbalanced study design. To take into account the possible gene-diet interaction effects, the interaction term was added in the model (SNPxdietary fat intake) (adjusted for age, sex and BMI). In this model, dietary fat intakes were included as continuous variables. Statistical analyses related to relative gene expression levels were conducted using the $2^{-\Delta\Delta CT}$ for the gene expression response as described by Livak *et al*. [30,31], separately by genotype according to dietary fat intake group. Pearson correlations were performed to observe associations between the gene expression response and the plasma TG response. 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 before correction for multiple testing and using a *p*-value < 0.05. All statistical analyses were performed using SAS statistical software version 9.3 [29].

Results

Characteristics of the Study Population, Genetic Variants and Dietary Intakes

Descriptive characteristics of the study participants are shown in Table 6.1. All tSNPs were in Hardy-Weinberg equilibrium. The selected tSNPs from Haploview software are presented in Figures 6.1 and 6.2 and supplementary material and the genetic information for each tSNP are shown in Table 6.2. For *RXRA* gene, 12 tSNPs covered 85% of the known genetic variability, for *CPT1A* gene, 9 tSNPs covered 85%, for *ACADVL* gene, 1 tSNP covered 100%, for *ACAA2* gene, 6 tSNPs covered 87%, for *ABCD2* gene, 8 tSNPs covered 85%, for *ACOX1* gene, 8 tSNPs covered 88% and for *ACAA1* gene, 3 tSNPs covered 83%.

Comparisons of dietary intakes pre- and post-supplementation are presented in Table 6.3. Briefly, proportions of the macronutrients remained mainly similar when considering dietary intakes expressed as a proportion of energy intake without taking into account *n*-3 PUFA supplements. However, when taking into account *n*-3 PUFA supplements, participants slightly decreased their carbohydrate intake (−1.9%) and SFA intakes (−0.8%) and increased their total fat (+2.7%) and PUFA intakes (+1.1%) ($p = 0.0009$, $p = 0.001$, $p < 0.0001$ and $p < 0.0001$, respectively). Protein and MUFA intakes remained similar ($p = 0.12$ and $p = 0.65$, respectively).

Associations between Dietary Fat Intakes and the Plasma TG Response

When observing the impact of dietary fat consumption on the plasma TG response (%) to a fish oil supplementation, a trend was observed only for SFA intake ($p = 0.08$) (adjusted for age, sex and BMI) (high SFA > 10.48% and low SFA ≤ 10.48%). Individuals with high SFA intakes had a smaller relative decrease in plasma TG levels following the intake of fish oil than individuals with low SFA intake (−8.77% ± 25.56% compared to −15.01% ± 26.01%). No significant differences were observed between SFA intake groups and baseline plasma TG levels ($p = 0.76$).

Associations between tSNPs, Relative Gene Expression Levels and the Plasma TG Response Following Fish Oil Supplementation

None of the tSNPs were associated with the plasma TG response. When observing the fold change gene expression in response to the fish oil supplementation (using the $2^{-\Delta\Delta CT}$), fold change in gene expression levels of *CPT1A* were inversely related to the relative delta TG ($r = -0.15$, $p = 0.03$). These results suggest that a greater increase in *CPT1A* gene expression was associated with a more important reduction in plasma TG following the intake of fish oil. No relationships were observed for the other genes (data not shown).

Gene-Diet Interaction Effects on the Plasma TG Response and on the Gene Expression Response Following Fish Oil Supplementation

Significant gene-diet interaction effects on the plasma TG response are presented in Table 6.4. Briefly, one tSNP (rs11185660) within *RXRA* gene interacted with total fat intakes, three tSNPs (rs10881576, rs12339187 and rs11185660) within *RXRA* gene interacted with SFA intakes and one tSNP (rs17583163) within *ACOX1* gene interacted with PUFA intakes to affect the plasma TG response. Figure 6.3 illustrates the interaction effect on the plasma TG response according to genotype and dietary fat intake group (low or high). Briefly, for the tSNP rs11185660, C/C homozygotes with high SFA intakes (a trend was also observed for total fat intakes) increased their plasma TG levels following the intake of fish oil whereas those with low SFA intakes decreased their plasma TG levels. Among C/T heterozygotes and T/T homozygotes, the decrease in plasma TG levels following the *n*-3 PUFA supplementation was comparable with either a high or a low SFA intake. For the tSNP rs12339187, carriers of the minor G allele with low SFA intakes had a greater decrease in plasma TG levels compared to carriers of the G allele with high SFA intakes or to A/A homozygotes with high or low SFA intakes.

None of the gene interaction effect had an impact on gene expression response following the fish oil supplementation. However, when participants were first stratified on the basis of dietary fat intakes and then on the basis of the genotype a few differences were observed.

As shown in Table 6.5, the fold change in *RXRA* gene expression levels due to the fish oil supplementation was different for T/T homozygotes of rs11185660. For this genotype, individuals with high total fat intakes had a mean fold change due to the fish oil supplementation of −1.08 (post-compared to pre-supplementation) compared to a mean fold change of 1.05 for individuals with low total fat intakes (p = 0.01). No significant differences were observed for C/C and C/T genotypes (p = 0.52 and p = 0.86, respectively). For the tSNP rs12339187, a trend was observed for A/A homozygotes (p = 0.06). For these individuals, when SFA intakes were high, *RXRA* gene expression levels slightly decreased with the fish oil supplementation and increased with low SFA intakes.

Discussion

Macronutrient intakes before the fish oil supplementation were comparable to intakes reported among Canada's population [32,33]. During this study, macronutrient intakes remained mostly constant. However, participants spontaneously reduced their energy intakes which may be related to the reduction observed in the intake of carbohydrates. The intake of DHA has been associated with reductions in energy intake among free living healthy men [34]. This effect was explained by an increase in the release of appetite hormone cholecystokinin [34]. This spontaneous reduction in energy intakes could also be caused by the Hawthorne effect, which involves that participants reduced their energy intakes only by knowing that they were in a study [35].

Among factors modulating the plasma TG response to fish oil intake, SFA intakes may be an important factor. The relative decrease in plasma TG levels of individuals with high SFA intakes was almost reduced by half compared to individuals with low SFA intakes (−9% compared to −15%). Moreover, there were no differences in baseline plasma TG levels between SFA intake groups. As mentioned previously, high SFA intakes increase intrahepatic TG levels which could enhance hepatic VLDL-TG secretion [6–8]. Contrary to PUFA, SFA increases the activity of *hepatic nuclear factor 4-α* (*HNF4A*) which is a transcription factor acting as a homodimer to activate several hepatic genes encoding apolipoproteins, including apoB [36,37]. It has been also observed that a diet high in SFA increases plasma TG levels possibly via an increase in apolipoprotein C-III gene (*APOC3*) mediated by *HNF4A* gene [37]. Globally, the impacts of SFA on the regulation of lipid metabolism could partly counteract the plasma TG lowering effects of *n*-3 PUFA which could lead to smaller decreases after the intake of fish oil, as observed in this study.

Following fish oil intake, only the genetic variability within *RXRA* and *ACOX1* genes seemed to be associated with differences in the plasma TG response (using the relative difference in plasma TG between post-supplementation and pre-supplementation). The impact of the presence of these intronic SNPs is unknown. However, gene expression levels tended to be different according to the genotype for two of these SNPs depending on

dietary fat intakes. Intronic SNPs could also be in LD with other functional SNPs or depending on splicing events, some of these SNPs may be in translated regions. The modulation of the activity of genes or encoded enzymes related to fatty acid β-oxidation may modulate fatty acid availability for VLDL-TG hepatic production, therefore contributing to modulate plasma TG levels [7,38,39]. The protein encoded by *RXRA* gene forms a heterodimer with PPARA transcription factor and affects the expression of many genes involved in fatty acid β-oxidation [40]. Fatty acids have been shown to be natural PPARA ligands [41]. Both SFA and unsaturated fatty acids are able to bind with PPARA, but long-chain *n*-3 PUFAs seem to be the most potent activators [41]. Moreover, RXRA may bind other transcription factors or act as a homodimer on other pathways, which could also have an impact on lipid metabolism [40]. A few studies have reported associations with SNPs or haplotypes within *RXRA* gene with plasma TG levels or the metabolic syndrome [17,19]. In this study, a few SNPs within *RXRA* gene interacted with dietary fats and were associated with the plasma TG response following fish oil intake (rs11185660, rs10881576 and rs12339187). Peloso *et al*. [18] have observed a decreased risk of having low HDL-C and coronary artery disease among carriers of the minor C allele compared to homozygotes T/T of rs11185660. However, dietary fat intakes were not taken into account. In this study, the T/T genotype of rs11185660 depending on dietary fat intakes was also associated with differences in gene expression levels following fish oil intake. *ACOX1* gene encodes for the first enzyme in peroxisomal fatty acid β-oxidation and is regulated by the PPARA transcription factor [42]. To our knowledge, none of the SNPs within the *ACOX1* gene have been studied in the context of lipid metabolism. One gene-diet interaction effect on the plasma TG response was observed with rs17583163 and PUFA intakes. Dietary fish oil has been shown to induce *ACOX1* gene expression in the liver, skeletal muscle and heart [43] which was not observed in this study (data not shown). Various transcripts have been reported for this gene that are likely to be differently regulated by PUFA intakes and possibly explaining the lack of association in the present study with an assay targeting three transcripts. Alternatively, the impact of dietary fish oil may be dependent on the intake of other dietary lipids and genetic variants.

The *CPT1A* gene encodes for an essential transporter required for the initiation of fatty acid β-oxidation in the mitochondria [44]. In this study, a greater increase in *CPT1A* relative gene expression levels following the intake of fish oil was associated with a more important reduction in plasma TG levels. *CPT1A* gene expression levels have been previously reported to increase following the intake of *n*-3 PUFA [45,46]. Radler *et al*. [46] have observed that the intake of a yogurt composed of *n*-3 PUFA, polyphenols and L-carnitine induced *CPT1A* gene expression among overweight moderately hyperlipidemic individuals. However, in this study mean *CPT1A* gene expression levels remained unchanged following the intake of fish oil (data not shown). This may be attributable to the healthy status of the study participants which could lead to too subtle differences in gene expression levels to be detected.

In this study, the presence of certain SNPs within genes involved in fatty acid β-oxidation depending on dietary fat intakes modulated the plasma TG response following fish oil intake. Because of the potentially small impacts of the gene-diet interaction effects among healthy individuals on the plasma TG response following fish oil, data was shown before correction for multiple testing. However, this could lead to false positive results. Thus, these results need to be replicated in order to properly determine relevant gene-diet interaction effects.

Conclusion

Globally, gene-diet interaction effects with *RXRA* and *ACOX1* genes were observed on the plasma TG response to fish oil intake. An increase in *CPT1A* gene expression was associated with a more important decrease in plasma TG levels. Moreover, higher SFA intakes tended to decrease the plasma TG response to fish oil. In conclusion, these results indicate that gene-diet interaction effects may modulate the response of plasma TG levels to fish oil intake, and contribute to the explanation of the inter-individual variability observed.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002, *106*, 3143–3421.
2. Elder, S.J.; Lichtenstein, A.H.; Pittas, A.G.; Roberts, S.B.; Fuss, P.J.; Greenberg, A.S.; McCrory, M.A.; Bouchard, T.J., Jr.; Saltzman, E.; Neale, M.C. Genetic and environmental influences on factors associated with cardiovascular disease and the metabolic syndrome. *J. Lipid Res.* 2009, *50*, 1917–1926.
3. Jermendy, G.; Horvath, T.; Littvay, L.; Steinbach, R.; Jermendy, A.L.; Tarnoki, A.D.; Tarnoki, D.L.; Metneki, J.; Osztovits, J. Effect of genetic and environmental influences on cardiometabolic risk factors: A twin study. *Cardiovasc. Diabetol.* 2011, *10*, 96.
4. Sacks, F.M.; Katan, M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. *Am. J. Med.* 2002, *113*, 13S–24S.
5. Singh, A.; Schwartzbard, A.; Gianos, E.; Berger, J.S.; Weintraub, H. What should we do about Hypertriglyceridemia in Coronary Artery Disease Patients? *Curr. Treat. Options Cardiovasc. Med.* 2013, *15*, 104–117.
6. Sullivan, S. Implications of diet on nonalcoholic fatty liver disease. *Curr. Opin. Gastroenterol.* 2010, *26*, 160–164.
7. Lottenberg, A.M.; Afonso, M.S.; Lavrador, M.S.; Machado, R.M.; Nakandakare, E.R. The role of dietary fatty acids in the pathology of metabolic syndrome. *J. Nutr. Biochem.* 2012, *23*, 1027–1040.
8. Fabbrini, E.; Magkos, F.; Mohammed, B.S.; Pietka, T.; Abumrad, N.A.; Patterson, B.W.; Okunade, A.; Klein, S. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc. Natl. Acad. Sci. USA* 2009, *106*, 15430–15435.
9. Caslake, M.J.; Miles, E.A.; Kofler, B.M.; Lietz, G.; Curtis, P.; Armah, C.K.; Kimber, A.C.; Grew, J.P.; Farrell, L.; Stannard, J.; *et al*. Effect of sex and genotype on cardiovascular biomarker response to fish oils: The FINGEN Study. *Am. J. Clin. Nutr.* 2008, *88*, 618–629.
10. Madden, J.; Williams, C.M.; Calder, P.C.; Lietz, G.; Miles, E.A.; Cordell, H.; Mathers, J.C.; Minihane, A.M. The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. *Annu. Rev. Nutr.* 2011, *31*, 203–234.
11. Cormier, H.; Rudkowska, I.; Paradis, A.M.; Thifault, E.; Garneau, V.; Lemieux, S.; Couture, P.; Vohl, M.C. Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an *n*-3 PUFA supplementation. *Nutrients* 2012, *4*, 1026–1041.

12. Shearer, G.C.; Savinova, O.V.; Harris, W.S. Fish oil—How does it reduce plasma triglycerides? *Biochim. Biophys. Acta* 2012, *1821*, 843–851.

13. Jump, D.B. *n*-3 Polyunsaturated fatty acid regulation of hepatic gene transcription. *Curr. Opin. Lipidol.* 2008, *19*, 242–247.

14. Pyper, S.R.; Viswakarma, N.; Yu, S.; Reddy, J.K. PPARalpha: Energy combustion, hypolipidemia, inflammation and cancer. *Nucl. Recept. Signal.* 2010, *8*, e002.

15. Guo, Y.; Jolly, R.A.; Halstead, B.W.; Baker, T.K.; Stutz, J.P.; Huffman, M.; Calley, J.N.; West, A.; Gao, H.; Searfoss, G.H.; *et al*. Underlying mechanisms of pharmacology and toxicity of a novel PPAR agonist revealed using rodent and canine hepatocytes. *Toxicol. Sci.* 2007, *96*, 294–309.

16. Fourcade, S.; Savary, S.; Albet, S.; Gauthe, D.; Gondcaille, C.; Pineau, T.; Bellenger, J.; Bentejac, M.; Holzinger, A.; Berger, J.; *et al*. Fibrate induction of the adrenoleukodystrophy-related gene (ABCD2): Promoter analysis and role of the peroxisome proliferator-activated receptor PPARα. *Eur. J. Biochem.* 2001, *268*, 3490–3500.

17. Lima, L.O.; Almeida, S.; Hutz, M.H.; Fiegenbaum, M. *PPARA*, *RXRA*, *NR1I2* and *NR1I3* gene polymorphisms and lipid and lipoprotein levels in a Southern Brazilian population. *Mol. Biol. Rep.* 2013, *40*, 1241–1247.

18. Peloso, G.M.; Demissie, S.; Collins, D.; Mirel, D.B.; Gabriel, S.B.; Cupples, L.A.; Robins, S.J.; Schaefer, E.J.; Brousseau, M.E. Common genetic variation in multiple metabolic pathways influences susceptibility to low HDL-cholesterol and coronary heart disease. *J. Lipid Res.* 2010, *51*, 3524–3532.

19. Shi, H.; Yu, X.; Li, Q.; Ye, X.; Gao, Y.; Ma, J.; Cheng, J.; Lu, Y.; Du, W.; Du, J.; *et al*. Association between PPAR-gamma and RXR-α gene polymorphism and metabolic syndrome risk: A case-control study of a Chinese Han population. *Arch. Med. Res.* 2012, *43*, 233–242.

20. Lemas, D.J.; Wiener, H.W.; O'Brien, D.M.; Hopkins, S.; Stanhope, K.L.; Havel, P.J.; Allison, D.B.; Fernandez, J.R.; Tiwari, H.K.; Boyer, B.B. Genetic polymorphisms in carnitine palmitoyltransferase 1A gene are associated with variation in body composition and fasting lipid traits in Yup'ik Eskimos. *J. Lipid Res.* 2012, *53*, 175–184.

21. Robitaille, J.; Houde, A.; Lemieux, S.; Perusse, L.; Gaudet, D.; Vohl, M.C. Variants within the muscle and liver isoforms of the carnitine palmitoyltransferase I (CPT1) gene interact with fat intake to modulate indices of obesity in French-Canadians. *J. Mol. Med.* 2007, *85*, 129–137.

22. Lohman, T.; Roche, A.; Martorell, R. *Anthropometric Standardization Reference Manual*; Human Kinetics Publishers: Champaign, IL, USA, 1988; pp. 39–80.

23. McNamara, J.R.; Schaefer, E.J. Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin. Chim. Acta* 1987, *166*, 1–8.

24. Burstein, M.; Samaille, J. On a rapid determination of the cholesterol bound to the serum α- and β-lipoproteins. *Clin. Chim. Acta* 1960, *5*, 609.

25. Albers, J.J.; Warnick, G.R.; Wiebe, D.; King, P.; Steiner, P.; Smith, L.; Breckenridge, C.; Chow, A.; Kuba, K.; Weidman, S.; *et al*. Multi-laboratory comparison of three heparin-Mn^{2+} precipitation procedures for estimating cholesterol in high-density lipoprotein. *Clin. Chem.* 1978, *24*, 853–856.

26. Friedewald, W.T.; Levy, R.I.; Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* 1972, *18*, 499–502.

27. Laurell, C.B. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* 1966, *15*, 45–52.

28. Livak, K.J. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet. Anal.* 1999, *14*, 143–149.

29. *SAS/STAT® software*, version 9.3; SAS Institute Inc.: Cary, NC, USA, 2011.

30. Schmittgen, T.D.; Livak, K.J. Analyzing real-time PCR data by the comparative C(T) method. *Nat. Protoc.* 2008, *3*, 1101–1108.

31. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001, *25*, 402–408.

32. Vue d'ensemble des Habitudes Alimentaires des Canadiens. Available online: http://www5.statcan.gc.ca/bsolc/olc-cel/olc-cel?catno=82-620-MIF2006002&lang=fra (accessed on 3 March 2013).

33. Les Adultes Canadiens Comblent-Ils leur Besoins en Nutriments Uniquement Grâce à L'alimentation? Available online :http://www.hc-sc.gc.ca/fn-an/surveill/nutrition/commun/art-nutr-adult-fra.php (accessed on 5 May 2013).

34. Harden, C.J.; Jones, A.N.; Maya-Jimenez, T.; Barker, M.E.; Hepburn, N.J.; Garaiova, I.; Plummer, S.F.; Corfe, B.M. Effect of different long-chain fatty acids on cholecystokinin release *in vitro* and energy intake in free-living healthy males. *Br. J. Nutr.* 2012, *108*, 755–758.

35. Holden, J.D. Hawthorne effects and research into professional practice. *J. Eval. Clin. Pract.* 2001, *7*, 65–70.

36. Hayhurst, G.P.; Lee, Y.H.; Lambert, G.; Ward, J.M.; Gonzalez, F.J. Hepatocyte nuclear factor 4α (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol. Cell Biol.* 2001, *21*, 1393–1403.

37. Ceccarelli, V.; Nocentini, G.; Riccardi, C.; Ayroldi, E.; Di, N.P.; Roberti, R.; Binaglia, L.; Vecchini, A. Effect of dietary saturated fatty acids on HNF-4α DNA binding activity and ApoCIII mRNA in sedentary rat liver. *Mol. Cell Biochem.* 2011, *347*, 29–39.

38. Harris, W.S.; Bulchandani, D. Why do omega-3 fatty acids lower serum triglycerides? *Curr. Opin. Lipidol.* 2006, *17*, 387–393.

39. Mozaffarian, D.; Wu, J.H. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J. Am. Coll. Cardiol.* 2011, *58*, 2047–2067.

40. Ahuja, H.S.; Szanto, A.; Nagy, L.; Davies, P.J. The retinoid X receptor and its ligands: Versatile regulators of metabolic function, cell differentiation and cell death. *J. Biol. Regul. Homeost. Agents* 2003, *17*, 29–45.

41. Xu, H.E.; Lambert, M.H.; Montana, V.G.; Parks, D.J.; Blanchard, S.G.; Brown, P.J.; Sternbach, D.D.; Lehmann, J.M.; Wisely, G.B.; Willson, T.M.; *et al*. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell* 1999, *3*, 397–403.

42. Varanasi, U.; Chu, R.; Huang, Q.; Castellon, R.; Yeldandi, A.V.; Reddy, J.K. Identification of a peroxisome proliferator-responsive element upstream of the human peroxisomal fatty acyl coenzyme A oxidase gene. *J. Biol. Chem.* 1996, *271*, 2147–2155.

43. Baillie, R.A.; Takada, R.; Nakamura, M.; Clarke, S.D. Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: A mechanism for decreased body fat deposition. *Prostaglandins Leukot. Essent. Fatty Acids* 1999, *60*, 351–356.

44. McGarry, J.D.; Sen, A.; Esser, V.; Woeltje, K.F.; Weis, B.; Foster, D.W. New insights into the mitochondrial carnitine palmitoyltransferase enzyme system. *Biochimie* 1991, *73*, 77–84.

45. Flachs, P.; Horakova, O.; Brauner, P.; Rossmeisl, M.; Pecina, P.; Franssen-van, H.N.; Ruzickova, J.; Sponarova, J.; Drahota, Z.; Vlcek, C.; *et al*. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce β-oxidation in white fat. *Diabetologia* 2005, *48*, 2365–2375.

46. Radler, U.; Stangl, H.; Lechner, S.; Lienbacher, G.; Krepp, R.; Zeller, E.; Brachinger, M.; Eller-Berndl, D.; Fischer, A.; Anzur, C.; *et al*. A combination of (omega-3) polyunsaturated fatty acids, polyphenols and L-carnitine reduces the plasma lipid levels and increases the expression of genes involved in fatty acid oxidation in human peripheral blood mononuclear cells and HepG2 cells. *Ann. Nutr. Metab.* 2011, *58*, 133–140.

Table 6.1 Descriptive characteristics of the study cohort.

Variables	Men (*n* = 96)	Women (*n* = 112)	Means ± SD *
Age (years)	31.2 ± 8.1	30.5 ± 9.2	30.8 ± 8.7
BMI (kg/m^2)	27.5 ± 3.6	28.2 ± 3.8	27.8 ± 3.7
Waist circumference (cm)	94.9 ± 11.0	92.0 ± 10.4	93.3 ± 10.8
Systolic blood pressure (mmHg)	118.09 ± 11.40	106.79 ± 13.29 (*n* = 111)	112.03 ± 13.64 (*n* = 207)
Diastolic blood pressure (mmHg)	70.53 ± 9.13	68.68 ± 9.19 (*n* = 111)	69.54 ± 9.19 (*n* = 207)
Fasting glucose (mmol/L)	5.09 ± 0.44	4.83 ± 0.56	4.95 ± 0.52 (*n* = 208)
Fasting insulin (pmol/L)	79.50 ± 32.19 (*n* = 94)	85.04 ± 38.20	82.51 ± 35.61 (*n* = 206)
Total-C (mmol/L)	4.80 ± 0.99	4.83 ± 1.02	4.82 ± 1.01
LDL-C (mmol/L)	2.91 ± 0.87 (*n* = 95)	2.70 ± 0.86	2.79 ± 0.87 (*n* = 207)
HDL-C (mmol/L)	1.29 ± 0.31	1.61 ± 0.39	1.46 ± 0.39
Triglycerides (mmol/L)	1.32 ± 0.74	1.15 ± 0.53	1.23 ± 0.64
ApoB (g/L)	0.89 ± 0.25 (*n* = 95)	0.84 ± 0.25	0.86 ± 0.25 (*n* = 207)

*Mean ± standard deviation (SD).

Table 6.2 The selected single-nucleotide polymorphisms within *RXRA*, *CPT1A*, *ACADVL*, *ABCD2*, *ACOX1* and *ACAA1* genes.

Genes	dbSNP No [1]	Sequence [2]	Position	MAF	Genotype Frequency		
RXRA	rs10881576	GCGGGTG[C/T]GGACCGC	Intron	0.28	C/C (*n* = 106) 0.510	C/T (*n* = 86) 0.414	T/T (*n* = 16) 0.077
	rs7871655	CAGAATT[C/G]CGGGTGA	Intron	0.26	G/G (*n* = 110) 0.529	C/G (*n* = 87) 0.418	C/C (*n* = 11) 0.053
	rs12339187	GGACCAG[A/G]TGTTTTA	Intron	0.17	A/A (*n* = 143) 0.688	A/G (*n* = 60) 0.289	G/G (*n* = 5) 0.024
	rs11185660	CTGTGTC[C/T]CTGGAGA	Intron	0.27	T/T (*n* = 109) 0.524	C/T (*n* = 87) 0.418	C/C (*n* = 12) 0.058
	rs11103473	TCTCTCC[A/T]AACTATT	Intron	0.36	A/A (*n* = 81) 0.389	A/T (*n* = 105) 0.505	T/T (*n* = 22) 0.106
	rs10776909	GTGGGGA[C/T]TTTGAGT	Intron	0.23	C/C (*n* = 120) 0.577	C/T (*n* = 80) 0.385	T/T (*n* = 8) 0.039
	rs12004589	GCTCCCT[G/T]CATGGCC	Intron	0.08	G/G (*n* = 178) 0.856	G/T (*n* = 28) 0.135	T/T (*n* = 2) 0.010
	rs3132301	TGCTGAG[C/T]CCCCCAG	Intron	0.22	C/C (*n* = 125) 0.601	C/T (*n* = 76) 0.365	T/T (*n* = 7) 0.034
	rs1805352	ATAGGGA[A/C]AAACCTG	Intron	0.31	A/A (*n* = 97) 0.466	A/C (*n* = 95) 0.457	C/C (*n* = 16) 0.077
	rs3132294	GAACACT[A/G]TGAACCG	Intron	0.23	G/G (*n* = 121) 0.582	A/G (*n* = 77) 0.370	A/A (*n* = 10) 0.048
	rs1805343	CTTGCCC[A/G]GCCCTCA	Intron	0.37	A/A (*n* = 85) 0.409	A/G (*n* = 93) 0.447	G/G (*n* = 30) 0.144

	rs1045570	CGTGGCC[G/T]CAGGTGC	3′UTR	0.16	G/G (*n* = 146) 0.702	G/T (*n* = 57) 0.274	T/T (*n* = 5) 0.024
CPT1A	rs3019598	GTGCCCC[C/T]GTTACCT	Intron	0.35	C/C (*n* = 88) 0.423	C/T (*n* = 93) 0.447	T/T (*n* = 27) 0.130
	rs897048	GCTGTCA[C/G]ACCGGGC	Intron	0.19	C/C (*n* = 134) 0.644	C/G (*n* = 68) 0.327	G/G (*n* = 6) 0.029
	rs7942147	GGACACC[A/C]TGTGGCA	Intron	0.16	C/C (*n* = 144) 0.692	A/C (*n* = 60) 0.289	A/A (*n* = 4) 0.019
	rs4930248	TCAGGGT[C/T]GCTTTGG	Intron	0.44	T/T (*n* = 62) 0.298	C/T (*n* = 108) 0.519	C/C (*n* = 38) 0.183
	rs11228364	CTTCGAG[C/T]GCAGATC	Intron	0.10	C/C (*n* = 169) 0.813	C/T (*n* = 36) 0.173	T/T (*n* = 3) 0.014
	rs11228368	CCAGAAG[A/G]GGGCACA	Intron	0.50	G/G (*n* = 52) 0.250	A/G (*n* = 105) 0.505	A/A (*n* = 51) 0.245
	rs10896371	CTCGTTC[C/T]CACAAAT	Intron	0.14	T/T (*n* = 153) 0.736	C/T (*n* = 51) 0.245	C/C (*n* = 4) 0.019
	rs1017640	CTGGCCA[C/T]GTAATCA	Intron	0.10	C/C (*n* = 169) 0.813	C/T (*n* = 37) 0.178	T/T (*n* = 2) 0.010
	rs613084	TTCAGTG[A/C]CACACCC	Intron	0.35	C/C (*n* = 89) 0.428	A/C (*n* = 93) 0.447	A/A (*n* = 26) 0.125
ACADVL	rs2017365	GGCACAT[A/G]GTCTCTG	NearGene-5	0.38	A/A (*n* = 81) 0.389	A/G (*n* = 96) 0.462	G/G (*n* = 31) 0.149
ACAA2	rs529556	ACTTTTT[C/T]AGGACTC	Intron	0.43	T/T (*n* = 76) 0.365	C/T (*n* = 85) 0.409	C/C (*n* = 47) 0.226
	rs10502901	AAGCTAA[A/T]CTGTGTG	Intron	0.06	T/T	A/T	A/A

					(*n* = 184) 0.885	(*n* = 24) 0.115	(*n* = 0) 0.000
	rs631536	ATTGACT[A/G]TGGTTAC	Intron	0.14	A/A (*n* = 150) 0.721	A/G (*n* = 56) 0.269	G/G (*n* = 2) 0.010
	rs1942421	CTGTTCT[C/T]TCTTAAC	Intron	0.36	C/C (*n* = 91) 0.438	C/T (*n* = 84) 0.404	T/T (*n* = 33) 0.159
	rs2276168	AGTATCA[A/T]CACAAGG	Intron	0.23	A/A (*n* = 128) 0.615	A/T (*n* = 66) 0.317	T/T (*n* = 14) 0.067
	rs7237253	CCTTATA[A/G]TCATATT	3′UTR	0.10	A/A (*n* = 170) 0.817	A/G (*n* = 36) 0.173	G/G (*n* = 2) 0.010
ABCD2	rs4072006	GAGAATG[A/G]CTAGAGG	NearGene-5	0.13	G/G (*n* = 159) 0.764	A/G (*n* = 46) 0.221	A/G (*n* = 3) 0.014
	rs10877201	CTATAAT[C/T]CTTTAAC	Intron	0.20	C/C (*n* = 132) 0.635	C/T (*n* = 68) 0.327	T/T (*n* = 8) 0.039
	rs12582802	GAGGTTT[A/G]TTTCCAA	Intron	0.06	A/A (*n* = 186) 0.894	A/G (*n* = 21) 0.101	G/G (*n* = 1) 0.005
	rs4294600	ACTAAAT[A/G]TCACTCA	3′UTR	0.12	G/G (*n* = 161) 0.774	A/G (*n* = 44) 0.212	A/A (*n* = 3) 0.014
	rs11172696	AGGGAAA[C/T]ATTGTAT	Intron	0.08	T/T (*n* = 177) 0.851	C/T (*n* = 29) 0.139	C/C (*n* = 2) 0.010
	rs10877173	ACAAGTT[C/T]GGCTTTT	Intron	0.07	T/T (*n* = 184) 0.885	C/T (*n* = 21) 0.101	C/C (*n* = 3) 0.014
	rs7133376	GGTAAAG[A/G]TGTATGA	Intron	0.20	G/G (*n* = 132) 0.635	A/G (*n* = 67) 0.322	A/A (*n* = 9) 0.043
	rs7968837	ATATTAA[A/C]TTTACCA	3′UTR	0.27	C/C (*n* = 109)	A/C (*n* = 86)	A/A (*n* = 13)

					0.524	0.414	0.063
ACOX1	rs10852766	AAGAAAG[C/T]GCTCAGT	Intron	0.43	C/C (*n* = 74) 0.356	C/T (*n* = 90) 0.433	T/T (*n* = 44) 0.212
	rs3744033	GCCTTCA[A/G]GGAGAAG	Intron	0.17	A/A (*n* = 142) 0.683	A/G (*n* = 60) 0.289	G/G (*n* = 6) 0.029
	rs12430	TCCCAGA[C/T]GTAGCAC	3′UTR	0.11	C/C (*n* = 165) 0.793	C/T (*n* = 39) 0.188	T/T (*n* = 4) 0.019
	rs8065144	AAGCCTC[A/G]AAAATGG	Intron	0.36	A/A (*n* = 89) 0.428	A/G (*n* = 90) 0.433	G/G (*n* = 29) 0.139
	rs11651351	CTATTGC[C/T]GATCTCC	Intron	0.05	C/C (*n* = 188) 0.904	C/T (*n* = 20) 0.096	T/T (*n* = 0) 0.000
	rs3643	GTAGTTT[C/T]GCTTACC	3′UTR	0.12	T/T (*n* = 166) 0.798	C/T (*n* = 36) 0.173	C/C (*n* = 6) 0.029
	rs7213998	TCTGAAA[C/T]GTCAGAG	Intron	0.11	C/C (*n* = 169) 0.813	C/T (*n* = 34) 0.164	T/T (*n* = 5) 0.024
	rs17583163	GATTTCC[C/T]CTGATGA	Intron	0.08	T/T (*n* = 176) 0.846	C/T (*n* = 31) 0.149	C/C (*n* = 1) 0.005
ACAA1	rs5875	TACCATG[A/T]CATCAGT	3′UTR	0.14	T/T (*n* = 155) 0.745	A/T (*n* = 48) 0.231	A/A (*n* = 5) 0.024
	rs2239621	CCTTCTA[C/T]TCCTATG	Intron	0.32	C/C (*n* = 97) 0.466	C/T (*n* = 90) 0.433	T/T (*n* = 21) 0.101
	rs156265	TGGCCTT[C/G]TCCTTCT	Exon (missense Glu→Asp)	0.16	C/C (*n* = 149) 0.716	C/G (*n* = 53) 0.255	G/G (*n* = 6) 0.029

[1]SNP reference id from dbSNP Short Genetic Variations NCBI Reference Assembly; [2] Gene sequence from dbSNP Short Genetic Variations NCBI Reference Assembly.

Table 6.3 Dietary intakes pre-supplementation and post-supplementation.

Dietary Intakes	Pre-Supplementation (*n* = 207)	Post-Supplementation (*n* = 208)		*p*-Value (Without *n*-3 PUFA) *	*p*-Value (With *n*-3 PUFA) *
	Without *n*-3 PUFA Supplements	Without *n*-3 PUFA Supplements	With *n*-3 PUFA Supplements		
Energy (kcal)	2273 ± 590	2144 ± 566	2186 ± 566	<0.0001	0.006
Carbohydrate (%)	50.5 ± 7.2	49.4 ± 7.7	48.6 ± 7.8	<0.05	0.0009
Protein (%)	17.4 ± 3.3 (*n* = 206)	17.5 ± 3.4	17.0 ± 3.2	0.66	0.12
Total fat (%)	32.6 ± 6.0	33.3 ± 6.4	35.3 ± 6.3	0.15	<0.0001
SFA (%)	11.2 ± 3.6	11.5 ± 3.3	10.4 ± 3.0	0.13	0.001
MUFA (%)	11.9 ± 2.8	12.0 ± 3.2	12.0 ± 3.3	0.45	0.65
PUFA (%)	5.9 ± 2.0	5.8 ± 2.1	7.0 ± 2.1	0.56	<0.0001

**p*-Values provided by a paired *t*-test.

Table 6.4 Gene-diet interaction effects on the plasma TG response.

Gene	tSNP	Genotype	β (Interaction Term)[1]	*P* Genotype[2]	*P* Dietary Fat Intake[2]	*P* Interaction Effect[2]
Total fat intakes (%)						
RXRA	rs11185660	C/C C/T T/T	3.70 ± 1.16 1.02 ± 0.61 0	0.004	0.0009	0.004
Saturated fat intakes (%)						
RXRA	rs10881576	T/T C/T C/C	8.52 ± 2.61 1.84 ± 1.21 0	0.007	0.0004	0.004
	rs12339187	A/G + G/G A/A	3.20 ± 1.25 0	0.02	0.005	0.01
	rs11185660	C/C C/T T/T	9.39 ± 2.66 1.69 ± 1.23 0	0.003	<0.0001	0.002
Polyunsaturated fat intakes (%)						
ACOX1	rs17583163	C/C + C/T T/T	6.79 ± 2.34 0	0.02	0.09	0.004

[1]Homozygotes for the major allele is the reference group; [2] *p*-values were determined with an ANOVA using dietary fat intakes as continuous values adjusted for age, sex and BMI.

Table 6.5 Gene expression response according to dietary fat intake and genotype.

Gene	SNP	Genotype	Total Fat Intake [1]		*p* [2]
			Low (≤35.23%)	**High (>35.23%)**	
RXRA	rs11185660	C/C	1.12-fold (*n* = 7)	−1.14-fold (*n* = 5)	0.52
		C/T	−1.05-fold (*n* = 43)	−1.04-fold (*n* = 44)	0.86
		T/T	1.05-fold (*n* = 54)	−1.08-fold (*n* = 53)	0.01
Gene	**SNP**	**Genotype**	**Saturated Fat Intake [1]**		***p*** [2]
			Low (≤10.48%)	**High (>10.48%)**	
RXRA	rs12339187	A/G + G/G	−1.07-fold (*n* = 29)	−1.05-fold (*n* = 36)	0.92
		A/A	1.04-fold (*n* = 74)	−1.06-fold (*n* = 67)	0.06

[1] The fold change represents post-supplementation relative gene expression levels compared to pre-supplementation relative gene expression levels. Fold change = $2^{-\Delta\Delta CT} = 2^{-(\text{post-supplementation } \Delta CT - \text{pre-supplementation } \Delta CT)}$; [2] *p* values were calculated with an ANOVA adjusted for age, sex and BMI.

Figure 6.1 LD plot of *RXRA* gene.

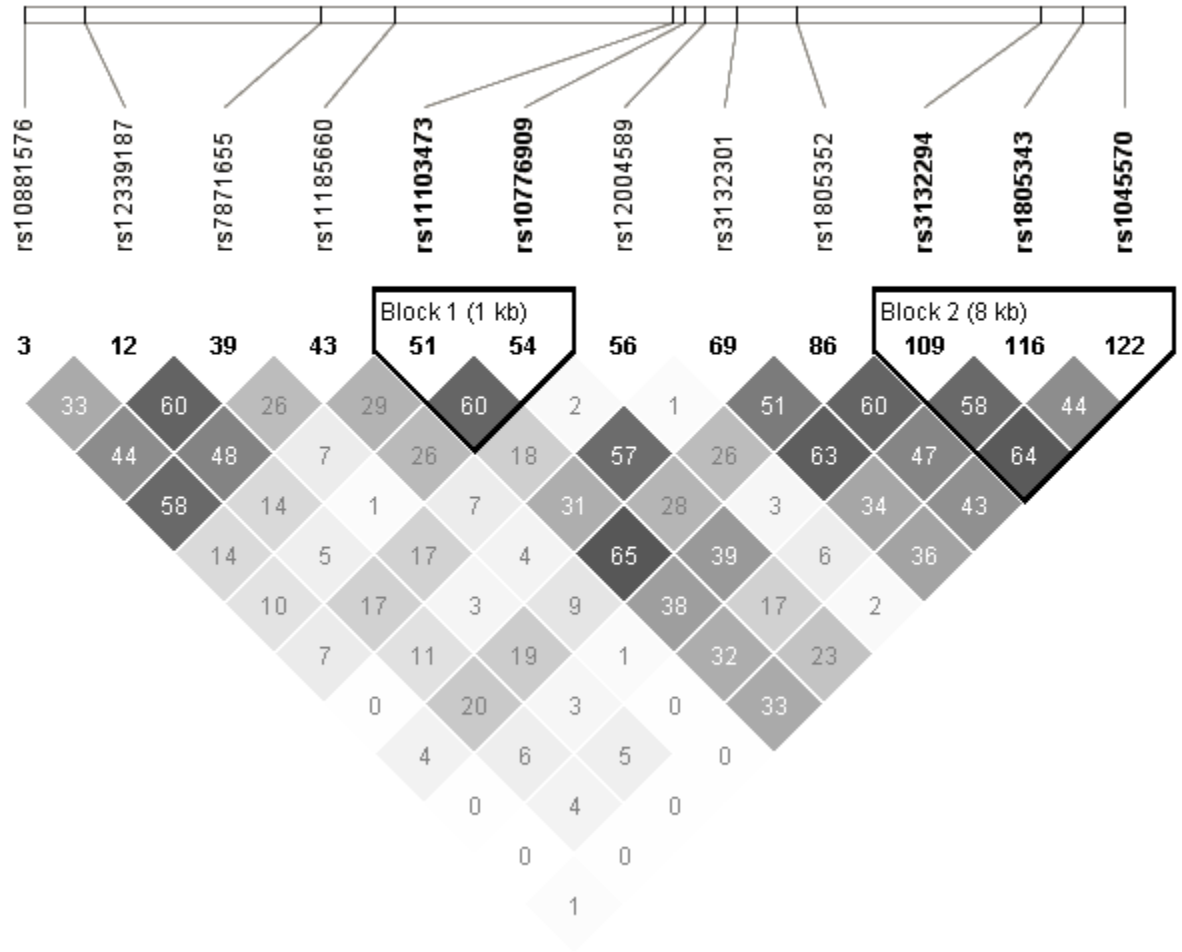


Figure 6.2 LD plot of *ACOX1* gene.

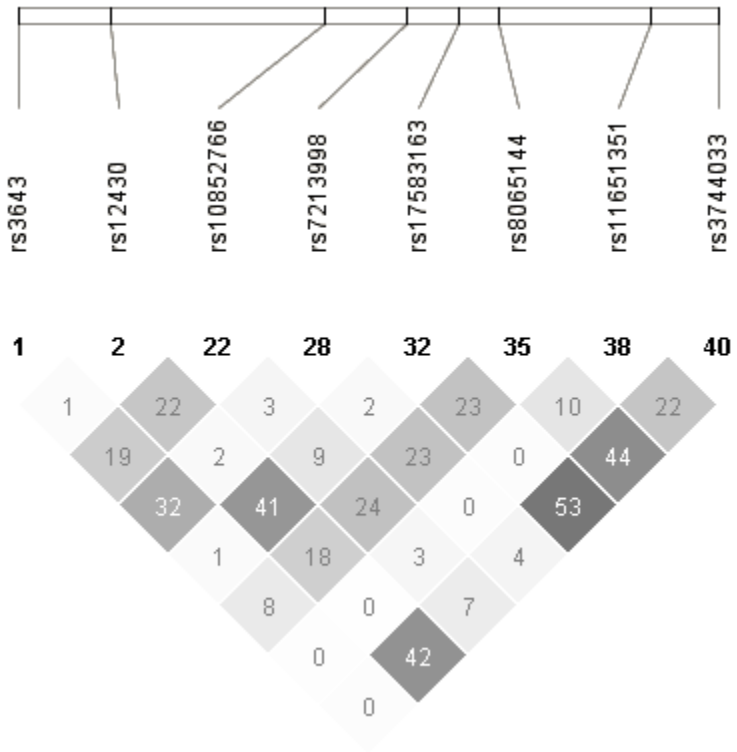
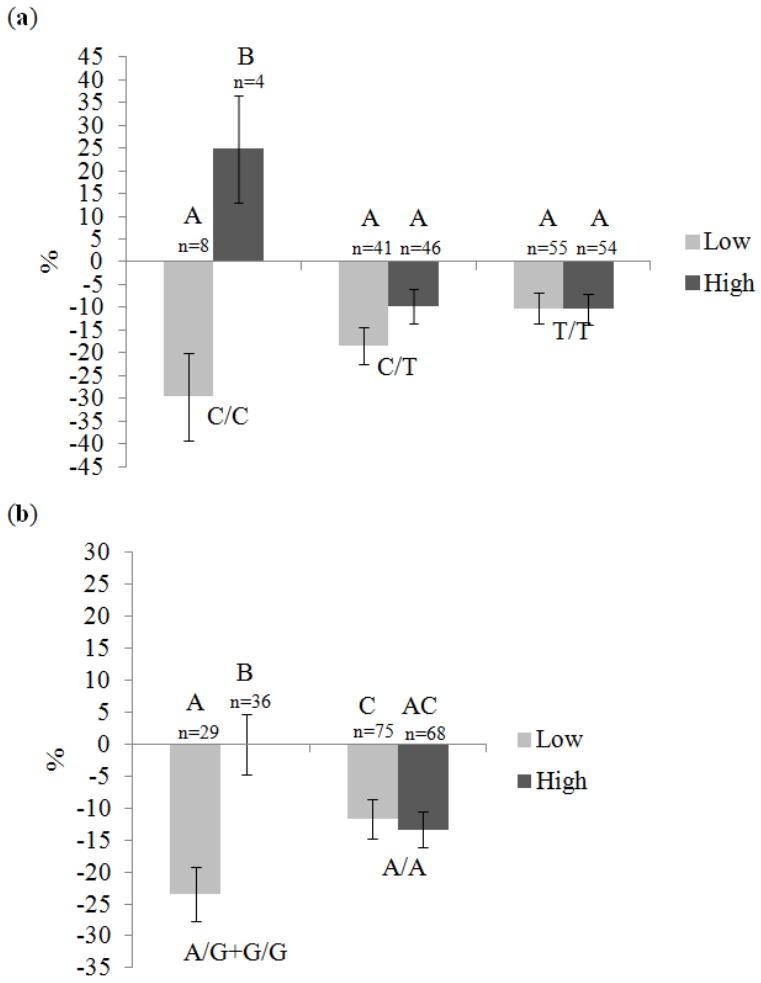


Figure 6.3 The plasma TG response following fish oil intake according to genotype of *RXRA* gene and dietary fat intakes.



Means ± SE. Means with different letters are significantly different (assessed by an ANOVA). Dietary fats are separated according to the median value (Low or High). (**a**) rs11185660 and saturated fat intakes (≤10.48% or >10.48%), and (**b**) rs12339187 and saturated fat intakes (≤10.48% or >10.48%).

Supplementary Information

Figure 6.S1 LD plot of *CPT1A* gene.

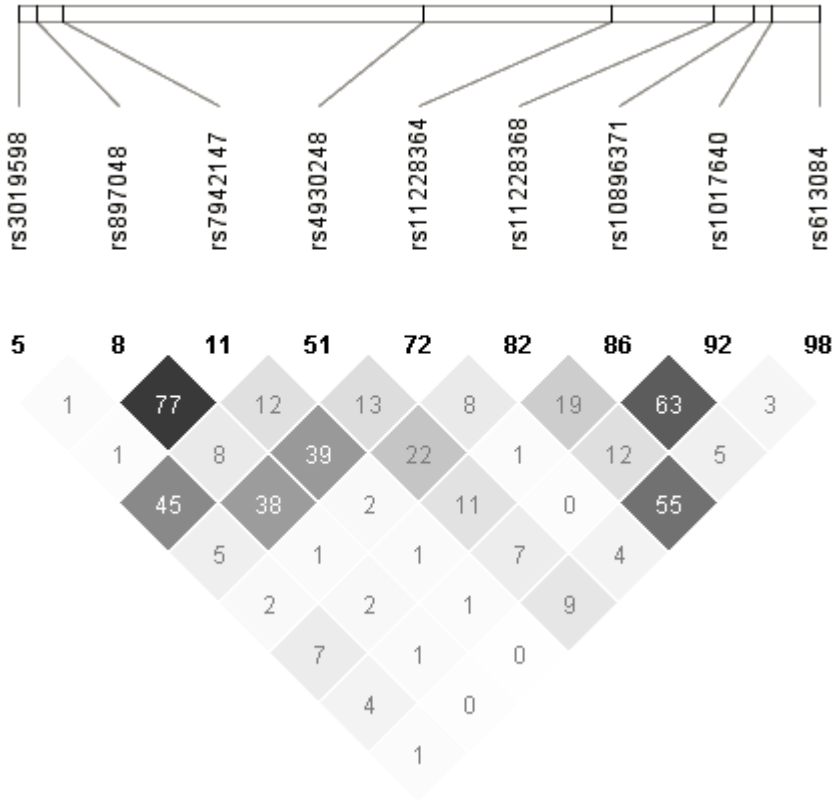


Figure 6.S2 LD plot of *ACAA2* gene.

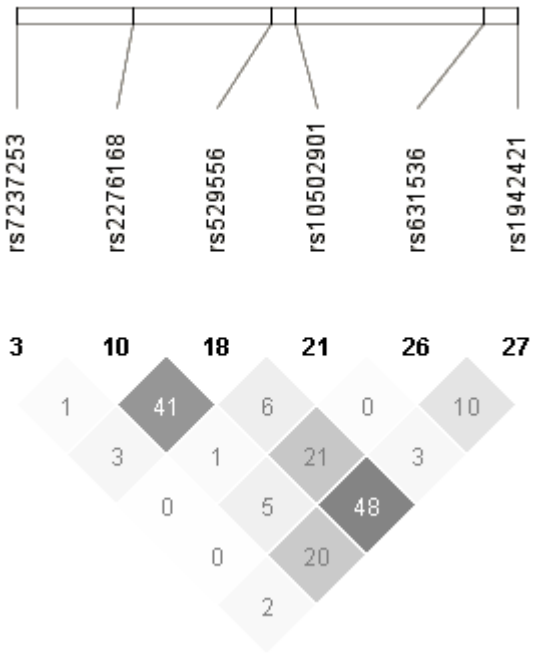


Figure 6.S3 LD plot of *ABCD2* gene.

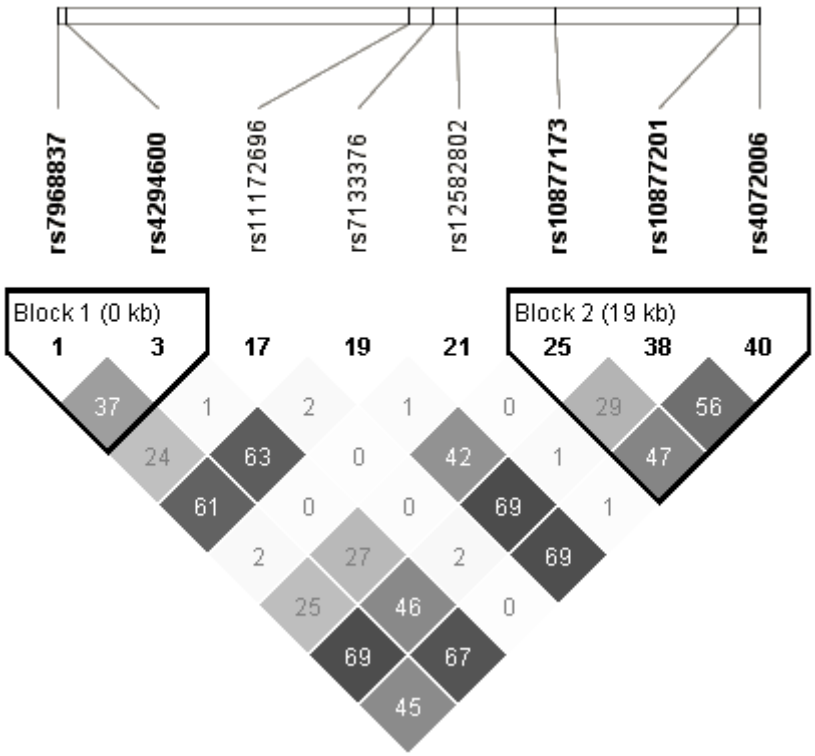
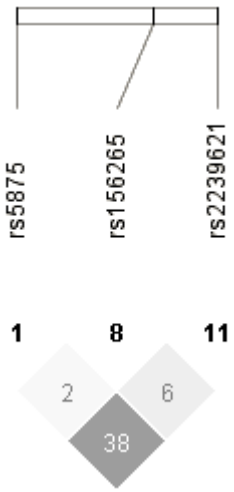


Figure 6.S4 LD plot of *ACAA1* gene.



Chapitre 7

Un effet d'interaction entre le gène de la glucokinase et les apports alimentaires en glucides module la réponse des triglycérides plasmatiques suite à une supplémentation en huile de poisson

Annie Bouchard-Mercier, Iwona Rudkowska, Simone Lemieux, Patrick Couture et Marie-Claude Vohl

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An interaction effect between *glucokinase* gene variation and carbohydrate intakes modulates the plasma triglyceride response to a fish oil supplementation

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Running head: Glucokinase gene, carbohydrate intake and the plasma triglyceride response to a fish oil supplementation

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Résumé

Introduction : Une importante variabilité interindividuelle dans la réponse des triglycérides plasmatiques (TG) suite à une supplémentation en huile de poisson a été observée. **Objectif** : Étudier les effets d'interaction gène-diète entre des polymorphismes nucléotidiques simples (SNPs) présents dans le gène de la *glucokinase* (*GCK*) et les apports alimentaires en glucides. **Méthodes** : Deux cent huit participants ont été recrutés dans la région de Québec. Les participants ont complété une supplémentation de 6 semaines en huile de poisson (5g d'huile de poisson/jour : 1,9-2,2g AEP et 1,1g ADH). Treize SNPs présents dans le gène *GCK* ont été génotypés en utilisant la méthodologie TAQMAN. **Résultats** : Un effet d'interaction gène-diète sur la réponse des TG plasmatiques a été observé avec le SNP rs741038 et les apports en glucides ajusté pour l'âge, le sexe et l'IMC (p=0,008). Afin de comparer la réponse des TG plasmatiques entre les génotypes et en fonction des apports alimentaires en glucides, les participants ont été divisés selon leur apport médian en glucides. Les homozygotes pour l'allèle mineure C du SNP rs741038 qui consommaient des apports élevés en glucides >48,59% avaient une plus grande diminution dans les concentrations de TG plasmatiques que les homozygotes C/C qui avaient de faibles apport en glucides (<0,05) et également que les autres génotypes peu importe leur consommation de glucides. **Conclusion** : La réponse des TG plasmatiques à une supplémentation en huile de poisson pourrait être modulée par des effets d'interaction gène-diète impliquant le gène *GCK* et les apports alimentaires en glucides.

Abstract

Introduction: A large inter-individual variability in the plasma triglyceride (TG) response to fish oil consumption has been observed. **Objective**: To investigate the gene-diet interaction effects between single-nucleotide polymorphisms (SNPs) within *glucokinase* (*GCK*) gene and dietary carbohydrate intakes (CHO) on the plasma TG response to a fish oil supplementation. **Methods**: Two hundred and eight participants were recruited in the greater Quebec City area. The participants completed a 6-week fish oil supplementation (5g fish oil/day: 1.9-2.2g EPA and 1.1g DHA). Thirteen SNPs within *GCK* gene were genotyped using TAQMAN methodology. **Results**: A gene-diet interaction effect on the plasma TG response was observed with rs741038 and CHO adjusted for age, sex and BMI ($p=0.008$). In order to compare the plasma TG response between genotypes according to CHO, participants were divided according to median CHO. Homozygotes of the minor C allele of rs741038 with high CHO >48.59% had a greater decrease in their plasma TG concentrations following the intake of fish oil ($p<0.05$) than C/C homozygotes with low CHO and also than the other genotypes either with high or low CHO. **Conclusion**: The plasma TG response to a fish oil supplementation may be modulated by gene-diet interaction effects involving *GCK* gene and CHO.

Introduction

Glucokinase (*GCK*) gene encodes for GCK or hexokinase type IV which phosphorylates glucose to glucose-6-phosphate (Massa et al. 2011). *GCK* gene is expressed mainly in the liver and in pancreatic beta cells but also in neurons, the pituitary and the entero-endocrine K and L cells (Massa et al. 2011). GCK has a glucose-sensor role and regulates glucose disposal towards glycogenesis and TG synthesis (Iynedjian 2009). It is partly regulated by GCKR which forms an inhibitory complex with GCK at low glucose concentrations because of its binding with fructose-6-phosphate (Choi et al. 2013). At high glucose concentrations such as after a meal high in carbohydrates (CHO), fructose-1-phosphate binds with GCKR and releases GCK which enhances glucose metabolism leading to a greater glycolytic flux (Girard et al. 1997b; Iynedjian 2009). A few single-nucleotide polymorphisms (SNPs) within GCK have been reported to have an impact on type 2 diabetes risk, glycemia, β-cell function and plasma TG concentrations (Hu et al. 2010; Sotos-Prieto et al. 2013; Wang et al. 2013; Fu et al. 2013; Asselbergs et al. 2012).

The hypotriglyceridemic impact of marine omega-3 polyunsaturated fatty acids (n-3 PUFA) at doses around 3g or more is well known (De 2011). This effect is even observed among individuals with plasma TG concentrations within normal values (<1.7mmol/L) and at lower marine n-3 PUFA doses (<2g) (Caslake et al. 2008). However, an important inter-individual variability in the plasma TG response to marine n-3 PUFA has been reported (Caslake et al. 2008; Cormier et al. 2012a; Minihane et al. 2000). Approximately 30% of the individuals do not lower their plasma TG concentrations following the intake of marine n-3 PUFA (Cormier et al. 2012a; Madden et al. 2011). The reduction in *de novo* lipogenesis pathway is one of the mechanisms which may explain the hypotriglyceridemic effect of n-3 PUFA (Harris et al. 2006). Among healthy individuals, the proportion of free fatty acids from *de novo* lipogenesis contributing to the free fatty acids pool that are incorporated into the VLDL-TG assembly is relatively small (~5%) (Vedala et al. 2006). However, among hypertriglyceridemic obese individuals with or without type 2 diabetes, the contribution of *de novo* lipogenesis increases to around 13-14% (Vedala et al. 2006). *De novo* lipogenesis is modulated by high dietary carbohydrate (CHO) intakes which increase the activity of

GCK, the production of malonyl-CoA and acetyl-CoA, therefore stimulating *de novo* lipogenesis (Girard et al. 1997a; Iynedjian 2009). Thus, we hypothesised that SNPs within *GCK* gene could have an impact on the plasma TG response to a fish oil supplementation and these impacts could be modulated by gene-diet interaction effects with dietary CHO.

Methods

Participants

A total of 254 unrelated participants were recruited to participate in this clinical trial from the greater Quebec City metropolitan area between September 2009 and December 2011 through advertisements in local newspapers as well as by electronic messages sent to university students/employees (Bouchard-Mercier et al. 2013). Briefly, to be eligible, participants had to be non-smokers and free of any thyroid or metabolic disorders requiring treatment such as diabetes, hypertension, severe dyslipidemia, and coronary heart disease. A total of 210 participants completed the n-3 PUFA supplementation period. However, plasma TG concentrations were available for 208 participants, thus the analyses were conducted on 208 participants. No differences in the descriptive characteristics were observed between the participants included in these analyses and the participants who did not complete the study or for which plasma TG concentrations values were missing. The experimental protocol was approved by the ethics committees of Laval University Hospital Research Center and Laval University. This clinical trial was registered at clinicaltrials.gov (NCT01343342). Informed written consent was obtained from all the study participants.

Study design and diets

The study design and diets have been described previously (Bouchard-Mercier et al. 2013). Briefly, participants followed a run-in period of 2 weeks. Individual dietary instructions were given by a trained dietitian to achieve the recommendations from Canada's Food Guide in order to standardize dietary intakes. Some specifications were given regarding the n-3 PUFA dietary intake: not exceed two fish or seafood servings per week, prefer white flesh fishes instead of fatty fishes (examples were given) and avoid enriched n-3 PUFA dietary products such as some milks, juices, breads and eggs. Subjects were also asked to limit their alcohol consumption during the protocol; two regular drinks per week were allowed. In addition, subjects were not allowed to take n-3 PUFA supplements (such as flaxseed), vitamins or natural health products during the protocol.

After the 2-week run-in period, each participant received a bottle containing n-3 PUFA capsules for the next 6 weeks. They were instructed to take five capsules (1 g of fish oil/capsule) per day (Ocean Nutrition, Nova Scotia, Canada), providing a total of 5 g of fish oil (1.9-2.2g EPA and 1.1 g DHA) per day.

Participants completed two 3-d food records, before and after the n-3 PUFA supplementation period. Thus, the first food record reflected dietary intakes of the week prior to the beginning of the supplementation period and for the second food record the week prior to the end of the supplementation period. All foods and beverages consumed on 2 representative weekdays and 1 weekend day were weighed or estimated and recorded in food diaries. 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.

Biochemical parameters

The morning after a 12-hour overnight fast and 48-h alcohol abstinence, blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA. Plasma was separated by centrifugation (2500 x g for 10 minutes at 4°C), samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (total-C) and plasma TG concentrations were measured using enzymatic assays (Burstein and Samaille 1960; McNamara and Schaefer 1987). Infranatant (d >1.006 g/ml) with heparin-manganese chloride was used to precipitate VLDL and LDL and then determine HDL-cholesterol concentrations (HDL-C) (Albers et al. 1978). The equation of Friedewald was used to estimate LDL-cholesterol concentrations (LDL-C) (Friedewald et al. 1972). Non-HDL-C was calculated by subtracting HDL-C from total-C.

SNPs selection and genotyping

As described previously (Bouchard-Mercier et al. 2013), SNPs were selected with the International HapMap Project SNP database (HapMap Data Rel 28 Phase II+III, August 10, on National Center for Biotechnology Information (NCBI) B36 assembly, dbSNP b126).

The chromosomal region selected was chr7:44149895-44198063, which included 2500bp upstream and 500bp downstream of *GCK* gene. Tag SNPs (tSNPs) were determined with the tagger procedure in HaploView software version 4.2 with minor allele frequency (MAF) of >0.05 and pairwise tagging $R^2 \geq 0.80$. For each gene a minimum of 85% of the most common SNPs had to be captured by tSNPs. Afterwards, as shown in Figure 7.1, linkage disequilibrium (LD) plot were generated with Haploview software version 4.2. All tSNPs were genotyped within INAF laboratories with the TAQMAN methodology (Livak 1999), as described previously (Cormier et al. 2012b). Briefly, genotypes were determined using ABI Prism SDS version 2.0.5 (Applied Biosystem, Foster City, CA, USA). All SNPs were successfully genotyped with a call rate of 100% (n=208).

Statistical analyses

Hardy-Weinberg equilibrium was tested with the ALLELE procedure of SAS version 9.3 using Fisher's exact test (P<0.01). When the genotype frequency for homozygous individuals of the minor allele was <5%, carriers (heterozygotes and homozygotes) of the minor allele were grouped. Variables non-normally distributed were logarithmically transformed (BMI, plasma TG and insulin). Differences were assessed using analyses of variance (ANOVA) with the GLM procedure in SAS and the type 3 sum of squares for unbalanced study design. To examine the impact of gene-diet interaction effects on the plasma TG response, the interaction term (SNP*CHO) was added into the ANOVA model. The plasma TG response was calculated as followed: ((post-supplementation plasma TG - pre-supplementation plasma TG)/pre-supplementation plasma TG*100). The model was adjusted for the effects of age, sex and BMI. To take into account the effects of multiple testing the simpleM procedure described by Gao *et al*. (Gao et al. 2008) was used. The number of effective independent test determined by the procedure of Gao *et al*. (Gao et al. 2008), was 8 and only one environmental factor (CHO) was studied. Thus, p-values lower than 6.25×10^{-3} were considered significant (p=0.05/(8*1)). All statistical analyses were performed using SAS statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Descriptive characteristics of the study population and tSNPs

As previously observed (Thifault et al. 2013), the baseline, pre- and post-supplementation descriptive characteristics including dietary intakes of the study participants are presented in Table 7.1. Briefly, BMI, fasting glucose and HDL-C slightly increased ($p=0.005$, $p=0.0003$ and $p=0.005$, respectively) whereas plasma TG decreased ($p<0.0001$) following the fish oil supplementation. For dietary intakes, total daily energy intakes (including fish oil supplements) and CHO (expressed as percentage of energy intakes) decreased ($p=0.006$ and $p=0.0009$, respectively) and total fat intakes (expressed as percentage of energy intakes) increased ($p=<0.0001$) following the fish oil supplementation.

The selected SNPs are presented in Online Resource 1. All SNPs were in Hardy-Weinberg equilibrium ($p<0.01$). Figure 7.1 presents the LD plot. Briefly, thirteen tSNPs covered 86% of the known genetic variability within *GCK* gene (including 2500bp upstream and 500bp downstream *GCK* gene).

The impact of CHO and tSNPs on pre-supplementation plasma TG concentrations

No gene-diet interaction effects on pre-supplementation plasma TG concentrations were observed (data not shown). Pre-supplementation CHO intake was positively associated with pre-supplementation plasma TG concentrations ($r=0.19$, $p=0.007$). Pre-supplementation plasma TG concentrations according to genotype are presented in Table 7.2. Briefly, no differences in plasma TG concentrations were observed according to genotypes. A trend ($p=0.07$) was observed for rs3757838, for which individuals carrying the A allele had lower plasma TG concentrations than T/T homozygotes.

The impact of tSNPs, CHO and gene-diet interaction effects on the plasma TG response

A trend for an inverse relationship between the plasma TG response and post-supplementation CHO was observed (r=-0.14, p=0.05). This association was also observed between the plasma TG response and pre-supplementation CHO (r=-0.19, p=0.008). As shown in Table 7.3, gene-diet interaction effects on the plasma TG response were observed with rs741038 and post-supplementation CHO expressed as percentage of energy consumption and adjusted for age, sex and BMI (p=0.008) and was significant after correction for multiple testing only when pre-supplementation plasma TG concentrations were included into the model as a confounding variable (p=0.001). A trend for a gene-diet interaction effect was also observed for rs1990458 (p=0.07) when age, sex and BMI were included in the model. None of the other SNPs were associated with the plasma TG response either alone or in interaction with CHO. To further explore the impact of rs741038 and CHO, participants were divided on the basis of CHO using the median value and genotype. As shown in Figure 7.2, homozygotes for the minor C allele of rs741038 with high CHO >48.59% had a greater decrease in plasma TG concentrations following the intake of fish oil than C/C homozygotes with low CHO intake (p=0.002), than C/T heterozygotes with either low or high CHO (p=0.03 and p=0.005, respectively) and than T/T homozygotes with either low or high CHO intakes (p=0.02 and p=0.04, respectively).

Discussion

In this study, gene-diet interaction effects on the plasma TG response to fish oil were observed between *GCK* gene and CHO. The intake of CHO modified the plasma TG response to fish oil depending on the genotype of rs741038 within *GCK* gene. For C/C homozygotes, low CHO intakes were associated with an increase in plasma TG concentrations whereas when CHO intakes were high they had the greatest decrease in their plasma TG concentrations following the fish oil supplementation.of all genotype groups. The SNP rs741038 is an intronic SNP with no potential regulatory impact and is not in LD with rs1799884 or rs2070971 which have been associated previously to plasma TG levels, fasting glucose and/or type 2 diabetes risk (Asselbergs et al. 2012; Holmkvist et al. 2008; Sotos-Prieto et al. 2013; Webster et al. 2009; Hu et al. 2010; Wang et al. 2013). However, it is possible that rs741038 is in LD with other unknown functional SNPs. Moreover, the association between rs1799884 and plasma TG concentrations was not confirmed by our results (Sotos-Prieto et al. 2013). Hishida *et al*. (2012) have investigated the impact of gene-diet interaction effect on the risk of dyslipidemia defined as elevated plasma TG and/or low HDL-C concentrations and did not observe interaction effects with *GCK* gene rs1799884 and the intakes of energy, fat, CHO or alcohol. A recent meta-analysis has shown that the association between rs1799884 and type 2 diabetes varies according to ethnic populations and could be more important among Caucasians than among Asians (Fu et al. 2013). In a large-scale gene-centric meta-analysis, the intronic SNP rs2070971 within *GCK* gene was associated with plasma TG concentrations (Asselbergs et al. 2012).

In the liver, *GCK* gene expression is regulated by the presence of insulin (Iynedjian et al. 1988; Iynedjian 2009). This mechanism involves the long-term regulation of *GCK* gene transcription and translation (Moore et al. 2012). The induction of *GCK* gene expression by insulin may be modulated by dietary components such as dietary PUFA which decrease *GCK* gene expression induction (Jump et al. 1994). Moreover, the long term consumption of a diet high in fat and in fructose among dogs has shown to also decrease GCK protein and its activity in the liver as well as glycogen storage, but not mRNA abundance (Coate et al. 2010; Coate et al. 2011; Moore et al. 2012). It has been observed that *GCK* mRNA

abundance is positively correlated with the liver TG content (Peter et al. 2011). An increased *GCK* gene expression was also positively associated with expression levels of lipogenic enzymes and *de novo* lipogenesis index (Peter et al. 2011). It has been observed that compared to a high-fat/low-CHO diet, a low-fat/high-CHO diet led to increases in *de novo* lipogenesis which was correlated with the increase in plasma TG concentrations among both normoinsulinemic and hyperinsulinemic individuals (Schwarz et al. 2003). In this study, there was a positive correlation between pre-supplementation CHO and plasma TG concentrations. However, following the intake of fish oil, an inverse relationship between CHO (both pre- and post-supplementation) and the plasma TG response was observed. Thus, it is possible that higher pre-supplementation CHO lead to higher GCK activity and therefore, enhances the potential of fish oil to decrease its activity and affect plasma TG concentrations.

The variability observed in the plasma TG response to fish oil could be attributable to factors such as poor compliance. However, compliance rates were high (94%) (Bouchard-Mercier et al. 2013), thus poor compliance could not explain the variability observed. Sex is another factor that may modulate the plasma TG response to fish oil (Caslake et al. 2008), however no sex effect (or statistical interaction with sex) was observed in this cohort (Thifault et al. 2013). In our previous work, differences between responders and non-responders to fish oil intake according to the plasma TG response in the modulation of the expression levels of many genes involved in glycerophospholipid, sphingolipid, arachidonic acid and linoleic acid metabolisms were observed (Rudkowska et al. 2012). It is possible that pre-supplementation gene expression levels, depending on the dietary intakes or other environmental parameters may affect the plasma TG response to fish oil.

Conclusion

A gene-diet interaction effect between *GCK* gene and CHO on the plasma TG response following fish oil intake among overweight individuals has been observed. For homozygotes of the minor allele, higher CHO intakes led to important decreases in plasma TG concentrations following the fish oil supplementation. However, when their intake of CHO was lower, they slightly increased their plasma TG concentrations. Still, for homozygotes of the minor allele with lower CHO intakes, plasma TG concentrations were within normal values. Thus, they are unlikely to be at increased cardiovascular disease risk following fish oil supplementation. These results need to be replicated among other populations. In the future, considering dietary carbohydrate intakes and the genetic background before recommending fish oil to reduce plasma TG concentrations may be of importance to identify subgroups of the population that may not respond as predicted. These results reinforce the importance of considering inter-individual variability in the response to fish oil supplementation. It will also be necessary to achieve a global understanding of the impacts of several gene-diet interaction effects on the plasma TG response to fish oil before taking this knowledge into clinical practice.

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Authors' contributions

IR, SL, PC, and MCV designed research; ABM conducted research with the research professionals; IR, SL, PC and MCV provided essential reagents or provided essential materials; ABM analyzed data and performed statistical analysis; ABM wrote paper; ABM, IR, SL, PC and MCV had primary responsibility for final content; All authors read and approved the final manuscript.

Compliance with Ethics Guidelines

Competing interests

Annie Bouchard-Mercier declares no conflict of interest.
Iwona Rudkowska declares no conflict of interest.
Simone Lemieux declares no conflict of interest.
Patrick Couture declares no conflict of interest.
Marie-Claude Vohl declares no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

References

Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L, Breckenridge C, Chow A, Kuba K, Weidman S, Arnett H, Wood P, Shlagenhaft A (1978) Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. Clin Chem 24:853-856

Asselbergs FW, Guo Y, van Iperen EP, Sivapalaratnam S, Tragante V, Lanktree MB, et al (2012) Large-scale gene-centric meta-analysis across 32 studies identifies multiple lipid loci. Am J Hum Genet 91:823-838

Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC (2013) Polymorphisms, de novo lipogenesis and plasma triglyceride response following fish oil supplementation. J Lipid Res 54:2866-2873

Burstein M, Samaille J (1960) [On a rapid determination of the cholesterol bound to the serum alpha- and beta-lipoproteins]. Clin Chim Acta 5:609

Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, Kimber AC, Grew JP, Farrell L, et al (2008) Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr 88:618-629

Choi JM, Seo MH, Kyeong HH, Kim E, Kim HS (2013) Molecular basis for the role of glucokinase regulatory protein as the allosteric switch for glucokinase. Proc Natl Acad Sci USA 110:10171-10176

Coate KC, KraftG, Lautz M, Smith M, Neal DW, Cherrington AD (2011) A high-fat, high-fructose diet accelerates nutrient absorption and impairs net hepatic glucose uptake in response to a mixed meal in partially pancreatectomized dogs. J Nutr 141:1643-1651

Coate KC, Scott M, Farmer B, Moore MC, Smith M, Roop J, Neal DW, Williams P, Cherrington AD (2010) Chronic consumption of a high-fat/high-fructose diet renders the liver incapable of net hepatic glucose uptake. Am J Physiol Endocrinol Metab 299:E887-E898

Cormier H, Rudkowska I, Paradis AM, Thifault E, Garneau V, Lemieux S, Couture P, Vohl MC (2012a). Association between Polymorphisms in the Fatty Acid Desaturase Gene Cluster and the Plasma Triacylglycerol Response to an n-3 PUFA Supplementation. Nutrients 4:1026-1041

De CR (2011) n-3 fatty acids in cardiovascular disease. N Engl J Med 364:2439-2450

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 18:499-502

Fu D, Cong X, Ma Y, Cai H, Cai M, Li D, Lv M, Yuan X, Huang Y, Lv Z (2013) Genetic polymorphism of glucokinase on the risk of type 2 diabetes and impaired glucose regulation: evidence based on 298,468 subjects. PLoS One 8:e55727

Gao X, Starmer J, Martin ER (2008) A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet Epidemiol 32:361-369

Girard J, Ferre P, Foufelle F (1997a) Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. Annu Rev Nutr 17:325-352

Girard J, Ferre P, Foufelle F (1997b) Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. Annu Rev Nutr 17:325-352

Harris WS, Bulchandani D (2006) Why do omega-3 fatty acids lower serum triglycerides? Curr Opin Lipidol 17:387-393

Hishida A, Morita E, Naito M, Okada R, Wakai K, Matsuo K, Nakamura K, Takashima N et al (2012) Associations of apolipoprotein A5 (APOA5), glucokinase (GCK) and glucokinase regulatory protein (GCKR) polymorphisms and lifestyle factors with the risk of dyslipidemia and dysglycemia in Japanese - a cross-sectional data from the J-MICC Study. Endocr J 59:589-599

Holmkvist J, Almgren P, Lyssenko V, Lindgren CM, Eriksson KF, Isomaa B, Tuomi T, Nilsson P, Groop L (2008) Common variants in maturity-onset diabetes of the young genes and future risk of type 2 diabetes. Diabetes 57:1738-1744

Hu C, Zhang R, Wang C, Yu W, Lu J, Ma X, Wang J, Jiang F, Tang S, Bao Y, Xiang K, Jia W (2010) Effects of GCK, GCKR, G6PC2 and MTNR1B variants on glucose metabolism and insulin secretion. PLoS One 5:e11761

Iynedjian PB (2009) Molecular physiology of mammalian glucokinase. Cell Mol Life Sci 66:27-42

Iynedjian PB, Gjinovci A, Renold AE (1988) Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. J Biol Chem 263:740-744

Jump DB, Clarke SD, Thelen A, Liimatta M (1994) Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. J Lipid Res 35:1076-1084

Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 14:143-149

Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H, Mathers JC, Minihane AM (2011) The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. Annu Rev Nutr 31:203-234

Massa ML, Gagliardino JJ, Francini F (2011) Liver glucokinase: An overview on the regulatory mechanisms of its activity. IUBMB Life 63:1-6

McNamara JR, Schaefer EJ (1987) Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. Clin Chim Acta 166:1-8

Minihane AM, Khan S, Leigh-Firbank EC, Talmud P, Wright JW, Murphy MC, Griffin BA, Williams CM (2000) ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. Arterioscler Thromb Vasc Biol 20:1990-1997

Moore MC, Coate KC, Winnick JJ, An Z, Cherrington AD (2012) Regulation of hepatic glucose uptake and storage in vivo. Adv Nutr 3:286-294

Nettleton JA, McKeown NM, Kanoni S, Lemaitre RN, Hivert MF, Ngwa J, van Rooij FJ, Sonestedt E, Wojczynski MK, Ye Z et al (2010). Interactions of dietary whole-grain intake with fasting glucose- and insulin-related genetic loci in individuals of European descent: a meta-analysis of 14 cohort studies. Diabetes Care 33, 2684-2691.

Perez-Martinez P, Delgado-Lista J, Garcia-Rios A, Mc MJ, Gulseth HL, Ordovas JM, Shaw DI, Karlstrom B, Kiec-Wilk B et al (2011) Glucokinase regulatory protein genetic variant interacts with omega-3 PUFA to influence insulin resistance and inflammation in metabolic syndrome. PLoS One 6:e20555

Peter A, Stefan N, Cegan A, Walenta M, Wagner S, Konigsrainer A, Konigsrainer I, Machicao F, Schick F, Haring HU, Schleicher E (2011) Hepatic glucokinase expression is associated with lipogenesis and fatty liver in humans. J Clin Endocrinol Metab 96:E1126-E1130

Rudkowska I, Paradis AM, Thifault E, Julien P, Barbier O, Couture P, Lemieux S, Vohl MC (2012) Differences in metabolomic and transcriptomic profiles between responders and non-responders to an n-3 polyunsaturated fatty acids (PUFAs) supplementation. Genes Nutr 8:411-423

Schwarz JM, Linfoot P, Dare D, Aghajanian K (2003) Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets. Am J Clin Nutr 77:43-50

Sotos-Prieto M, Guillen M, Vicente SJ, Portoles O, Guillem-Saiz P, Ignacio GJ, Qi L, Corella D (2013) Relevant associations of the glucokinase regulatory protein/glucokinase gene variation with TAG concentrations in a high-cardiovascular risk population: modulation by the Mediterranean diet. Br J Nutr 109:193-201

Thifault E, Cormier H, Bouchard-Mercier A, Rudkowska I, Paradis AM, Garneau V, Ouellette C, Lemieux S, Couture P, Vohl MC (2013) Effects of Age, Sex, Body Mass Index and APOE Genotype on Cardiovascular Biomarker Response to an n-3 Polyunsaturated Fatty Acid Supplementation. J Nutrigenet Nutrigenomics 6:73-82

Vedala A, Wang W, Neese RA, Christiansen MP, Hellerstein MK (2006) Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and de novo lipogenesis in humans. J Lipid Res 47:2562-2574

Wang H, Liu L, Zhao J, Cui G, Chen C, Ding H, Wang DW (2013) Large scale meta-analyses of fasting plasma glucose raising variants in GCK, GCKR, MTNR1B and G6PC2 and their impacts on type 2 diabetes mellitus risk. PLoS One 8:e67665

Webster RJ, Warrington NM, Weedon MN, Hattersley AT, McCaskie PA, Beilby JP, Palmer LJ, Frayling TM (2009) The association of common genetic variants in the APOA5, LPL and GCK genes with longitudinal changes in metabolic and cardiovascular traits. Diabetologia 52:106-114

Table 7.1 Descriptive characteristics of the study participants.

Variables	Baseline (n=208)	Pre (n=208)	Post (n=208)	P[a]
Age (years)	30.82±8.66	-	-	
Sex (men/women)	96/112	-	-	
BMI (Kg/m^2)	27.84±3.73	27.81±3.74	27.90±3.85	0.005
Fasting glucose (mmol/L)	4.95±0.52	4.95±0.46	5.05±0.49	0.0003
Fasting insulin (pmol/L)	82.51±35.61 (n=206)	87.15±75.70	83.63±40.81 (n=207)	0.98
Total-C (mmol/L)	4.82±1.01	4.75±0.90	4.72±0.94	0.48
LDL-C (mmol/L)	2.79±0.87 (n=207)	2.76±0.81	2.78±0.85	0.42
HDL-C (mmol/L)	1.46±0.39	1.44±0.36	1.47±0.40	0.005
Triglycerides (mmol/L)	1.23±0.64	1.21±0.63	1.02±0.52	<0.0001
Dietary intakes				
Energy (kcal)	-	2273±590 (n=207)	2186±566	0.006
Fat intake (%)	-	32.59±6.04 (n=207)	35.30±6.27	<0.0001
CHO (%)	-	50.51±7.16 (n=207)	48.61±7.75	0.0009
Protein intake (%)	-	17.36±3.32 (n=206)	16.95±3.17	0.12

Means±SD.
[a]P-values were calculated with paired t-tests.

Table 7.2 Pre-supplementation plasma TG concentrations according to genotype of tSNPs within *GCK* gene.

tSNPs	Genotype	Plasma TG concentrations (mmol/L)	P[a]
rs2268573	C/C	1.26±0.69	0.90
	A/C	1.19±0.65	
	A/A	1.17±0.47	
rs2908297	G/G	1.22±0.66	0.97
	A/A+A/G	1.17±0.47	
rs2971676	C/C	1.21±0.65	0.76
	T/T+C/T	1.21±0.45	
rs758989	A/A	1.27±0.74	0.53
	A/G	1.21±0.63	
	G/G	1.12±0.47	
rs12673242	T/T	1.22±0.66	0.81
	C/C+C/T	1.18±0.47	
rs2908290	C/C	1.27±0.75	0.80
	C/T	1.13±0.49	
	T/T	1.22±0.51	
rs2284777	A/A	1.23±0.61	0.17
	G/G+A/G	1.15±0.67	
rs2300584	T/T	1.24±0.69	0.68
	C/C+C/T	1.15±0.50	
rs1990458	G/G	1.16±0.65	0.13
	A/G	1.18±0.56	
	A/A	1.42±0.77	
rs741038	T/T	1.26±0.63	0.48
	C/T	1.18±0.62	
	C/C	1.11±0.65	
rs1799884	G/G	1.24±0.65	0.28
	A/A+A/G	1.15±0.59	
rs2908277	C/C	1.22±0.64	0.87
	T/T+C/T	1.18±0.58	
rs3757838	T/T	1.24±0.65	0.07
	A/A+A/T	1.00±0.36	

Means±SD.
[a]P-values of the GLM models are adjusted for age, sex and BMI.

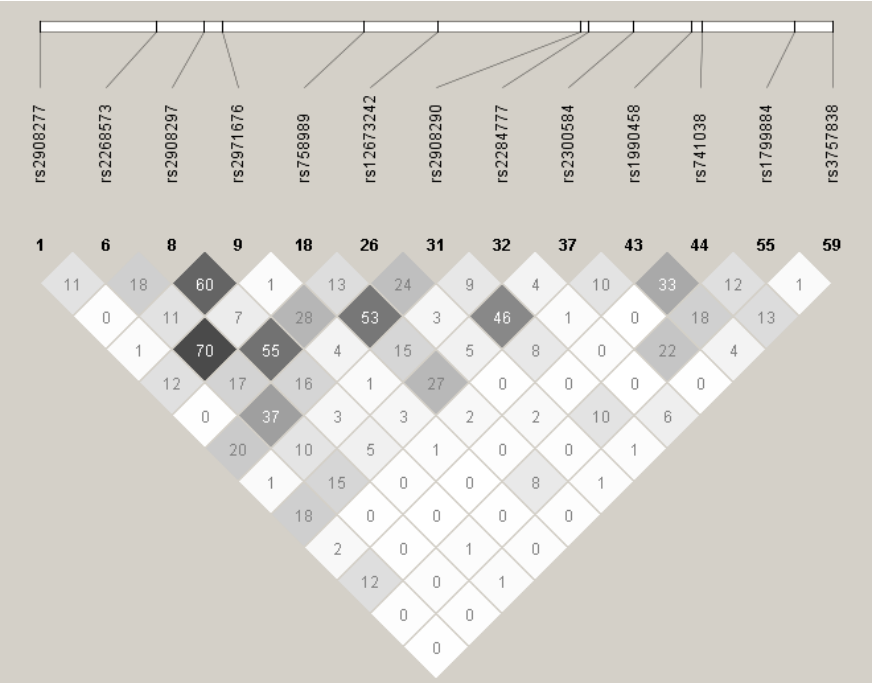
Table 7.3 Gene-diet interaction effects on the plasma TG response to fish oil.

tSNPs	Genotype	β (interaction term)	P genotype[a]	P CHO[a]	P interaction effect[a]
rs2268573	C/C	0			
	A/C	0.0740±0.5332	0.94	0.05	0.96
	A/A	-0.1084±0.6796			
rs2908297	G/G	0	0.54	0.27	0.40
	A/A+A/G	0.4819±0.5730			
rs2971676	C/C	0	0.73	0.23	0.70
	T/T+C/T	0.2427±0.6239			
rs758989	A/A	0			
	A/G	0.4084±0.5660	0.62	0.04	0.67
	G/G	-0.0393±0.5978			
rs12673242	T/T	0	0.14	0.68	0.09
	C/C+C/T	1.0556±0.6198			
rs2908290	C/C	0			
	C/T	0.3690±0.4906	0.65	0.07	0.65
	T/T	-0.2902±0.8764			
rs2284777	A/A	0	0.48	0.04	0.50
	G/G+A/G	-0.3345±0.4975			
rs2300584	T/T	0	0.38	0.04	0.36
	C/C+C/T	-0.4474±0.4880			
rs1990458	G/G	0			
	A/G	1.1604±0.5029	0.09	0.07	0.07
	A/A	0.4946±0.7105			
rs741038	T/T	0			
	C/T	0.3766±0.4779	**0.01**	0.0004	**0.008**
	C/C	-2.4272±0.9010			
rs1799884	G/G	0	0.73	0.05	0.78
	A/A+A/G	-0.1385±0.4847			
rs2908277	C/C	0	0.97	0.08	0.83
	T/T+C/T	-0.1151±0.5440			
rs3757838	T/T	0	0.18	0.03	0.19
	A/A+A/T	-1.0834±0.8202			

Means±SD.

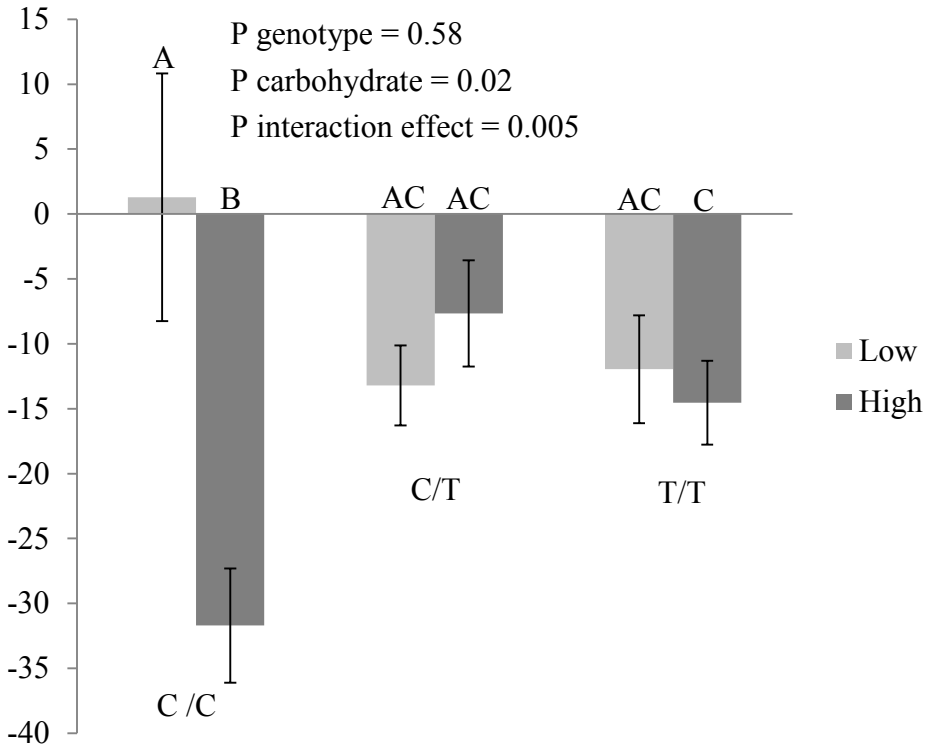
[a]P-values of the GLM models are adjusted for age, sex and BMI.

Figure 7.1 Linkage disequilibrium (LD) plot of tSNPs within *GCK* gene.



LD plots were generated by HaploView software version 4.2 using R^2 LD values.

Figure 7.2 The plasma TG response (%) according to median CHO and rs741038 genotype.



The plasma TG response following fish oil supplementation (means±SE). Means with different letters are significantly different (assessed by an ANOVA). CHO are separated according to the median value: ≤48.59 (Low in light color) and >48.59% (High in dark color). Number of participants: low CHO and C/C genotype (n=13), high CHO and C/C genotype (n=10), low CHO and C/T genotype (n=44), high CHO and C/T genotype (n=49), low CHO and T/T genotype (n=47), high CHO and T/T genotype (n=45).

Chapitre 8

La variabilité génétique présente dans le gène *SREBF1* module la réponse des concentrations d'insuline à jeun suite à une supplémentation en huile de poisson

Annie Bouchard-Mercier, Iwona Rudkowska, Simone Lemieux, Patrick Couture, Louis Pérusse et Marie-Claude Vohl

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***SREBF1* gene variations modulate insulin sensitivity in response to a fish oil supplementation**

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Trial registration: NCT01343342. http://clinicaltrials.gov/show/NCT01343342

Résumé

Introduction : Une importante variabilité interindividuelle dans la réponse de la sensibilité à l'insuline suite à une supplémentation en huile de poisson a été observée. **Objectif** : Examiner les associations entre des polymorphismes nucléotidiques simples (SNPs) présents dans le gène *sterol regulatory element binding transcription factor 1* (*SREBF1*) et la réponse de la sensibilité à l'insuline suite à une supplémentation en huile de poisson. **Méthodes** : Les participants (n=210) ont été recrutés dans la région de Québec et ont suivi un protocole de supplémentation en huile de poisson de 6 semaines (5g d'huile de poisson/jour : 1,9-2,2 AEP; 1,1g ADH). La sensibilité à l'insuline a été calculée avec l'indice *quantitative insulin sensitivity check index* (QUICKI). Trois SNPs présents dans le gène *SREBF1* ont été génotypés selon la méthodologie TAQMAN. **Résultats** : Les trois SNPs (rs12953299, rs4925118 and rs4925115) couvraient 100% de la variabilité génétique connue du gène *SREBF1*. Aucun de ces SNPs n'était associé avec les concentrations d'insuline de départ (rs12953299, rs4925118 et rs4925115) (p=0,29, p=0,20 et p=0,70, respectivement) ni à l'indice QUICKI de départ (p=0,20, p=0,18 et p=0,76, respectivement). Les trois SNPs (rs12953299, rs4925118 et rs4925115) étaient associés à la réponse des concentrations d'insuline (p=0,01, p=0,005 et p=0,004, respectivement) et les SNPs rs12953299 de même que rs4925115 étaient associés avec la réponse de la sensibilité à l'insuline (p=0,009 et p=0,01, respectivement) suite à la supplémentation en huile de poisson, indépendamment des effets de l'âge, du sexe et de l'IMC. **Conclusion** : La variabilité génétique présente dans le gène *SREBF1* aurait un impact sur la réponse de l'insuline et la sensibilité à l'insuline suite à une supplémentation en huile de poisson.

Abstract

Background: An important inter-individual variability in the response of insulin sensitivity following a fish oil supplementation has been observed. **Objective**: To examine the associations between single nucleotide polymorphisms (SNPs) within *sterol regulatory element binding transcription factor 1* (*SREBF1*) gene and the response of insulin sensitivity to a fish oil supplementation. **Design**: Participants (n=210) were recruited in the greater Quebec City area and followed a 6-week fish oil supplementation protocol (5g/day: 1.9-2.2g EPA; 1.1g DHA). Insulin sensitivity was assessed by the quantitative insulin sensitivity check index (QUICKI). Three tag SNPs (tSNPs) within *SREBF1* gene were genotyped according to TAQMAN methodology. **Results**: Three tSNPs (rs12953299, rs4925118 and rs4925115) covered 100% of the known genetic variability within *SREBF1* gene. None of the three tSNPs was associated with either baseline fasting insulin concentrations (rs12953299, rs4925118 and rs4925115) (p=0.29, p=0.20 and p=0.70, respectively) or QUICKI (p=0.20, p=0.18 and p=0.76, respectively). The three tSNPs were associated with differences in the response of plasma insulin levels (p=0.01, p=0.005 and p=0.004, respectively) and rs12953299 as well as rs4925115 were associated with the insulin sensitivity response (p=0.009 and p=0.01, respectively) to the fish oil supplementation, independently of the effects of age, sex and BMI. **Conclusions**: The genetic variability within *SREBF1* gene has an impact on the insulin sensitivity in response to a fish oil supplementation.

Introduction

Sterol regulatory element binding transcription factor 1 (*SREBF1*) gene encodes a transcription factor which is a main regulator of lipid metabolism, the sterol regulatory element-binding protein-1c (SREBP-1c) [1]. *SREBF1* gene is expressed in multiple tissues including liver, white and brown adipose tissue, adrenal gland and to a lower extent in pancreatic β-cell [2,3]. Insulin induces the expression of *SREBF1* gene in adipose tissue, liver and muscle cells [1]. In pancreatic β-cell, it has been observed that SREBP-1c modulates insulin secretion potentially through a mechanism involving lipotoxicity [3,4]. *SREBF1* gene expression is also regulated by dietary intakes. An insulin independent effect has been demonstrated with different types of sugar on *SREBF1* gene expression induction [5]. A high saturated fat (SFA) diet increases *SREBF1* gene expression both in the liver and in pancreatic β-cell [3,6] whereas a diet high in polyunsaturated fat (PUFA) decreases *SREBF1* gene expression [3,7,8]. Among diet induced obese mice, the intake of fish oil reduces *SREBF1* gene expression levels in the liver and modifies the expression of other genes involved in lipid metabolism [9]. Eicosapentaenoic acid (EPA) was shown to inhibit SREBP-1 maturation [10] and to restore insulin secretion after suppression by palmitate through an SREBP-1c dependent mechanism [11].

A meta-analysis of genome wide scans in European populations showed linkage with type 2 diabetes in the 17p11 region, which comprises *SREBF1* gene [12]. Single nucleotide polymorphisms (SNPs) within *SREBF1* gene have been associated with type 2 diabetes, insulin resistance, obesity and blood lipid levels [13-18]. Thus, the genetic variability within *SREBF1* gene may play a role in insulin resistance or type 2 diabetes. The intake of fish oil may have a favorable impact on insulin sensitivity. Among fructose-induced hypertriglyceridemic and insulin resistant male rhesus macaques, the intake of 4g/day of fish oil prevented the development of hypertriglyceridemia and insulin resistance [19]. Studies observing rodents have also observed a beneficial effect of fish oil on insulin sensitivity [20,21]. However, among humans these effects have not been consistent [22-26]. Some studies even observed an increase in fasting insulin concentrations and/or fasting glucose concentrations [27,28]. These inconsistencies in results could be partly due to

genetic variants within transcription factors such as *SREBF1* gene. To our knowledge this is the first study to examine the associations between SNPs within *SREBF1* gene and the plasma insulin and glucose response to a fish oil supplementation.

Methods

Participants

Methods related to this study cohort have been previously described [29]. Briefly, a total of 254 unrelated participants from the greater Quebec City metropolitan area were recruited to participate in this clinical trial 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, participants had to be non-smokers and without any thyroid or metabolic disorders requiring treatment, for example diabetes, hypertension, severe dyslipidemia, and/or coronary heart disease. A total of 210 participants completed the fish oil supplementation period. However, fasting insulin and glucose concentrations were obtained only for 207 participants. The experimental protocol was approved by the ethics committees of Laval University Hospital Research Center and Laval University. This clinical trial was registered at clinicaltrials.gov (NCT01343342). Informed written consent was obtained from all the study participants.

Study design and diets

The study design and diets have been described previously [29]. Briefly, participants followed a run-in period of 2 weeks. Individual dietary instructions were given by a trained dietitian to achieve the recommendations from Canada's Food Guide. After the 2-week run-in period, each participant received a bottle containing fish oil capsules for the next 6 weeks. They were instructed to take five capsules (1 g of fish oil/capsule) per day (Ocean Nutrition, Nova Scotia, Canada), providing a total of 5 g of fish oil (1.9-2.2 g EPA and 1.1 g docosahexaenoic acid (DHA)) per day. Compliance was assessed from the return of bottles and by measuring erythrocyte membranes and plasma phospholipids fatty acid (FA) composition

Biochemical parameters

The morning after a 12-hour overnight fast and 48-h alcohol abstinence, blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA. Blood

samples were collected at screening, baseline, pre-supplementation (two weeks after baseline) and post-supplementation (six weeks after pre-supplementation). Plasma was separated by centrifugation (2500 x g for 10 minutes at 4°C), samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (total-C) and plasma triglyceride concentrations were measured using enzymatic assays [30,31]. Infranatant (d >1.006 g/ml) with heparin-manganese chloride was used to precipitate VLDL and LDL and then determine HDL-cholesterol concentrations (HDL-C) [32]. The equation of Friedewald was used to estimate LDL-cholesterol concentrations (LDL-C) [33]. Non-HDL-C was calculated by subtracting HDL-C from total-C. Fasting insulinemia was measured by radioimmunoassay with polyethylene glycol separation [34]. Fasting glucose concentrations were enzymatically measured [35]. The quantitative insulin sensitivity check index (QUICKI) was used to verify the insulin sensitivity calculated as following: 1/(log(insulin(mU/L)) + log(glucose (mg/dL))) [36]. QUICKI has been reported to be more reproducible than the common homeostasis model assessment (HOMA) of insulin resistance [37] and has an excellent linear correlation with glucose clamp estimates among various types of health conditions (healthy, obesity, insulin resistance, diabetes and hypertension) [38].

SNPs selection and genotyping

As described previously [29], SNPs were selected with the International HapMap Project SNP database (HapMap Data Rel 28 Phase II+III, August 10, on National Center for Biotechnology Information (NCBI) B36 assembly, dbSNP b126). Tag SNPs (tSNPs) were determined with the tagger procedure in HaploView software version 4.2 with minor allele frequency (MAF) of >0.05 and pairwise tagging $R^2 \geq 0.80$. Afterwards, as shown in Figure 8.1, linkage disequilibrium (LD) plot were generated with Haploview software version 4.2. Figure 8.1 also illustrates the high LD between the chosen tSNP rs4925115 and the well described rs2297508 located in exon 18c of *SREBF1* gene [13,16,17]. All tSNPs were genotyped within INAF laboratories with the TAQMAN methodology [39], as described previously [40]. Briefly, genotypes were determined using ABI Prism SDS version 2.0.5 (Applied Biosystem, Foster City, CA, USA). All SNPs were successfully genotyped.

Fatty acid composition of erythrocyte membranes and plasma phospholipids

As described previously [41], FA composition was measured in erythrocyte membranes by gas chromatographic analysis. Methods to extract plasma phospholipids have been described elsewhere [29]. Plasma lipids (PL) were extracted according to a modified Folch method [42]. Capillary gas chromatography was used to obtain FA profiles [43]. FA profiles both in erythrocyte membranes and plasma phospholipids were expressed as the relative percentage areas of total FAs.

Gene expression assessment

Blood samples (pre- and post- supplementation) were collected into an 8-ml Cell Preparation Tube (CPT) (Becton Dickinson, Oakville, On, Canada). Gene expression levels were measured in peripheral blood mononuclear cells (PBMCs), which are considered a valid proxy measure for many tissues including the liver [44,45]. Peripheral blood mononuclear cells (PBMCs) were separated by centrifugation (1500 X g, 20 min, at room temperature) and washed according to the manufacturer's instructions. Total RNA was extracted with RNeasy Plus Mini Kit (QIAGEN, Mississauga, On, Canada) according to manufacturer's protocol. Spectrophotometric quantification was realised with NanoDrop 2000C UV-Vis Spectrophotometer (Thermo Scientific) and cDNA was generated using 400 ng of total RNA with the High Capacity cDNA Reverse Transcription Kit (Life Technologies™). cDNA was mixed with TaqMan OpenArray® Real-Time PCR Master Mix (#4462164, Life Technologies™). The assays used were as follows: Hs01088691_m1 (*SREBF1*) and *GAPDH* Hs99999905_m1 as the housekeeping gene. All assays used the same fluorescent reporter probe (FAM dye labeled). All samples were run in triplicate on a QuantStudio™ 12K Flex Real-Time PCR (RT-PCR) System (Life Technologies™) using 48-well plates TaqMan® OpenArray® RT PCR Inventoried Format 18. The RT-PCR results were analysed with ExpressionSuite software v1.0.1 (Life Technologies™).

Replication cohort

Seven hundred (700) Caucasians aged between 18 and 55 years were recruited in the Quebec City metropolitan region. Recruitment occurred between 2004 and 2006 through

public advertisements (local newspapers and electronic messages) sent to university and hospital employees, as described previously [46]. A trained research assistant took anthropometric measures. A registered dietitian administered a validated food frequency questionnaire (FFQ) to assess dietary intakes [47]. *SREBF1* c.*619C>G (rs2297508) was genotyped using the TAQMAN methodology [39].

Statistical analyses

The Hardy-Weinberg equilibrium was tested with the ALLELE procedure of SAS version 9.3 using Fisher's exact test ($P<0.01$). When the genotype frequency for homozygous individuals of the minor allele was <5%, carriers (heterozygotes and homozygotes) of the minor allele were grouped.

Non-normally distributed variables were logarithmically transformed. Fasting insulin concentration values higher or lower than means ± 3 multiplied by standard deviation (SD) were considered as outliers (n=6), thus 201 participants were kept for the statistical analyses. Differences were assessed using analyses of variance (ANOVA) with the GLM procedure in SAS and the type 3 sum of squares for unbalanced study design. The fasting insulin response (delta) was calculated as followed: ((post-supplementation insulin concentrations minus pre-supplementation insulin concentrations)/pre-supplementation insulin concentrations*100). The same model was used to test the associations with fasting glucose concentrations and insulin sensitivity (QUICKI). Each model was adjusted for the effects of age, sex and BMI. To take into account the impact of multiple testing, the simpleM method described by Gao *et al*. [48] was utilised. Briefly, this method considers the impacts of LD between SNPs and has been demonstrated as efficient and accurate comparatively to permutation-based corrections [48]. First, the composite LD correlation matrix was derived from the data set. Then, eigenvalues were calculated using the SAS PRINCOMP procedure and the number of effective independent tests was inferred so that the corresponding eigenvalues explain 99.5% of the variation in SNP data or the variables (fasting glucose, insulin and QUICKI), as proposed by Gao *et al*. [48]. According to Gao's method, the number of effective independent tests for the three SNPs was 2 and for the

three traits (fasting glucose, insulin and QUICKI) was 2. The final step applies the Bonferroni correction formula to calculate the adjusted point-wise significance level, which was defined as α_G=0.05/(2X2) (effective independent tests). Thus, p-values <0.0125 were considered significant (p=0.05/(4)). All statistical analyses were performed using SAS statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

All tSNPs were in Hardy-Weinberg equilibrium. Three tSNPs covered 100% of the known genetic variability within *SREBF1* gene [29]. As presented in Figure 8.1, two of the selected tSNPs were in moderate to high LD with the exonic SNP rs2297508 (rs12953299 (R^2=0.62) and rs4925115 (R^2=0.95)).

Baseline characteristics of the study participants are shown in Table 8.1. Before the fish oil supplementation period, no differences in fasting insulin concentrations according to genotypes were observed for the three tSNPs (rs12953299, rs4925118 and rs4925115) (p=0.29, p=0.20 and p=0.70, respectively). Also there were no differences in either fasting glucose concentrations according to genotypes of rs12953299, rs4925118 and rs4925115 (p=0.16, p=0.64 and p=0.22, respectively) or for insulin sensitivity (QUICKI) values (p=0.20, p=0.18 and p=0.76, respectively).

Globally, the fish oil supplementation did not modify fasting insulin levels (pre-supplementation insulin concentrations: 77.68±29.27pmol/L; post-supplementation insulin concentrations: 79.02±30.00pmol/L) (p=0.52)) but did slightly increase fasting glucose levels from 4.95±0.44 mmol/L to 5.04±0.49 mmol/L after the fish oil supplementation period (p=0.0002), as previously reported [49]. An important inter-individual variability has been observed in the response of fasting insulin concentrations, ranging from a decrease of -53.00% to an increase of +135.19%. Briefly, 110 individuals decreased (relative change≤0%) and 91 increased (relative change>0%) their fasting insulin concentrations. Globally, the mean change in insulin concentrations was 5.14%±29.97%. The insulin sensitivity (QUICKI) was not modified by the fish oil supplementation (p=0.19).

No differences according to genotypes of *SREBF1* gene were observed in the response of fatty acid n-3 PUFA (EPA, DHA and total n-3 PUFA) phospholipid content to the fish oil supplementation, except for EPA concentrations in erythrocyte membranes according to rs4925118 genotypes (p=0.02) (T/T+C/T=147.66±79.91% (n=4); C/C=236.48±73.00%

(n=23)). A trend (p=0.07) was also observed for pre-supplementation EPA content in erythrocyte membranes according to rs4925118 genotypes (T/T+C/T=0.88±0.33% (n=4); C/C=0.68±0.22% (n=24)).

SREBF1 gene expression levels were not modified by the fish oil supplementation (p=0.85). As shown in Table 8.2, no differences were observed in the response of *SREBF1* gene expression levels to fish oil supplementation between genotypes of rs12953299, rs4925118 and rs4925115 (p=0.59, p=0.47 and p=0.25, respectively).

As shown in Figure 8.2, the response of fasting insulin concentrations ((post-supplementation insulin concentrations minus pre-supplementation insulin concentrations)/pre-supplementation insulin concentrations*100) following the fish oil supplementation was different according to genotypes of the three tSNPs (rs12953299, rs4925118 and rs4925115) within *SREBF1* gene, adjusted for age, sex and BMI (p=0.01, p=0.005 and p=0.004, respectively). For rs12953299, A/A homozygotes increased by 15.27±32.03% their fasting insulin concentrations (from 70.15±21.49pmol/L to 79.76±20.31pmol/L) following the fish oil supplementation which was different from the response of insulin concentrations for A/G heterozygotes with 1.18±30.06% (from 81.01±30.47pmol/L to 79.12±29.99pmol/L) and G/G homozygotes with 3.86±26.39% (from 77.93±31.89pmol/L to 78.24±30.31pmol/L). For rs4925118, T allele carriers (T/T+C/T) increased by 14.13±36.18% their fasting insulin concentrations (from 76.12±27.39pmol/L to 84.13±32.10pmol/L) following the fish oil supplementation which was different from the response of insulin concentrations for C/C homozygotes with 0.64±25.29% (from 78.46±30.23pmol/L to 76.47±28.68pmol/L). For rs4925115, A/A homozygotes increased by 19.48±34.01% their fasting insulin concentrations (from 73.52±23.00pmol/L to 86.39±32.34pmol/L) following the fish oil supplementation which was different from the response of insulin concentrations for A/G heterozygotes with 3.36±29.25% (from 76.76±28.87pmol/L to 76.54±27.89pmol/L) and G/G homozygotes with 0.60±27.00% (from 81.40±32.67pmol/L to 79.30±31.93pmol/L).

The response of fasting glucose concentrations was not different according to genotypes of the tSNPs (rs12953299, rs4925118 and rs4925115). The insulin sensitivity (QUICKI) was associated with the tSNPs rs12953299 and rs4925115 (p=0.009 and p=0.01, respectively) but not with rs4925118 (p=0.16). For rs12953299, the insulin sensitivity (QUICKI) for A/A homozygotes decreased by -1.96±4.11% (from 0.342±0.017 to 0.335±0.019) following the fish oil supplementation which was different from the response of insulin sensitivity (QUICKI) for A/G heterozygotes with 0.38±4.80% (from 0.336±0.020 to 0.337±0.021) and G/G homozygotes with 3.86±26.39% (from 0.338±0.018 to 0.337±0.018). For rs4925115, the insulin sensitivity (QUICKI) for A/A homozygotes decreased by -2.30±4.19% (from 0.340±0.017 to 0.332±0.019) following the fish oil supplementation which was different from the response of insulin sensitivity (QUICKI) for A/G heterozygotes with -0.14±4.51% (from 0.338±0.019 to 0.337±0.020) and G/G homozygotes with 0.36±4.50% (from 0.336±0.019 to 0.337±0.020). The potential effects of total dietary n-3 PUFA intakes in the participants' habitual diets were included as a confounding variable in these models and did not modify the associations observed (data not shown).

Replication cohort

Two significant gene-diet interaction effects on QUICKI (insulin sensitivity index) were observed, the first one between rs2297508 and dietary intakes of omega-3 (n-3) PUFA (in grams) (p=0.03) and the second between rs2297508 and dietary PUFA intakes (in grams) (p=0.03). Both models were adjusted for the effects of age, sex, BMI and energy intakes. No gene-diet interaction effects were observed on fasting insulin or glucose concentrations. Table 8.3 presents the results for these two gene-diet interaction effects on QUICKI.

Discussion

Participants of this cohort were overweight but generally considered as healthy according to lipid concentration values [50]. Fasting glucose concentrations were within normal values [51]. The QUICKI index indicated a probable borderline insulin resistance state among these participants [36,38,52]. An important inter-individual variability in the response of fasting insulin concentrations was observed. This wide inter-individual variability in HOMA-insulin sensitivity (IS) response in this cohort has been previously described [53]. In this study, effects of tSNPs within *SREBF1* gene on the fasting insulin and insulin sensitivity responses were observed after the fish oil supplementation.

SREBF1 gene is an important transcription factor regulating many genes involved in the lipid metabolism and also in insulin induced glucose metabolism [1]. Moreover, the expression of *SREBF1* gene is significantly affected by dietary intakes, including fish oil [3,5-9]. Therefore, *SREBF1* gene is an interesting candidate for the study of inter-individual variability in the response of fasting insulin concentrations to a fish oil supplementation. Whether these impacts are mediated through the effects of SREBP-1c within hepatocytes and/or pancreatic cells is unknown. In the liver, increased SREBP-1c concentrations have been shown to repress the transcription of *insulin receptor substrate 2* (*IRS2*) gene which led to a detrimental impact on insulin sensitivity, a fatty liver and a production of VLDL enriched in triglycerides [54]. IRS2 mediates insulin signaling in the liver [54]. Insulin in the liver activates glycogen synthesis, inhibits hepatic glucose output and promotes lipogenesis [54]. The induction of SREBP-1c in mice resulted in impaired secretion and glucose intolerance, as reviewed by Shimano *et al*. [3]. SREBP-1c may also affect insulin secretion of pancreatic β-cell through a mechanism involving uncoupling protein-2 (UCP2) [55]. A sterol regulatory element (SRE) has been discovered in the promoter region of *UCP2* gene [55]. An increase in *UCP2* gene expression is associated with a lower efficacy of glucose-induced insulin secretion [56]. In the present study no differences in *SREBF1* gene expression were observed following the fish oil supplementation. Studies examining the impacts of PUFA, fish oil or EPA on *SREBF1* gene regulation have been conducted among mice or *in vitro* with human cells [5-11]. It is possible that the dose used for the

supplementation in this study was insufficient to observe an effect on *SREBF1* gene expression. However, the activity of lipogenic target genes of SREBP-1c transcription factor is not only regulated by *SREBF1* mRNA abundance. For example, Tanaka *et al*. [10] did not observe reduced *SREBF1* mRNA levels but rather an inhibition of the maturation of SREBP-1c. Thus, the fish oil supplementation in the present study may have had effects on the insulin response through posttranslational modifications of SREBP-1c and its impact on subsequent target genes. However, we cannot rule out the possibility that significant differences may have been observed if expression levels were directly measured in hepatocytes [57].

The overall effects of fish oil intakes on insulin resistance, glycemic control and the risk of type 2 diabetes appears to be negligible, as recently reviewed by Wu *et al*. [58]. However, the authors observed a large heterogeneity. Thus, it is possible that for some individuals the impacts of fish oil intake on the risk of type 2 diabetes or other related biologic parameters may be beneficial and for some other individuals detrimental. Quite a few studies have observed a slight increase in fasting glucose concentrations after fish oil intake [59]. It has been observed that the reduction in plasma triglyceride concentrations induced by n-3 PUFA intake may be partly induced by the increased use of glycerol for gluconeogenesis which may explain increases in fasting glucose concentrations [60,61]. The increase in fasting glucose concentrations was also observed in this cohort [49].

In the present study, homozygotes for minor alleles of the tSNPs rs12953299 and rs4925115 were associated with an increase in fasting insulin concentrations and a decrease in insulin sensitivity assessed by QUICKI after a fish oil supplementation compared to the other genotypes. For the tSNP rs4925118 only a difference in the fasting insulin response was observed, carriers of the T allele increased their fasting insulin concentrations after a fish oil supplementation compared to C/C homozygotes. Thus, for these genotypes the impact of fish oil on insulin sensitivity may be detrimental. For the other genotypes, the fish oil supplementation had a minor impact which may be less likely to increase the risk of type 2 diabetes. The genetic variability within transcription factors such as *SREBF1* gene, which are affected by fish oil intake may be the key to understanding the variability

observed in the response of fasting insulin concentrations and insulin sensitivity [62]. SNPs within *SREBF1* gene have been frequently associated with type 2 diabetes or insulin resistance. A meta-analysis of four European genome screens found the strongest linkage with type 2 diabetes on chromosome 17p11.2-q22 where is located *SREBF1* gene [12]. One SNP (rs2297508) within *SREBF1* gene has been reported across a few populations to be associated with the risk of type 2 diabetes [13,14,16,17]. Felder *et al*. [17] found that the C/G and the G/G genotypes of rs2297508 had a ~1.4-fold higher risk of type 2 diabetes. In a French cohort, rs2297508 was also associated with obesity and type 2 diabetes independently of the obesity status [13]. Moreover, the SNP rs2297508 was related to sex-specific differences in the response of lipid and insulin concentrations as well as in HOMA-IR to a diet high in carbohydrates [63]. In their study, Zhang et al. [63] observed that the C allele of rs2297508 was associated with more favorable impacts on plasma triglyceride, fasting insulin and HOMA-IR than the G allele. Our results from the replication cohort also indicate that homozygotes C/C of rs2297508 may have a beneficial impact on insulin sensitivity following fish oil supplementation compared to the other genotypes. A large-scale gene-centric meta-analysis identified *SREBF1* gene as a type 2 diabetes loci among Europeans, with rs4925115 being the most significantly associated SNP [15]. The three tSNPs (rs12953299, rs4925115 and rs4925118) covered 100% of the known genetic variability within *SREBF1* gene. However, two of these tSNPs were in moderate LD (rs12953299 and rs4925115, R^2=0.68) and were also in moderate to high LD with the most studied *SREBF1* gene SNP rs2297508 (R^2=0.62 and R^2=0.95, respectively). Since SNPs within *SREBF1* gene including the promoter region, are in moderate to high LD, we cannot rule out the possibility that the tSNPs examined herein may also be in LD with SNPs within *SREBF1* gene promoter region which could affect its expression [64]. In the replication cohort, the SNP rs2297508 from *SREBF1* gene interacted with both dietary n-3 PUFA and PUFA intakes to affect insulin sensitivity (assessed by QUICKI). This SNP is located in exon 18c and is synonymous (Gly952Gly). Thus, these results may indicate that the genetic variability within *SREBF1* gene has an impact on the response of insulin sensitivity to n-3 PUFA and/or PUFA intakes.

Conclusion

To our knowledge this study was the first to examine associations between SNPs within *SREBF1* gene and the response of fasting insulin and insulin sensitivity to a fish oil supplementation. In this study, the genetic variability within *SREBF1* gene was associated with differences in the response of insulin and insulin sensitivity to a fish oil supplementation. *SREBF1* gene may be an important candidate to study in order to understand the discrepancies observed in the impacts of fish oil on insulin resistance. Clinical trials taking into account the genetic variability within *SREBF1* gene and observing the impact of fish oil supplementation on insulin and insulin sensitivity are warranted. Moreover, the identification of individuals with a beneficial or adverse response to fish oil is important in order to appropriately recommend its supplementation.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

IR, SL, PC, LP and MCV designed research; ABM conducted research with the research professionals; IR, SL, PC, LP and MCV provided essential reagents or provided essential materials; ABM analyzed data and performed statistical analysis; ABM wrote paper; ABM, IR, SL, PC, LP and MCV had primary responsibility for final content; All authors read and approved the final manuscript.

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References

1. Eberle D, Hegarty B, Bossard P, Ferre P, Foufelle F: SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 2004, 86: 839-848.
2. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS: Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J Clin Invest* 1997, 99: 838-845.
3. Shimano H, Amemiya-Kudo M, Takahashi A, Kato T, Ishikawa M, Yamada N: Sterol regulatory element-binding protein-1c and pancreatic beta-cell dysfunction. *Diabetes Obes Metab* 2007, 9 Suppl 2: 133-139.
4. Takahashi A, Motomura K, Kato T, Yoshikawa T, Nakagawa Y, Yahagi N, Sone H, Suzuki H, Toyoshima H, Yamada N, Shimano H: Transgenic mice overexpressing nuclear SREBP-1c in pancreatic beta-cells. *Diabetes* 2005, 54: 492-499.
5. Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Tomita S, Sekiya M, Hasty A, Nakagawa Y, Sone H, Toyoshima H, Ishibashi S, Osuga J, Yamada N: Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. *Diabetes* 2004, 53: 560-569.
6. Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, Yang W, Pei L, Uldry M, Tontonoz P, Newgard CB, Spiegelman BM: Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell* 2005, 120: 261-273.
7. Sekiya M, Yahagi N, Matsuzaka T, Najima Y, Nakakuki M, Nagai R, Ishibashi S, Osuga J, Yamada N, Shimano H: Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. *Hepatology* 2003, 38: 1529-1539.
8. Hannah VC, Ou J, Luong A, Goldstein JL, Brown MS: Unsaturated fatty acids down-regulate srebp isoforms 1a and 1c by two mechanisms in HEK-293 cells. *J Biol Chem* 2001, 276: 4365-4372.
9. Wakutsu M, Tsunoda N, Shiba S, Muraki E, Kasono K: Peroxisome proliferator-activated receptors (PPARs)-independent functions of fish oil on glucose and lipid metabolism in diet-induced obese mice. *Lipids Health Dis* 2010, 9: 101.
10. Tanaka N, Zhang X, Sugiyama E, Kono H, Horiuchi A, Nakajima T, Kanbe H, Tanaka E, Gonzalez FJ, Aoyama T: Eicosapentaenoic acid improves hepatic steatosis independent of PPARalpha activation through inhibition of SREBP-1 maturation in mice. *Biochem Pharmacol* 2010, 80: 1601-1612.
11. Kato T, Shimano H, Yamamoto T, Ishikawa M, Kumadaki S, Matsuzaka T, Nakagawa Y, Yahagi N, Nakakuki M, Hasty AH, Takeuchi Y, Kobayashi K, Takahashi A, Yatoh S, Suzuki H, Sone H, Yamada N: Palmitate impairs and eicosapentaenoate restores insulin secretion through regulation of SREBP-1c in pancreatic islets. *Diabetes* 2008, 57: 2382-2392.

12. Demenais F, Kanninen T, Lindgren CM, Wiltshire S, Gaget S, Dandrieux C, Almgren P, Sjögren M, Hattersley A, Dina C, Tuomi T, McCarthy MI, Froguel P, Groop LC: A meta-analysis of four European genome screens (GIFT Consortium) shows evidence for a novel region on chromosome 17p11.2-q22 linked to type 2 diabetes. *Hum Mol Genet* 2003, 12: 1865-1873.

13. Eberle D, Clement K, Meyre D, Sahbatou M, Vaxillaire M, Le Gall A, Ferré P, Basdevant A, Froquel P, Foufelle F: SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. *Diabetes* 2004, 53: 2153-2157.

14. Laudes M, Barroso I, Luan J, Soos MA, Yeo G, Meirhaeghe A, Logie L, Vidal-Puig A, Schafer AJ, Wareham NJ, O'Rahilly S: Genetic variants in human sterol regulatory element binding protein-1c in syndromes of severe insulin resistance and type 2 diabetes. *Diabetes* 2004, 53: 842-846.

15. Saxena R, Elbers CC, Guo Y, Peter I, Gaunt TR, Mega JL, Lanktree MB, Tare A, Castillo BA, Li YR, Johnson T, Bruinenberg M, Gilbert-Diamond D, Rajagopalan R, Voight BF, Balasubramanyam A, Barnard J, Bauer F, Baumert J, Bhangale T, Böhm BO, Braund PS, Burton PR, Chandrupatla HR, Clarke R, Cooper-DeHoff RM, Crook ED, Davey-Smith G, Day IN, de Boer A *et al.*: Large-scale gene-centric meta-analysis across 39 studies identifies type 2 diabetes loci. *Am J Hum Genet* 2012, 90: 410-425.

16. Grarup N, Stender-Petersen KL, Andersson EA, Jorgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Schmitz O, Hansen T, Pedersen O: Association of variants in the sterol regulatory element-binding factor 1 (SREBF1) gene with type 2 diabetes, glycemia, and insulin resistance: a study of 15,734 Danish subjects. *Diabetes* 2008, 57: 1136-1142.

17. Felder TK, Oberkofler H, Weitgasser R, Mackevics V, Krempler F, Paulweber B, Patsch W: The SREBF-1 locus is associated with type 2 diabetes and plasma adiponectin levels in a middle-aged Austrian population. *Int J Obes (Lond)* 2007, 31: 1099-1103.

18. Liu JX, Liu J, Li PQ, Xie XD, Guo Q, Tian LM, Ma XQ, Zhang JP, Liu J, Gao JY: Association of sterol regulatory element-binding protein-1c gene polymorphism with type 2 diabetes mellitus, insulin resistance and blood lipid levels in Chinese population. *Diabetes Res Clin Pract* 2008, 82: 42-47.

19. Bremer AA, Stanhope KL, Graham JL, Cummings BP, Ampah SB, Saville BR, Havel PJ: Fish Oil Supplementation Ameliorates Fructose-Induced Hypertriglyceridemia and Insulin Resistance in Adult Male Rhesus Macaques. *J Nutr* 2013.

20. Lanza IR, Blachnio-Zabielska A, Johnson ML, Schimke JM, Jakaitis DR, Lebrasseur NK, Jensen MD, Sreekumaran Nair K, Zabielski P: Influence of fish oil on skeletal muscle mitochondrial energetics and lipid metabolites during high-fat diet. *Am J Physiol Endocrinol Metab* 2013, 304: E1391-E1403.

21. Gonzalez-Periz A, Horrillo R, Ferre N, Gronert K, Dong B, Moran-Salvador E, Titos E, Martinez-Clemente M, Lopez-Parra M, Arroyo V, Claria J: Obesity-induced

insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. *FASEB J* 2009, 23: 1946-1957.

22. Ogawa S, Abe T, Nako K, Okamura M, Senda M, Sakamoto T, Ito S, DIMS Study Group: Eicosapentaenoic acid improves glycemic control in elderly bedridden patients with type 2 diabetes. *Tohoku J Exp Med* 2013, 231: 63-74.

23. Lopez-Huertas E: The effect of EPA and DHA on metabolic syndrome patients: a systematic review of randomised controlled trials. *Br J Nutr* 2012, 107 Suppl 2: S185-S194.

24. Tierney AC, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WH, Paniaqua JA, Golabek-Leszczynska I, Defoort C, Williams CM, Karslström B, Vessby B, Dembinska-Kiec A, Lopez-Miranda J, Blaak EE, Drevon CA, Gibney MJ, Lovegrove JA, Roche HM: Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European randomized dietary intervention study. *Int J Obes (Lond)* 2011, 35: 800-809.

25. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV: The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N Engl J Med* 1993, 328: 238-244.

26. Jafari T, Fallah AA, Azadbakht L: Role of dietary n-3 polyunsaturated fatty acids in type 2 diabetes: a review of epidemiological and clinical studies. *Maturitas* 2013, 74: 303-308.

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

28. Woodman RJ, Mori TA, Burke V, Puddey IB, Watts GF, Beilin LJ: Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. *Am J Clin Nutr* 2002, 76: 1007-1015.

29. Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC: Polymorphisms, de novo lipogenesis, and plasma triglyceride response following fish oil supplementation. *J Lipid Res* 2013, 54: 2866-2873.

30. McNamara JR, Schaefer EJ: Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin Chim Acta* 1987, 166: 1-8.

31. Burstein M, Samaille J: [On a rapid determination of the cholesterol bound to the serum alpha- and beta-lipoproteins]. *Clin Chim Acta* 1960, 5: 609.

32. Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L, Breckenridge C, Chow A, Kuba K, Weidman S, Arnett H, Wood P, Shlagenhaft A: Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. *Clin Chem* 1978, 24: 853-856.

33. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972, 18: 499-502.

34. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 1971, 33: 732-738.

35. Richterich R, Dauwalder H: [Determination of plasma glucose by hexokinase-glucose-6-phosphate dehydrogenase method]. *Schweiz Med Wochenschr* 1971, 101: 615-618.

36. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ: Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000, 85: 2402-2410.

37. Antuna-Puente B, Faraj M, Karelis AD, Garrel D, Prud'homme D, Rabasa-Lhoret R, Bastard JP: HOMA or QUICKI: is it useful to test the reproducibility of formulas? *Diabetes Metab* 2008, 34: 294-296.

38. Muniyappa R, Lee S, Chen H, Quon MJ: Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab* 2008, 294: E15-E26.

39. Livak KJ: Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal* 1999, 14: 143-149.

40. 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: 1026-1041.

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

42. Shaikh NA, Downar E: Time course of changes in porcine myocardial phospholipid levels during ischemia. A reassessment of the lysolipid hypothesis. *Circ Res* 1981, 49: 316-325.

43. Kroger E, Verreault R, Carmichael PH, Lindsay J, Julien P, Dewailly E, Ayotte P, Laurin D: Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. *Am J Clin Nutr* 2009, 90: 184-192.

44. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA: The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J Lab Clin Med* 2006, 147: 126-132.

45. Rudkowska I, Raymond C, Ponton A, Jacques H, Lavigne C, Holub BJ, Marette A, Vohl MC: Validation of the use of peripheral blood mononuclear cells as surrogate model for skeletal muscle tissue in nutrigenomic studies. *OMICS* 2011, 15: 1-7.

46. Paradis AM, Godin G, Perusse L, Vohl MC: Associations between dietary patterns and obesity phenotypes. *Int J Obes (Lond)* 2009, 33: 1419-1426.

47. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S: Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. *Nutr J* 2004, 3: 13.

48. Gao X, Starmer J, Martin ER: A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008, 32: 361-369.

49. Thifault E, Cormier H, Bouchard-Mercier A, Rudkowska I, Paradis AM, Garneau V, Ouellette C, Lemieux S, Couture P, Vohl MC: Effects of age, sex, body mass index and APOE genotype on cardiovascular biomarker response to an n-3 polyunsaturated fatty acid supplementation. *J Nutrigenet Nutrigenomics* 2013, 6: 73-82.

50. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002, 106: 3143-3421.

51. Canadian Diabetes Association: Screening for and diagnosing diabetes [http://guidelines.diabetes.ca/ScreeningAndDiagnosis/Screening].

52. Hrebicek J, Janout V, Malincikova J, Horakova D, Cizek L: Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention. *J Clin Endocrinol Metab* 2002, 87: 144-147.

53. 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; 3:485-498

54. Ide T, Shimano H, Yahagi N, Matsuzaka T, Nakakuki M, Yamamoto T, Nakagawa Y, Takahashi A, Suzuki H, Sone H, Toyoshima H, Fukamizu A, Yamada N: SREBPs suppress IRS-2-mediated insulin signalling in the liver. *Nat Cell Biol* 2004, 6: 351-357.

55. Medvedev AV, Robidoux J, Bai X, Cao W, Floering LM, Daniel KW, Collins S: Regulation of the uncoupling protein-2 gene in INS-1 beta-cells by oleic acid. *J Biol Chem* 2002, 277: 42639-42644.

56. Chan CB, MacDonald PE, Saleh MC, Johns DC, Marban E, Wheeler MB: Overexpression of uncoupling protein 2 inhibits glucose-stimulated insulin secretion from rat islets. *Diabetes* 1999, 48: 1482-1486.

57. Price PT, Nelson CM, Clarke SD: Omega-3 polyunsaturated fatty acid regulation of gene expression. *Curr Opin Lipidol* 2000, 11: 3-7.

58. Wu JH, Micha R, Imamura F, Pan A, Biggs ML, Ajaz O, Djousse L, Hu FB, Mozaffarian D: Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. *Br J Nutr* 2012, 107 Suppl 2: S214-S227.

59. Friedberg CE, Janssen MJ, Heine RJ, Grobbee DE: Fish oil and glycemic control in diabetes. A meta-analysis. *Diabetes Care* 1998, 21: 494-500.

60. Puhakainen I, Ahola I, Yki-Jarvinen H: Dietary supplementation with n-3 fatty acids increases gluconeogenesis from glycerol but not hepatic glucose production in patients with non-insulin-dependent diabetes mellitus. *Am J Clin Nutr* 1995, 61: 121-126.

61. Kamolrat T, Gray SR, Thivierge MC: Fish oil positively regulates anabolic signalling alongside an increase in whole-body gluconeogenesis in ageing skeletal muscle. *Eur J Nutr* 2013, 52: 647-657.

62. Berger A, Mutch DM, German JB, Roberts MA: Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression. *Lipids Health Dis* 2002, 1: 2.

63. Zhang Z, Gong RR, Du J, Xiao LY, Duan W, Zhou XD, Fang DZ: Associations of the SREBP-1c gene polymorphism with gender-specific changes in serum lipids induced by a high-carbohydrate diet in healthy Chinese youth. *Appl Physiol Nutr Metab* 2011, 36: 226-232.

64. Nagata R, Nishio Y, Sekine O, Nagai Y, Maeno Y, Ugi S, Maegawa H, Kashiwagi A: Single nucleotide polymorphism (-468 Gly to A) at the promoter region of SREBP-1c associates with genetic defect of fructose-induced hepatic lipogenesis [corrected]. *J Biol Chem* 2004, 279: 29031-29042.

Table 8.1 Baseline characteristics (n=201).

Variables	Means ± SD	Pre-supplementation	Post-supplementation	P-value[1]
Age (years)	30.92±8.71	-	-	
Sex (men/women)	92/109	-	-	
BMI (Kg/m^2)	27.64±3.50	27.61±3.52	27.68±3.59	0.03
Waist circumference (cm)	Men: 94.5±10.5 Women: 91.5±10.2	Men: 94.4±10.3 Women: 91.5±9.9	Men: 94.8±10.3 Women: 91.4±10.2	0.10 0.65
Fasting glucose (mmol/L)	4.94±0.53	4.95±0.44	5.04±0.49	0.0002
Fasting insulin (pmol/L)	78.97±27.49 (n=199)	77.68±29.27	79.02±30.00	0.52
QUICKI	0.367±0.018 (n=199)	0.338±0.019	0.336±0.020	0.19
HOMA-IR	2.51±1.00 (n=199)	2.48±1.01	2.57±1.06	0.12
Total-C (mmol/L)	4.80±1.01	4.74±0.90	4.71±0.95	0.45
LDL-C (mmol/L)	2.78±0.87	2.75±0.81	2.77±0.86	0.40
HDL-C (mmol/L)	1.46±0.39	1.44±0.36	1.48±0.41	0.007
Triglycerides (mmol/L)	1.21±0.60	1.19±0.60	1.00±0.46	<0.0001

Means ± SD

[1]P-values were determined using a paired t-test and compared post-supplementation to pre-supplementation values.

Table 8.2 Gene expression response according to genotypes of SNPs within *SREBF1* gene.

SNPs	Genotype	Fold change[1]	P-value[2]
rs12953299	A/A (n=45)	1.04±0.36	0.59
	A/G (n=98)	1.05±0.27	
	G/G (n=55)	1.00±0.22	
rs4925118	T/T+C/T (n=66)	1.06±0.26	0.47
	C/C (n=132)	1.02±0.29	
rs4925115	A/A (n=32)	1.00±0.25	0.25
	A/G (n=103)	1.07±0.32	
	G/G (n=63)	1.00±0.23	

Means ± SD

[1]The fold change represents post-supplementation relative gene expression levels compared to pre-supplementation relative gene expression levels.

Fold change = $2^{-\Delta\Delta CT} = 2^{-(\text{post-supplementation } \Delta CT - \text{pre-supplementation } \Delta CT)}$.

[2]P-values were calculated with an ANOVA adjusted for age, sex and BMI.

Table 8.3 Gene-diet interaction effects on QUICKI between rs2297508 and PUFA intakes (total and n-3 PUFA).

	Dietary n-3 PUFA intake (in grams)[1]				**Dietary PUFA intake (in grams)[1]**			
Genotype	**β (Interaction term)**	**P Genotype**	**P Dietary fat intake**	**P Interaction effect**	**β (Interaction term)**	**P Genotype**	**P Dietary fat intake**	**P Interaction effect**
G/G (n=125)	-0.0676±0.0252	0.04	0.80	0.03	-0.0548±0.0205	0.03	0.22	0.03
C/G (n=297)	-0.0233±0.0194				-0.0204±0.0159			
C/C (n=242)	0				0			

Means ± SD

[1]ANOVA adjusted for age, sex, BMI and energy intakes.

Figure 8.1 Linkage disequilibrium (LD) plot of tSNPs within *SREBF1* gene.

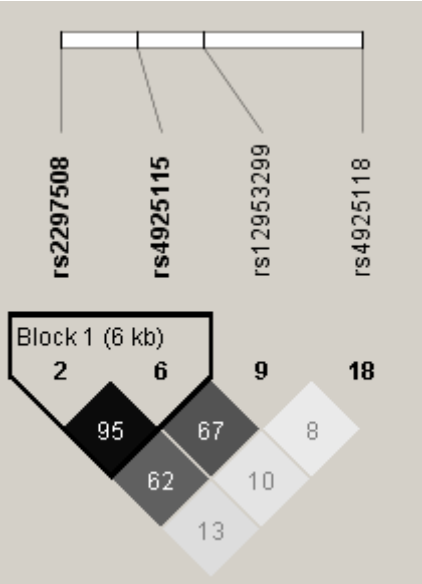
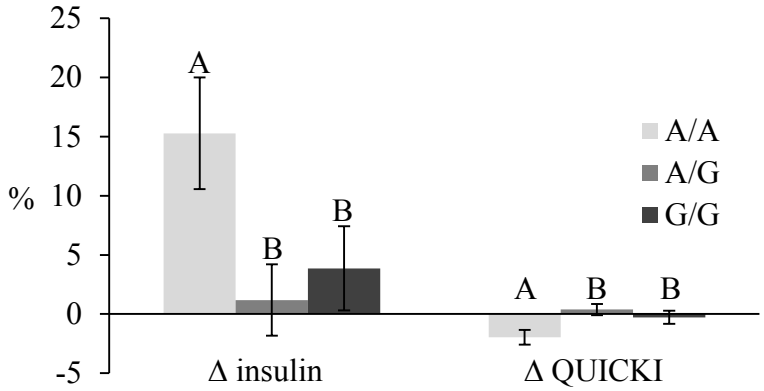
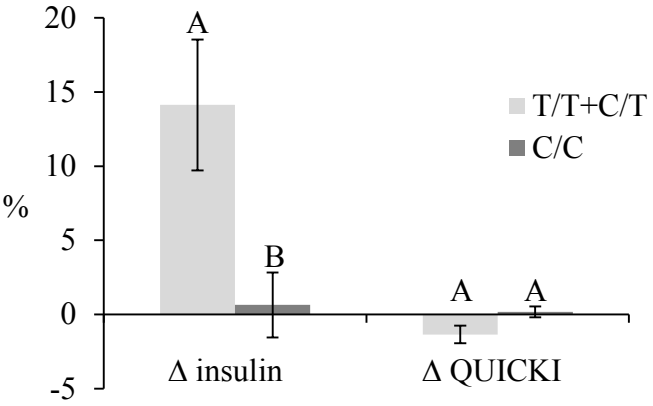


Figure legend. LD plots were generated by HaploView software version 4.2 using R^2 LD values.

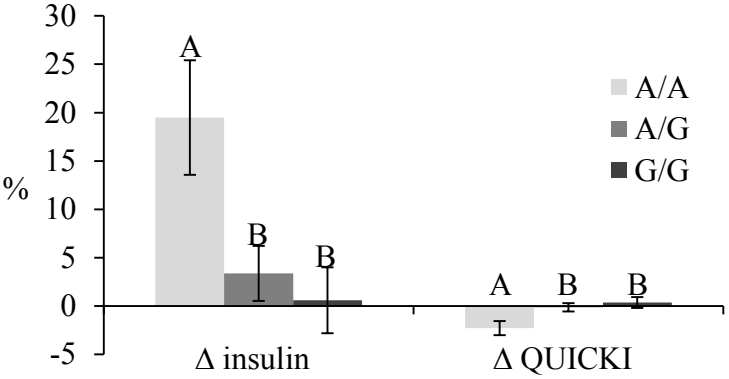
Figure 8.2 The relative response in fasting insulin concentrations and QUICKI index (insulin sensitivity) according to genotype.



a)



b)



c)

Figure legend. a) rs12953299 (A/A: n=46, A/G: n=100, G/G: n=55); P-value for delta insulin model: p=0.01; P-value for delta QUICKI model: p=0.009 b) rs4925118 (T/T+C/T: n=67, C/C: n=134); P-value for delta insulin model: p=0.005; P-value for delta QUICKI model: p=0.16 c) rs4925115 (A/A: n=33, A/G: n=105, G/G: n=63); P-value for delta insulin model: p=0.004; P-value

for delta QUICKI model: p=0.01. Delta values (relative change) were calculated as ((post-supplementation values minus pre-supplementation values)/pre-supplementation values*100). All differences were assessed with ANOVA adjusted for age, sex and BMI.

Chapitre 9

Polymorphismes, effets d'interactions gène-diète, concentrations de triglycérides plasmatiques et d'acides gras dans les phospholipides plasmatiques : une étude transversale

Annie Bouchard-Mercier, Frédéric Guénard, Louis Pérusse et Marie-Claude Vohl

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Polymorphisms, gene-diet interaction effects on plasma triglyceride and plasma phospholipid fatty acid concentrations: a cross-sectional study

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Trial registration: NCT01343342. http://clinicaltrials.gov/show/NCT01343342

Résumé

Introduction : Une importante varabilité interindividuelle dans la réponse des triglycérides plasmatiques (TG) à une supplémentation en huile de poisson a été observée. Une étude d'association sur le génome entier (GWAS) comparant les répondeurs et les non-répondeurs en fonction de la réponse des TG à une supplémentation en huile de poisson a révélé des SNPs potentiellement reliés à la réponse. **Objectif** : L'objectif de cette étude est d'examiner les associations de SNPs révélées dans un GWAS précédemment réalisé, avec les concentrations de TG et les concentrations d'acides gras dans les phospholipides du plasma dans une cohorte d'individus en santé. **Méthodes** : Sept cent (700) Caucasiens âgés entre 18 et 55 ans ont été recrutés dans la région de Québec entre 2004 et 2006 et les concentrations de lipides du plasma ont été mesurées. Les apports alimentaires ont été mesurés avec un questionnaire de fréquence alimentaire. Les SNPs identifiés précédemment dans une étude GWAS ont été génotypés selon la méthodologie TAQMAN. **Résultats** : Les participants étaient en santé, toutefois, l'IMC moyen était de 27,74±5,68Kg/m^2. Le SNP rs752088 du gène *NELL1* était associé aux concentrations de TG indépendamment de l'effet de l'âge, du sexe et de l'IMC (p=0,01). Les hétérozygotes A/G avaient des concentrations de TG plus élevées que les homozygotes A/A (1,30±0,83mmol/L comparé à 1,16±0,77mmol/L). Quelques SNPs étaient associés avec les concentrations d'acides gras dans les phospholipides du plasma. Un SNP près du gène *JADE1* (rs1216365 (p=0,04)) et des SNPs dans le gène *IQCJ-SCHIP1* (rs2621308 (p=0,04) et rs61332355 (p=0,005)) étaient associés avec les concentrations d'acides gras oméga-3 totales des phospholipides du plasma après ajustements pour les effets de l'âge, du sexe et de l'IMC. Le SNP rs61332355 (gène *IQCJ-SCHIP1*) était associé avec les concentrations d'acide docosahexaénoïque (ADH) des phospholipides du plasma (p=0,001) et le SNP rs6463808 (gène *NXPH1*) avec les concentrations d'acide eicosapentaénoïque (AEP) des phospholipides du plasma (p=0,03). **Conclusion** : La variabilité génétique présente dans des gènes tels que *NELL1*, *NXPH1*, *SLIT2*, *JADE1* et/ou *IQCJ-SCHIP1* modulerait les concentrations de TG et/ou les concentrations d'acides gras dans les phospholipides du plasma.

Abstract

Background: A large inter-individual variability in the plasma triglyceride (TG) response to fish oil supplementation has been reported. A genome-wide association study (GWAS) comparing responders to non-responders according to plasma TG changes to a fish oil supplementation revealed potential SNPs associated with the response. **Objective:** The objective of this study is to test the association of SNPs revealed in this previous GWAS study with plasma TG and phospholipid fatty acid concentrations in a cross-sectional cohort of healthy individuals. **Methods:** Seven hundred (700) Caucasians aged between 18 and 55 years were recruited in the Quebec City metropolitan area between 2004 and 2006 and plasma lipid concentrations measured. Food intakes were measured using a food frequency questionnaire. SNPs identified in a previous GWAS study were genotyped with the TAQMAN methodology. **Results:** Participants were healthy; however, the mean BMI was of 27.74±5.68Kg/m^2. The SNP rs752088 within *NELL1* gene was associated with plasma TG concentrations adjusted for the effects of age, sex and BMI (p=0.01). A/G heterozygotes had higher plasma TG concentrations than A/A homozygotes (1.30±0.83mmol/L compared to 1.16±0.77mmol/L). A few SNPs were associated with plasma phospholipid fatty acid content. A SNP near *JADE1* gene (rs1216365 (p=0.04)) and SNPs within *IQCJ-SCHIP1* gene (rs2621308 (p=0.04) and rs61332355 (p=0.005)) were associated with total omega-3 plasma phospholipid fatty acid content after adjustments for the effects of age, sex and BMI. The SNP rs61332355 (*IQCJ-SCHIP1* gene) was associated with docosahexaenoic acid (DHA) plasma phospholipid content (p=0.001) and the SNP rs6463808 (*NXPH1* gene) with eicosapentaenoic acid (EPA) plasma phospholipid content (p=0.03). **Conclusion:** The genetic variability within genes such as *NELL1*, *NXPH1*, *SLIT2*, JADE1 and/or *IQCJ-SCHIP1* may affect plasma TG and/or phospholipid fatty acid concentrations.

Introduction

The impact of genetic and environmental factors on cardiovascular disease risk has been known for a long time. Kuo [1] published a review in 1968 in which he hypothesised that environmental factors, including the diet, and the genetic profile may have equal weight on the risk to develop atherosclerosis. Elder *et al*. [2] have observed that the heritability for lipid risk factors (total cholesterol (total-C), very low-density lipoprotein-C (VLDL-C), low-density lipoprotein-C (LDL-C), high-density lipoprotein-C (HDL-C) and triglycerides (TG)) was around 56% to 77%. Other factors such as the diet and gene-diet interaction effects also have an impact on cardiovascular risk factors [3]. Depending on dietary intakes, individuals with different genetic profiles do not respond similarly to dietary interventions. A large inter-individual variability in the plasma TG response to fish oil intake has been reported [4-6]. Approximately, 30% of the individuals taking a fish oil supplementation do not lower their plasma TG concentrations [4-6].

Our research group has used a supplementation protocol with fish oil as a model to further investigate gene-diet interaction effects on cardiovascular disease risk factors. In particular, the plasma TG response has been investigated. A candidate gene approach was used to further understand the inter-individual variability observed in the cardiovascular disease risk factor response to fish oil supplementation [6-13]. Genome-wide association studies (GWAS) have helped finding more loci associated with cardiovascular diseases which are not associated with conventional risk factors [14]. In a recent GWAS study by our group, new loci discriminating individuals who lowered their plasma TG concentrations from individuals who did not lower their plasma TG concentrations following a fish oil supplementation were identified [15]. Thirteen new loci were identified. These loci comprised single-nucleotide polymorphisms (SNPs) within or near *Slit homolog 2 (Drosophila)* (*SLIT2*), *Jade family PHD finger 1* (*JADE1*), *IQ Motif Containing J-Schwannomin Interacting Protein 1 Fusion Protein* (*IQCJ-SCHIP1*), *Neurexophilin 1* (*NXPH1*), *NEL-like 1 (chicken)* (*NELL1*) and *V-myb avian myeloblastosis viral oncogene homolog* (*MYB*) genes. The objective of this study was to verify whether SNPs revealed in a GWAS performed on responders versus non-responders according to the plasma TG

response following a supplementation providing 5g per day of fish oil (1.9g EPA and 1.1g DHA) [15] were associated with plasma TG and phospholipid fatty acid concentrations in a cross-sectional study.

Methods

Subjects and study design

Seven hundred (700) Caucasians aged between 18 and 55 years were recruited in the Quebec City metropolitan area. Recruitment occurred between 2004 and 2006 through public advertisements (local newspapers and electronic messages) sent to university and hospital employees, as described previously [16]. A trained research assistant took anthropometric measures. A registered dietitian administered a validated food frequency questionnaire (FFQ) to assess dietary intakes [17]. This questionnaire was composed of 91-items and assessed dietary intakes of the past month. Information on omega-3 (n-3) supplement intake was also collected and included in total n-3 estimates. The Nutrition Data System for Research software version 4.03, developed by the Nutrition Coordination Center (University of Minnesota, Minneapolis, MN, 2002) and the Food and Nutrient Database 31, was used to analyse data obtained from FFQ. All subjects gave written consent to participate in this study which has been approved by the Ethics Committee of Laval University.

Anthropometric measurements

As previously described [16], body weight, height, and waist girth were measured according to the procedures recommended by the Airlie Conference [18]. BMI was calculated as weight per meter squared (Kg/m^2).

Biochemical parameters

The morning after a 12-hour overnight fast, blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA. Plasma was separated by centrifugation. Plasma total-C and TG were determined in plasma and lipoprotein fractions using OLYMPUS AU400e, Olympus America Inc., Melville, NY, USA. Infranatant (d >1.006 g/ml) with heparin-manganese chloride was used to precipitate LDL and then determine HDL-C [19]. The equation of Friedewald was used to estimate LDL-C concentrations [20].

SNPs selection and genotyping

SNPs were selected from a previously conducted GWAS study in our research group observing the differences in allele frequencies among responders and non-responders according to the plasma TG response following a fish oil supplementation [15]. This GWAS study allowed the identification of 13 SNPs. Two of these SNPs were in high linkage disequilibrium (R^2=100%) (rs2952724 and rs201419407). Thus, 12 SNPs were genotyped for this study. Genetic analyses were performed on genomic DNA isolated from human leukocytes. DNA was extracted from 200µl of buffy coat using the GenElute™ Blood Genomic DNA Kit (Sigma-Aldrich). Spectrophotometric quantification was realised with NanoDrop 2000C UV-Vis Spectrophotometer (Thermo Scientific). All selected SNPs (Table 9.1) within or near *SLIT2*, *JADE1*, *IQCJ-SCHIP1*, *NXPH1*, *NELL1*, and *MYB* genes were genotyped with the TAQMAN methodology [21] using the QuantStudio™ 12k Flex System with OpenArray™ Block (with Accufill™ System) of Life Technologies™ with TaqMan® OpenArray™ genotyping master mix. Genotypes were attributed using the QuantStudio™ 12K Flex Software v1.2.2 and the TaqMan® Genotyper software v1.3. Of the 700 participants, 676 subjects for whom DNA was available were genotyped. Genotype calling rates were all over 97% except for rs1449009 (93%) and rs2621309 (92%).

Fatty acid composition of plasma phospholipids

As previously described [22], following the collection of fasting blood samples into vacutainer tubes containing EDTA and centrifugation, samples were stored at -80°C for 2 to 5 years. Plasma phospholipids are biomarkers of the n-3 PUFA status over a few weeks or the last month and have been associated with mortality and coronary heart disease death among older adults as well as with type 2 diabetes [23-26]. Therefore, the plasma phospholipid fatty acid content of 200 participants randomly selected from this cohort were measured. Extraction of plasma lipids was made using a chloroform-methanol mixture (2:1, vol/vol). As described by Kröger *et al*. [27], total PL were separated by thin layer chromatography using a combination of isopropyl ether and acetic acid. Fatty acids of isolated plasma phospholipids were then methylated. Capillary gas chromatography was

used to obtain fatty acid profiles. Values of fatty acid concentrations are expressed in percent of total fatty acid in plasma phospholipids.

Statistical analyses

Hardy-Weinberg equilibrium was tested with the ALLELE procedure of SAS version 9.3 using Fisher's exact test ($P<0.01$). When the genotype frequency for homozygotes of the minor allele was <5%, carriers (heterozygotes and homozygotes) of the minor allele were grouped in order to have appropriate statistical power. Participants who had extreme values (mean±standard deviation multiplied by four) were excluded as well as one homeless and a pregnant women. Variables abnormally distributed were logarithmically transformed. The General Linear Model (GLM) using the type 3 sum of squares for unbalanced study design was used to assess differences according to genotype. To test for potential gene-diet interaction effects on TG, the interaction term was also added into the GLM model (total, saturated, monounsaturated, PUFA and n-3 PUFA fat intakes). The potential impact of the SNPs on plasma phospholipid fatty acid concentrations was also investigated with the GLM procedure. In our previous work, a genetic risk score was calculated from the sum of risk alleles [15]. To verify if this genetic risk score had also an impact within this larger cohort, we calculated for each participant their risk scores. Participants were further divided in three groups according to their genetic risk score (low -2 to 1; medium 2 to 4; high 5 to 8). The associations of these genetic risk score groups with plasma TG concentrations (including gene-diet interaction effects) and with plasma phospholipid fatty acid content were examined. SNPs tested for associations with complex diseases rarely account for a large proportion of variance, characterized by very low p-values ($p<0.001$). Moreover, since this study is an attempt to replicate results obtained in an existing cohort supplemented with fish oil using a pharmacological dose, expected differences may be smaller in a cohort where n-3 PUFA dietary intakes were provided from usual dietary habits of the participants. Therefore, we decided to present the results before correction for multiple testing and using a p-value < 0.05. All statistical analyses were performed using SAS statistical software version 9.3.

Results

Descriptive characteristics of the study participants are presented in Table 9.1. Briefly, participants were mainly overweight with a plasma lipid profile around normal values [28]. More specifically, 31% (n=211) of participants were obese (BMI≥30Kg/m^2), 34% (n=235) were overweight (BMI<30Kg/m^2≥25Kg/m^2) and 35% (n=245) had a BMI <25Kg/m^2. For plasma TG, 17% (n=120) of the participants had plasma TG ≥1.7mmol/L and 83% (n=571) had plasma TG <1.7mmol/L. All SNPs were in Hardy-Weinberg equilibrium. Further informations about the selected SNPs are presented in Table 9.2.

A few associations between the studied SNPs and plasma TG concentrations were observed. Associations with plasma TG were observed for rs1449009 and rs2621309 within *IQCJ-SCHIP1* gene (p=0.04 and p=0.05, respectively) and rs752088 within *NELL1* gene (p=0.03). When adding the effects of age, sex and BMI into the model only rs752088 within *NELL1* gene (p=0.01) was associated with plasma TG and a trend was observed for a SNP within *IQCJ-SCHIP1* gene (rs2621309) (p=0.10). For rs752088, A/G heterozygotes had higher plasma TG concentrations than A/A homozygotes (1.30±0.83mmol/L compared to 1.16±0.77mmol/L) (Figure 9.1). One gene-diet interaction effect on plasma TG concentrations was observed between total dietary fat intakes and rs752088 (p=0.04) which was no longer significant after adjustments for the effects of age, sex and BMI (p=0.21). Gene-diet interaction effects on plasma TG concentrations were also observed with PUFA intakes for rs752088 within *NELL1* gene and rs6463808 within *NXPH1* gene (p=0.03 for both) which were no longer significant after adjustments for the confounding effects of age, sex and BMI (p=0.08 and p=0.06, respectively). Gene-diet interaction effects with n-3 PUFA intakes on plasma TG for rs2952724 and rs2629715, both near *SLIT2* gene as well as rs752088 within *NELL1* gene (p=0.04, p=0.05, p=0.05, respectively) were observed (Table 9.3). These associations were no longer significant when including the effects of age, sex and BMI (p=0.45, p=0.48, p=0.37, respectively).

When stratifying individuals on the basis of plasma TG concentrations using the median value as a cut-off point (>0.99mmol/L (high) or ≤0.99mmol/L (low)), genotype frequencies

assessed by Fishers' exact test were different for rs2621309 within *IQCJ-SCHIP1* gene (p=0.04) and trends were observed for rs1449009 within *IQCJ-SCHIP1* gene (p=0.05) and rs752088 within *NELL1* gene (p=0.05). Briefly, for rs2621309, G/G homozygotes (n=41) had more frequently low plasma TG (68%) than the other genotypes (47% (C/G) (n=196) and 49% (C/C) (n=379).

A genetic risk score was calculated for each participant and three groups were formed (low -2 to 1; medium 2 to 4; high 5 to 8). According to their genetic risk score, plasma TG concentrations were not significantly different (p=0.53). Moreover, no gene-diet interaction effects on plasma TG concentrations were observed (data not shown).

Finally, in a subset of 200 participants, we verified whether SNPs were associated with plasma phospholipid fatty acid concentrations. A few SNPs were associated with n-3 PUFA concentrations within plasma phospholipids, rs1216365 (p=0.04) and rs931681 (p=0.05) both near *JADE1* gene, as well as rs2621308 (p=0.02), rs61332355 (p=0.001) and rs2621309 (p=0.03) within *IQCJ-SCHIP1* gene. As shown in Table 9.4, the associations with SNPs rs1216365 (*JADE1* gene), rs2621308 (*IQCJ-SCHIP1* gene) and rs61332355 (*IQCJ-SCHIP1* gene) remained significant after adjustments for age, sex and BMI (p=0.04, p=0.04 and p=0.005, respectively). The SNPs rs2621308, rs61332355 and rs2621309 within *IQCJ-SCHIP1* gene were associated with DHA plasma phospholipid concentrations (p=0.01, p=0.0002 and p=0.02, respectively) and only rs61332355 remained significant after adjustments for the effects of age, sex and BMI (p=0.001). The SNP rs6463808 within *NXPH1* gene was associated with eicosapentaenoic acid (EPA) Plasma phospholipid content (p=0.03) and results remained significant after adjustments for the effects of age, sex and BMI (p=0.03).

To obtain further insight into the SNPs that explained the most variance in total n-3 PUFA concentrations within plasma phospholipid, all 12 SNPs were entered into a regression model, as well as the confounding variables (age, sex and BMI). The model explained a total of 16.5% (R^2) of the variance in total n-3 PUFA concentrations within plasma

phospholipid. The SNP rs61332355 (*IQCJ-SCHIP1* gene) explained the most variance (partial R^2=6.1%), followed by age (partial R^2=4.2%), rs1216365 (partial R^2=3.2%) and sex (partial R^2=3.0%). The association between the genetic risk score and plasma phospholipid content has also been tested. As shown in Figure 9.2, the genetic risk score was associated with total n-3 PUFA, EPA and DHA plasma phospholipid content (p=0.004, p=0.01 and p=0.004, respectively). These associations remained significant after adjustments for the effects of age, sex and BMI (p=0.03 for all three associations).

Discussion

The objective of this study was to verify whether SNPs revealed in a GWAS performed on responders versus non-responders according to the plasma TG response following a supplementation providing 5g per day of fish oil (1.9g EPA and 1.1g DHA) were associated with plasma TG concentrations and plasma phospholipid fatty acid content in a cross-sectional study [15]. An association between rs752088 within *NELL1* gene and plasma TG concentrations was observed. Gene-diet interaction effects on plasma TG concentrations with total fat, PUFA and n-3 PUFA intakes estimated using a FFQ were observed with SNPs within or near *SLIT2* gene, *NXPH1* gene and *NELL1* gene. Associations with total n-3 PUFA, DHA and EPA plasma phospholipid content were also found.

The SNP rs752088 within *NELL1* gene was associated with plasma TG concentrations. This SNP (rs752088) also interacted with total fat, PUFA and n-3 PUFA intakes to affect plasma TG concentrations. The SNP rs752088 is intronic within *NELL1* gene and encodes for a protein (NELL1) within the cytoplasm containing epidermal growth factor (EGF)-like repeats [29]. NELL1 may play a role in the promotion of osteogenic differentiation as well as in bone formation and regeneration [30]. Osteoblast and adipocytes have a common precursor, mesenchymal stem cells (MSCs) [31]. It has been observed that NELL1 may act as an anti-adipogenic agent, possibly through the regulation of *peroxisome proliferator-activated receptor gamma* (*PPARG*) gene [32]. Only a few studies have examined SNPs within *NELL1* gene for their impacts on lipid concentrations. In a recent GWAS, two SNPs within *NELL1* gene (rs12279250 and rs4319515) were associated with changes in plasma TG concentrations following the intake of hydrochlorothiazide (HCTZ), an antihypertensive medication [33]. Another study, the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study, observed that several SNPs were associated with the response of lipids traits to fenofibrate, including rs4244549, an intronic SNP of *NELL1* gene, with the response of large HDL particle cholesterol concentrations and HDL particle size [34].

A gene-diet interaction effect on plasma TG concentrations was observed between rs6463808, a SNP located within an intronic region of *NXPH1* gene, and PUFA intakes. This SNP (rs6463808) was also associated with differences in EPA plasma phospholipid content. *NXPH1* gene encodes for a secreted protein forming a tight complex with alpha neurexins which promotes adhesion between dendrites and axons [35]. In the GOLDN study, the SNP rs10952132 near *NXPH1* gene proxy was associated with small LDL particle concentrations, LDL size, large HDL particle concentrations and HDL size [34]. A SNP, rs2349775, within an intronic region of *NXPH1* gene has been associated with irritable bowel disease [36]. The authors hypothesised that *NXPH1* gene may have an impact on the immune response by promoting low grade inflammation in the context of a gastrointestinal insult [36]. Among a Mexican American population, the SNP rs757705 within *NXPH1* gene was associated with the risk of type 2 diabetes [37]. Overall, the link between plasma lipid concentrations and *NXPH1* gene remains unknown. However, *NXPH1* gene function and its effects on lipid concentrations merit further investigation.

Gene-diet interaction effects were observed between n-3 PUFA intakes and two SNPs located near *SLIT2* gene on plasma TG concentrations. SLIT2 protein was shown to regulate angiogenesis, to repress lipopolysaccharide-induced inflammatory responses and to act as a regulator in vascular injury [38-40]. The SNP rs1379659 within the 3'untranslated region (UTR) of *SLIT2* gene has been associated with echocardiography traits, specifically the left ventricular diastolic dimension [41]. Another study observed an association for the SNP rs1379659 within *SLIT2* gene and the response of norepinephrine to physical activity [42]. The mechanisms by which SNPs within *SLIT2* gene may affect the plasma TG response to n-3 PUFA are unknown. However it is possible that SLIT2 may modulate the anti-inflammatory effects of n-3 PUFA, affect hepatic metabolism and indirectly plasma TG concentrations.

Two SNPs within *IQCJ-SCHIP1* gene were associated with plasma TG concentrations (rs1449009 and rs2621309) as well as three with total n-3 PUFA and DHA plasma phospholipid content (rs2621308, rs61332355 and rs2621309). *IQCJ-SCHIP1* is a complex of two adjacent genes which encode distinct proteins [43]. SCHIP1 has been shown to be

associated with neurofibromatosis type 2 (NF2) which is a tumor suppressor protein [44]. IQCJ-SCHIP1 could also participate in the regulation of axon initial segments and nodes of Ranvier [45]. Studies are warranted to further understand its possible impacts on plasma TG concentrations and plasma phospholipid fatty acid content.

JADE1, gene for apoptosis and differentiation, is located to the cell nucleus and has been reported to encode a protein that has an impact on endogenous histone acetyltransferase activity [46,47]. *JADE1* gene could also be a renal tumor suppressor by promoting apoptosis [48]. To our knowledge no study has investigated the link between *JADE1* gene and plasma lipid concentrations.

When all SNPs (10 SNPs with linkage disequilibrium <80%) were examined together using a genetic risk score according to the information provided by Rudkowska *et al*. [15], individuals with high genetic risk scores had lower n-3 PUFA, EPA and DHA plasma phospholipid content than individuals with medium genetic risk scores and trends were observed for individuals with low genetic risk scores. However, in our previous work, plasma phospholipid fatty acid content was not different pre-supplementation between responders and non-responders to fish oil supplementation according to the plasma TG response [15]. The impact of these SNPs on plasma phospholipid fatty acid content remains to be understood.

Conclusion

In this study, a few SNPs previously identified in a GWAS comparing frequencies between responders and non-responders according to the plasma TG response to a fish oil supplementation were associated with plasma TG concentrations and plasma phospholipid fatty acid content in a cross-sectional cohort of healthy individuals. Gene-diet interaction effects on plasma TG concentrations were also observed for dietary intakes of total fat, PUFA and n-3 PUFA. These results reinforce the importance of considering these novel associations in future studies investigating the inter-individual variability of plasma TG concentrations, its response to fish oil supplementation and plasma phospholipid fatty acid content. Studies to further understand the functions of these genes and their role in the lipid metabolism are also warranted.

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References

1. Kuo PT: Current metabolic-genetic interrelationship in human atherosclerosis, with therapeutic considerations. *Ann Intern Med* 1968, 68: 449-466.
2. Elder SJ, Lichtenstein AH, Pittas AG, Roberts SB, Fuss PJ, Greenberg AS *et al.*: Genetic and environmental influences on factors associated with cardiovascular disease and the metabolic syndrome. *J Lipid Res* 2009, 50: 1917-1926.
3. Perez-Martinez P, Phillips CM, Delgado-Lista J, Garcia-Rios A, Lopez-Miranda J, Perez-Jimenez F: Nutrigenetics, Metabolic Syndrome Risk and Personalized Nutrition. *Curr Vasc Pharmacol* 2013.
4. Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK *et al.*: Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. *Am J Clin Nutr* 2008, 88: 618-629.
5. Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H *et al.*: The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. *Annu Rev Nutr* 2011, 31: 203-234.
6. Cormier H, Rudkowska I, Paradis AM, Thifault E, Garneau V, Lemieux S *et al.*: Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an n-3 PUFA supplementation. *Nutrients* 2012, 4: 1026-1041.
7. Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC: Polymorphisms, de novo lipogenesis, and plasma triglyceride response following fish oil supplementation. *J Lipid Res* 2013, 54: 2866-2873.
8. Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC: An interaction effect between glucokinase gene variation and carbohydrate intakes modulates the plasma triglyceride response to a fish oil supplementation. *Genes Nutr* 2014, 9: 395.
9. Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC: Polymorphisms in Genes Involved in Fatty Acid beta-Oxidation Interact with Dietary Fat Intakes to Modulate the Plasma TG Response to a Fish Oil Supplementation. *Nutrients* 2014, 6: 1145-1163.
10. Thifault E, Cormier H, Bouchard-Mercier A, Rudkowska I, Paradis AM, Garneau V *et al.*: Effects of age, sex, body mass index and APOE genotype on cardiovascular biomarker response to an n-3 polyunsaturated fatty acid supplementation. *J Nutrigenet Nutrigenomics* 2013, 6: 73-82.
11. Ouellette C, Cormier H, Rudkowska I, Guenard F, Lemieux S, Couture P *et al.*: Polymorphisms in genes involved in the triglyceride synthesis pathway and marine omega-3 polyunsaturated fatty acid supplementation modulate plasma triglyceride levels. *J Nutrigenet Nutrigenomics* 2013, 6: 268-280.
12. Rudkowska I, Julien P, Couture P, Lemieux S, Tchernof A, Barbier O *et al.*: Cardiometabolic risk factors are influenced by Stearoyl-CoA Desaturase (SCD) -1 gene polymorphisms and n-3 polyunsaturated fatty acid supplementation. *Mol Nutr Food Res* 2014, 58: 1079-1086.

13. 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: 485-498.

14. McPherson R: From genome-wide association studies to functional genomics: new insights into cardiovascular disease. *Can J Cardiol* 2013, 29: 23-29.

15. Rudkowska I, Guenard F, Julien P, Couture P, Lemieux S, Barbier O *et al*.: Genome-wide association study of the plasma triglyceride response to an n-3 polyunsaturated fatty acid (PUFA) supplementation. *J Lipid Res* 2014.

16. Paradis AM, Godin G, Perusse L, Vohl MC: Associations between dietary patterns and obesity phenotypes. *Int J Obes (Lond)* 2009, 33: 1419-1426.

17. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S: Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. *Nutr J* 2004, 3: 13.

18. Callaway, C.W., W.C. Chumlea, C. Bouchard,J.H. Himes, T.G. Lohman, A.D. Martin, C.D. Mitchell, W.H. Mueller, A.F. Roche, V.D. Seefeldt. 1988. Standardization of Anthropometric Measurements, In: T. Lohman, A. Roche, R. Martorel. eds, The Airlie (VA) Consensus Conference, Human Kinetics Publishers, Champaign, IL. 39-80.

19. Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L *et al*.: Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. *Clin Chem* 1978, 24: 853-856.

20. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972, 18: 499-502.

21. Livak KJ: Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal* 1999, 14: 143-149.

22. Garneau V, Rudkowska I, Paradis AM, Godin G, Julien P, Perusse L *et al*.: Omega-3 fatty acids status in human subjects estimated using a food frequency questionnaire and plasma phospholipids levels. *Nutr J* 2012, 11: 46.

23. Hodson L, Skeaff CM, Fielding BA: Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* 2008, 47: 348-380.

24. Saadatian-Elahi M, Slimani N, Chajes V, Jenab M, Goudable J, Biessy C *et al*.: Plasma phospholipid fatty acid profiles and their association with food intakes: results from a cross-sectional study within the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr* 2009, 89: 331-346.

25. Mozaffarian D, Lemaitre RN, King IB, Song X, Huang H, Sacks FM *et al*.: Plasma phospholipid long-chain omega-3 fatty acids and total and cause-specific mortality in older adults: a cohort study. *Ann Intern Med* 2013, 158: 515-525.

26. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw KT, Wareham NJ *et al*.: Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. *Am J Clin Nutr* 2010, 92: 1214-1222.

27. Kroger E, Verreault R, Carmichael PH, Lindsay J, Julien P, Dewailly E *et al*.: Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. *Am J Clin Nutr* 2009, 90: 184-192.

28. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002, 106: 3143-3421.

29. NCBI. NELL1 NEL-like 1 (chicken) [Homo sapiens (human)]. 2013 [http://www.ncbi.nlm.nih.gov/gene/4745].

30. Zhang X, Zara J, Siu RK, Ting K, Soo C: The role of NELL-1, a growth factor associated with craniosynostosis, in promoting bone regeneration. *J Dent Res* 2010, 89: 865-878.

31. Graneli C, Karlsson C, Brisby H, Lindahl A, Thomsen P: The effects of PPAR-gamma inhibition on gene expression and the progression of induced osteogenic differentiation of human mesenchymal stem cells. *Connect Tissue Res* 2014, 1-14.

32. Liu Y, Chen C, He H, Wang D, E L, Liu Z *et al*.: Lentiviral-mediated gene transfer into human adipose-derived stem cells: role of NELL1 versus BMP2 in osteogenesis and adipogenesis in vitro. *Acta Biochim Biophys Sin (Shanghai)* 2012, 44: 856-865.

33. Del-Aguila JL, Beitelshees AL, Cooper-DeHoff RM, Chapman AB, Gums JG, Bailey K *et al*.: Genome-wide association analyses suggest NELL1 influences adverse metabolic response to HCTZ in African Americans. *Pharmacogenomics J* 2014, 14: 35-40.

34. Kraja AT, Borecki IB, Tsai MY, Ordovas JM, Hopkins PN, Lai CQ *et al*.: Genetic analysis of 16 NMR-lipoprotein fractions in humans, the GOLDN study. *Lipids* 2013, 48: 155-165.

35. NCBI. NXPH1 neurexophilin 1 [Homo sapiens (human)]. 2008. [http://www.ncbi.nlm.nih.gov/gene/30010]

36. Wouters MM, Lambrechts D, Knapp M, Cleynen I, Whorwell P, Agreus L *et al*.: Genetic variants in CDC42 and NXPH1 as susceptibility factors for constipation and diarrhoea predominant irritable bowel syndrome. *Gut* 2013.

37. Hayes MG, Pluzhnikov A, Miyake K, Sun Y, Ng MC, Roe CA *et al*.: Identification of type 2 diabetes genes in Mexican Americans through genome-wide association studies. *Diabetes* 2007, 56: 3033-3044.

38. Wang B, Xiao Y, Ding BB, Zhang N, Yuan X, Gui L *et al*.: Induction of tumor angiogenesis by Slit-Robo signaling and inhibition of cancer growth by blocking Robo activity. *Cancer Cell* 2003, 4: 19-29.

39. Zhao H, Anand AR, Ganju RK: Slit2-Robo4 pathway modulates lipopolysaccharide-induced endothelial inflammation and its expression is dysregulated during endotoxemia. *J Immunol* 2014, 192: 385-393.

40. Yuen DA, Robinson LA: Slit2-Robo signaling: a novel regulator of vascular injury. *Curr Opin Nephrol Hypertens* 2013, 22: 445-451.

41. Vasan RS, Larson MG, Aragam J, Wang TJ, Mitchell GF, Kathiresan S *et al*.: Genome-wide association of echocardiographic dimensions, brachial artery endothelial function and treadmill exercise responses in the Framingham Heart Study. *BMC Med Genet* 2007, 8 Suppl 1: S2.

42. Karoly HC, Stevens CJ, Magnan RE, Harlaar N, Hutchison KE, Bryan AD: Genetic Influences on Physiological and Subjective Responses to an Aerobic Exercise Session among Sedentary Adults. *J Cancer Epidemiol* 2012, 2012: 540563.

43. Kwasnicka-Crawford DA, Carson AR, Scherer SW: IQCJ-SCHIP1, a novel fusion transcript encoding a calmodulin-binding IQ motif protein. *Biochem Biophys Res Commun* 2006, 350: 890-899.

44. Goutebroze L, Brault E, Muchardt C, Camonis J, Thomas G: Cloning and characterization of SCHIP-1, a novel protein interacting specifically with spliced isoforms and naturally occurring mutant NF2 proteins. *Mol Cell Biol* 2000, 20: 1699-1712.

45. Martin PM, Carnaud M, Garcia del CG, Irondelle M, Irinopoulou T, Girault JA *et al*.: Schwannomin-interacting protein-1 isoform IQCJ-SCHIP-1 is a late component of nodes of Ranvier and axon initial segments. *J Neurosci* 2008, 28: 6111-6117.

46. Foy RL, Song IY, Chitalia VC, Cohen HT, Saksouk N, Cayrou C *et al*.: Role of Jade-1 in the histone acetyltransferase (HAT) HBO1 complex. *J Biol Chem* 2008, 283: 28817-28826.

47. Panchenko MV, Zhou MI, Cohen HT: von Hippel-Lindau partner Jade-1 is a transcriptional co-activator associated with histone acetyltransferase activity. *J Biol Chem* 2004, 279: 56032-56041.

48. Zhou MI, Foy RL, Chitalia VC, Zhao J, Panchenko MV, Wang H *et al*.: Jade-1, a candidate renal tumor suppressor that promotes apoptosis. *Proc Natl Acad Sci U S A* 2005, 102: 11035-11040.

Table 9.1 Descriptive characteristics of the study participants.

Variables	Means±SD
Age (years)	37.87±11.29 (n=691)
Sex (men/women)	284/407
BMI (Kg/m^2)	27.74±5.68 (n=691)
Waist circumference (cm)	Men: 96.8±14.8 (n=283) Women: 85.8±15.0 (n=407)
Total-C (mmol/L)	4.61±1.00 (n=648)
LDL-C (mmol/L)	2.88±0.96 (n=674)
HDL-C (mmol/L)	1.39±0.42 (n=674)
Triglycerides (mmol/L)	1.23±0.80 (n=674)

Means±SD

Table 9.2 The selected SNPs, minor allele and genotype frequencies.

Genes	SNPs	Position	MAF	Genotype frequency		
SLIT2	rs2952724	Intergenic (300 kp upstream of *SLIT2*)	0.286	T/T (n=61) 0.092	C/T (n=256) 0.387	C/C (n=344) 0.520
SLIT2	rs2629715	Intergenic (300 kb upstream of *SLIT2*)	0.283	T/T (n=59) 0.091	G/T (n=249) 0.384	G/G (n=341) 0.525
JADE1	rs1216352	Intergenic (300 kb upstream of *JADE-1*)	0.309	T/T (n=63) 0.096	C/T (n=280) 0.427	C/C (n=313) 0.477
JADE1	rs1216365	Intergenic (500 kb upstream of *JADE-1*)	0.353	C/C (n=82) 0.126	A/C (n=295) 0.454	A/A (n=273) 0.420
JADE1	rs931681	Intergenic (300 kb upstream of *JADE-1*)	0.348	C/C (n=78) 0.119	C/T (n=301) 0.459	T/T (n=277) 0.422
IQCJ-SCHIP1	rs2621308	Intron variant *IQCJ-SCHIP1*	0.229	T/T (n=41) 0.062	G/T (n=220) 0.334	G/G (n=398) 0.604
IQCJ-SCHIP1	rs1449009	Intron variant *IQCJ-SCHIP1*	0.239	G/G (n=43) 0.069	A/G (n=212) 0.340	A/A (n=369) 0.591
IQCJ-SCHIP1	rs61332355	Intron variant *IQCJ-SCHIP1*	0.165	A/A (n=26) 0.040	A/C (n=164) 0.250	C/C (n=465) 0.710
IQCJ-SCHIP1	rs2621309	Intron variant *IQCJ-SCHIP1*	0.226	G/G (n=41) 0.067	C/G (n=196) 0.318	C/C (n=379) 0.615
NXPH1	rs6463808	Intron variant *NXPH1*	0.181	A/A (n=24) 0.036	A/G (n=192) 0.290	G/G (n=447) 0.674
NELL1	rs752088	Intron variant *NELL1*	0.359	G/G (n=81) 0.123	A/G (n=313) 0.474	A/A (n=267) 0.404
MYB	rs6920829	Intron variant *MYB*	0.121	C/C (n=7) 0.011	C/T (n=146) 0.222	T/T (n=506) 0.768

Table 9.3 Gene-diet interaction effects on plasma TG concentrations.

Gene	SNPs		β (interaction term)	P genotype[1]	P dietary fat intakes[1]	P interaction effect[1]
Total fat intakes						
NELL1	rs752088	G/G A/G A/A	-0.0012±0.0078 0.0105±0.0045 0	0.12	0.58	0.04
Polyunsaturated fat intakes						
NXPH1	rs6463808	A/A+A/G G/G	0.2517±0.1140 0	0.02	0.42	0.03
NELL1	rs752088	G/G A/G A/A	-0.0518±0.2036 0.2740±0.1153 0	0.07	0.44	0.03
Omega-3 polyusaturated fat intakes						
SLIT2	rs2952724	T/T C/T C/C	0.3289±0.1459 0.1308±0.0786 0	0.03	0.0003	0.04
SLIT2	rs2629715	T/T G/T G/G	0.3391±0.1458 0.1073±0.0790 0	0.03	0.0006	0.05
NXPH1	rs6463808	A/A+A/G G/G	0.1554±0.0808 0	0.03	0.0003	0.06
NELL1	rs752088	G/G A/G A/A	0.0256±0.1300 0.1879±0.0786 0	0.27	0.03	0.05

[1]Unadjusted P-values generated from an ANOVA.

Table 9.4 Relative total n-3 PUFA, DHA and EPA content (%) of plasma phospholipids according to genotypes.

	SNPs	Genotype			P-value[1]	P-value[2]
Total n-3 PUFA (%)	rs1216352	T/T 5.11±1.23 (n=15)	C/T 5.32±1.25 (n=78)	C/C 5.74±1.60 (n=102)	0.08	0.11
	rs1216365	C/C 4.99±1.21 (n=17)	A/C 5.37±1.29 (n=88)	A/A 5.80±1.59 (n=89	0.04	0.04
	rs931681	C/C 4.99±1.21 (n=17)	C/T 5.36±1.29 (n=89)	T/T 5.78±1.60 (n=90)	0.05	0.05
	rs2621308	T/T 5.05±1.09 (n=10)	G/T 5.18±1.21 (n=68)	G/G 5.75±1.56 (n=118)	0.02	0.04
	rs1449009	C/C 5.14±1.12 (n=9)	C/T 5.24±1.28 (n=68)	T/T 5.71±1.46 (n=111)	0.07	0.11
	rs61332355	-	A/C 4.95±1.05 (n=50)	C/C 5.72±1.52 (n=145)	0.001	0.005
	rs2621309	G/G 5.05±1.09 (n=10)	C/G 5.22±1.18 (n=58)	C/C 5.77±1.58 (n=113)	0.03	0.07
	rs6463808	-	A/G 5.77±1.49 (n=68)	G/G 5.39±1.42 (n=128)	0.08	0.12
DHA (%)	rs2621308	T/T 2.82±0.68 (n=10)	G/T 3.02±0.86 (n=68)	G/G 3.36±0.89 (n=118)	0.01	0.06
	rs1449009	C/C 2.91±0.66 (n=9)	C/T 3.06±0.88 (n=68)	T/T 3.34±0.88 (n=111)	0.07	0.17
	rs61332355	-	A/C 2.83±0.72 (n=50)	C/C 3.36±0.90 (n=145)	0.0002	0.001
	rs2621309	G/G 2.82±0.68 (n=10)	C/G 3.03±0.88 (n=58)	C/C 3.36±0.90 (n=113)	0.02	0.08
EPA (%)	rs1216365	C/C 0.91±0.49 (n=17)	A/C 1.06±0.61 (n=88)	A/A 1.23±0.74 (n=89)	0.06	0.08
	rs931681	C/C 0.91±0.49 (n=17)	C/T 1.05±0.61 (n=89)	T/T 1.22±0.74 (n=90)	0.06	0.09
	rs61332355	-	A/C 0.97±0.48 (n=50)	C/C 1.17±0.72 (n=145)	0.06	0.09

	rs6463808	-	A/G 1.25±0.75 (n=68)	G/G 1.04±0.61 (n=128)	0.03	0.03

Means±SD.
[1]Unadjusted P-values generated from an ANOVA.
[2]P-values adjusted for the effects of age, sex and BMI.

Figure 9.1 Plasma triglyceride concentrations according to genotype of rs752088.

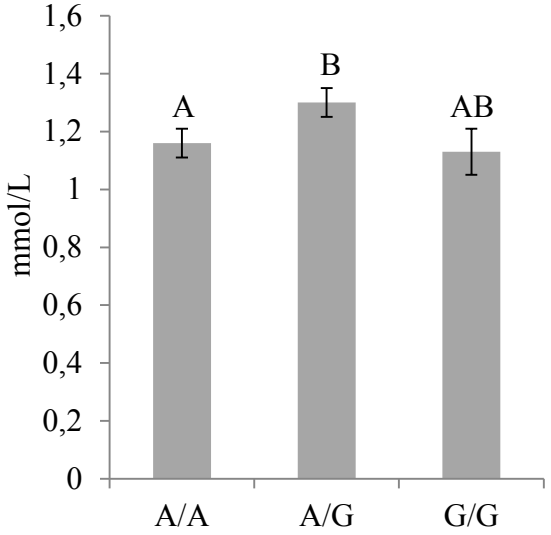

Figure legend. A/A homozygotes (n=265) 1.16±0.77mmol/L, A/G heterozygotes (n=309) 1.30±0.83mmol/L and G/G homozygotes (n=81) 1.13±0.70mmol/L. A/A homozygotes vs A/G heterozygotes (p=0.006), A/A homozygotes vs G/G homozygotes (p=0.88) and A/G heterozygotes vs G/G homozygotes (p=0.09). Means with different letters are significantly different. P-values were obtained with an ANOVA adjusted for the effects of age, sex and BMI. Means±SE.

Figure 9.2 Total n-3 PUFA, EPA and DHA plasma phospholipid relative content according to genetic risk score group.

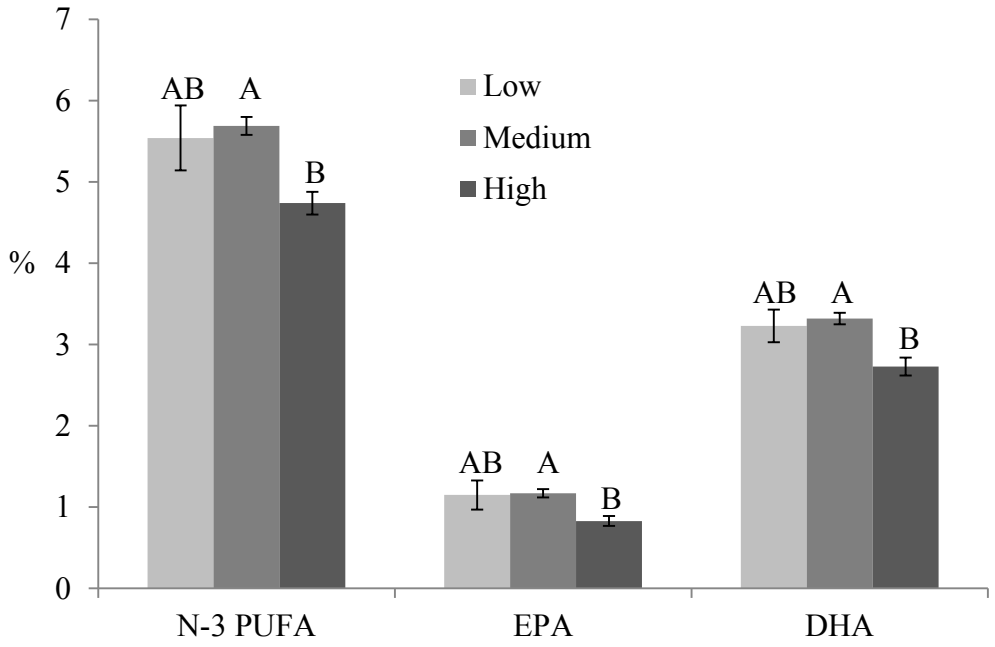

Figure legend. For total n-3 PUFA Low vs Medium genetic risk score group (p=0.73), Medium vs High (p=0.007) and Low vs High (p=0.08); For EPA Low vs Medium (p=0.38), Medium vs High (p=0.008) and Low vs High (p=0.20); For DHA Low vs Medium (p=0.97), Medium vs High (p=0.008) and Low vs High (p=0.05). Low risk group n=28, Medium risk group n=142 and High risk group n=30. Means with different letters are significantly different. P-values were obtained with an ANOVA adjusted for the effects of age, sex and BMI. Means±SE.

Chapitre 10

Les diététistes ont-ils l'intention ou discutent-ils déjà de nutrigénétique avec leurs patients/clients? Une application de la Théorie du comportement planifié

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Soumis à *Journal of Nutrition Education and Behavior*

Do registered dietitians intend to or actually discuss nutrigenetics with their patients/clients? An application of the Theory of planned behavior

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Résumé

Introduction : Les tests de nutrigénétique deviennent de plus en plus accessibles aux consommateurs. La plupart des études rapportent que les diététistes ne sont pas bien préparées à discuter de nutrigénétique avec leurs patients/clients. L'objectif principal de cette étude est d'examiner les facteurs affectant l'intention des diététistes de discuter de nutrigénétique avec leurs patients/clients. **Méthodes** : Un questionnaire basé sur la Théorie du comportement planifié (TCP) (attitude, norme subjective et perception de contrôle) a été développé et envoyé aux diététistes membres de l'Ordre professionnel des diététistes du Québec (OPDQ) par courriel. Des analyses par régressions multiples ont été réalisées afin d'examiner les déterminants de l'intention et du comportement. **Résultats** : Cent quarante et un (141) diététistes ont complété le questionnaire. Les participants étaient distribués uniformément à travers les groupes d'âge avec une proportion légèrement plus élevée pour le groupe des 26-30 ans et plus basse pour le groupe des 50 ans et plus. Sur une échelle de (-2 à 2 (de fortement en désaccord à fortement en accord), l'intention des diététistes de discuter de nutrigénétique avec leurs patients/clients était neutre avec une moyenne de -0,07±0,92. L'attitude était le construit de la TCP le plus fortement associé avec l'intention (β=0,66, $p<0,0001$), suivi de la perception de contrôle (β=0,33, $p<0,0001$) et de la norme subjective (β=0,21, $p=0,03$). Finalement, 13 diététistes sur 141 (~9%) avaient discuté de nutrigénétique avec leurs patients/clients dans les trois derniers mois. Seule la perception de contrôle expliquait une partie de la variance du comportement (β=0,20, $p<0,0001$). **Conclusion** : Les principaux déterminants de l'intention des diététistes de discuter de nutrigénétique avec leurs patients/clients ont été déterminés. Ce savoir pourra aider à concevoir un outil de formation à propos de la nutrigénétique pour les diététistes.

Abstract

Background: Nutrigenetic testing is becoming more available to the consumer. Most studies report that dietitians are not well prepared to discuss nutrigenetics with their patients/clients. The main objective of this study was to investigate factors affecting the intention of dietitians to discuss nutrigenetics with their patients/clients. **Methods:** A survey based on the Theory of planned behavior (TPB) (attitude, subjective norm and perceived behavioral control) was developed and sent by email to dietitians members of the *Ordre professionnel des diététistes du Québec* (OPDQ). Multiple regression analyses were performed to examine the determinants of intention and behavior. **Results:** One hundred and fourty one (141) dietitians completed the questionnaire. Participants were evenly distributed across age groups with a slightly higher proportion aged between 26 and 30 years old and fewer were 50 years old or more. On a scale from -2 to 2 (from strongly disagree to strongly agree), the intention of discussing nutrigenetics with patients/clients was neutral with a mean of -0.07±0.92. The TPB construct of attitude was the most strongly associated with intention (β=0.66, $p<0.0001$) followed by perceived behavioral control (β=0.33, $p<0.0001$) and subjective norm (β=0.21, $p=0.03$). Finally, 13 out of 141 dietitians (~9%) did actually practice the behavior which was to have discussed nutrigenetics with their patients/clients in the last three months. Only the perceived behavioral control contributed to explain the behavior (β=0.20, $p<0.0001$). **Conclusion:** Main determinants of the intention of dietitians to discuss nutrigenetics with their patients/clients were determined. This knowledge will help in the design of future educational content about nutrigenetics.

Introduction

Nutritional genomics which comprises nutrigenetics, nutrigenomics, and nutritional epigenomics, studies the relationships between nutrients and genes and its impacts on phenotypic outcomes, for example disease risk (Camp & Trujillo, 2014). Nutrigenetics is the study of *the role of DNA sequence variation in the responses to nutrients*, often referred to as gene-diet interaction effects (Bouchard & Ordovas, 2012). For example, the treatment of phenylketonuria (PKU), a monogenic disease, with a specific diet was the first application of nutrigenetics (Woolf *et al*., 1955). Nutrigenetics has also focused on further understanding the impacts of gene-diet interactions on chronic diseases and their related risk factors, such as cardiovascular disease and components of the metabolic syndrome (Phillips, 2013; Corella & Ordovas, 2012). Even though this science is promising, in order to personalise dietary recommendations given to individuals, groups of individuals and even to the population, several obstacles have to be overcome before implementing nutrigenetic-related recommendations into clinical practice (Phillips, 2013; Fenech *et al*., 2011; de Roos, 2013). Difficulties such as replicating the observed gene-diet interactions were encountered (Phillips, 2013). Moreover, nutrigenetic findings need to be validated within intervention trials prior to making personalised dietary recommendations (Fenech *et al*., 2011). Despite these facts, in 2008 approximately thirty companies were already offering direct-to-consumer nutrigenetic tests (Ries & Castle, 2008).

To ensure the successful knowledge translation of nutritional genomics to end-users such as dietitians, physicians, and patients, multiple professionals will have to work in synergy, from researchers to health care providers (Guttmacher *et al*., 2001). Dietitians are the health professionals with the strongest nutritional background and which could integrate nutrigenetic-based dietary recommendations into both clinical and public health practices (Patterson *et al*., 1999; Collins *et al*., 2013). However, surveys conducted among dietitians have revealed a lack of knowledge regarding nutrigenomics and nutrigenetics (Collins *et al*., 2013; Rosen *et al*., 2006; McCarthy *et al*., 2008; Whelan *et al*., 2008; Cormier *et al*., 2014). A majority of dietitians do not understand properly the basic concepts related to nutritional genomics (Rosen *et al*., 2006). Recent surveys conducted among dietitians from

the US, UK, Australia and Canada reported low levels of knowledge and/or confidence related to genetics and nutritional genomics, a lack of continuing professional education and a lack of educational experts in the field (Collins *et al*., 2013; Rosen *et al*., 2006; Cormier *et al*., 2014). High confidence levels in genetics and nutritional genomics are associated with increased reference to nutritional genomics into dietitians' practice (Collins *et al*., 2013; Whelan *et al*., 2008). Therefore the need for adequate education regarding nutritional genomics is required.

The Theory of planned behavior (TPB) has been used successfully among health professionals including dietitians to predict the adoption of specific behaviors or the intention related to the adoption of the behavior (Godin *et al*., 2008; Chase *et al*., 2003). The TPB has also been used as a theoretical framework to build intervention programs promoting health behaviors and effective continuing education programs for health professionals (Beaulieu & Godin, 2012; Casper, 2007; Edwards *et al*., 2007; Perkins *et al*., 2007). Overall, the main objective of this study was to investigate factors affecting the intention of dietitians to discuss nutrigenetics with their patients/clients.

Methods

Study population and sample

An invitation to participate in a cross-sectional survey was sent by email to all members of the *Ordre professionnel des diététistes du Québec* (OPDQ) (n=2 823) between September 2013 and November 2013. Members of the OPDQ are registered dietitians with a valid licence to practice in the province of Quebec, Canada. To be eligible, participants had to be members of the OPDQ, to be able to answer the survey in French as well as to have access to a computer and an Internet connection. Participants were asked to complete the survey online. Participants with >10% of unanswered TPB specific questions were excluded.

Elicitation study

In order to determine the salient beliefs from our study population prior to the development of the quantitative TPB questionnaire, an elicitation study was conducted with a sample of 23 dietitians' members of the OPDQ. The participants were asked to complete a questionnaire comprising six open-ended questions. Following the TPB theoretical constructs, questions referred to dietitians perceived advantages and disadvantages (attitudes) with regard to discussing nutrigenetics with their patients/clients, the individuals or groups they perceived as favourable or unfavourable (subjective norm) towards the action of discussing nutrigenetics with their patients/clients, and the barriers as well as the facilitating factors (perceived behavioral control) they perceived about discussing nutrigenetics with their patients/clients. In order to clarify the term «nutrigenetics», a short definition was provided at the beginning of the questionnaire as well as short definitions of the terms «nutritional genomics» and «nutrigenomics». Nutrigenetics was presented as the study of how the genetic profile (gene variants) may influence the response to dietary intakes of an individual or a group of individuals.

A content analysis was conducted separately by two researchers in order to extract salient modal beliefs common to this population according to the methodology described by Gagné and Godin (Gagne & Godin, 1999). Both researchers each created separately three

lists (behavioral, normative and control beliefs). Beliefs expressing the same ideas were grouped and the frequency of each belief was noted. Researchers then agreed on the classification and labelling of the themes extracted. Most popular themes were kept until 75% of the total number of beliefs was reached. To elaborate the items assessing the main variables of the TPB (attitude, subjective norm, perceived behavioral control), guidelines provided by Gagné and Godin were followed (Gagne & Godin, 1999).

Data collection procedure

An approbation letter was sent to the OPDQ to ask for their approval to distribute the survey to their members via an electronic message (September 11th 2013). Two reminders were sent via electronic messages four and six weeks after the first email (October 16th 2013 and November 5th 2013). The survey was distributed via a secured link to SurveyMonkey. This project was approved by the Ethics Committee of Laval University.

Instrument development and validation

After the elicitation study, the questionnaire was developed following guidelines provided by Ajzen (Ajzen, 2006) as well as by Gagné and Godin (Gagne & Godin, 1999). The questionnaire was a self-administered questionnaire including fourty-seven (47) questions. The estimated time to complete the survey was around ten minutes. The first section assessed informed consent to participate in this study and sociodemographic questions, followed by short definitions of «nutritional genomics», «nutrigenomics» and «nutrigenetics». The second section assessed components based on the TPB. The behavior was assessed with one direct question: «During the last three months I have discussed nutrigenetics with my patients/clients» which they answered by either selecting «yes» or «no». Intention was measured with three items beginning with either «I plan to…», «I intend to…» and «I want to…» and ending with «…to discuss nutrigenetics with my patients/clients» (five-point scale: -2=strongly disagree; 2=strongly agree). Attitude (direct construct) was measured by means of four items composed of pairs of adjectives, appearing after the following sentence: «To discuss nutrigenetics with my patients/clients would be… (five-point scale: -2=Very deleterious to 2=Very beneficial; -2=Very unpleasant to 2=Very

pleasant; -2=Very bad to 2=Very good; -2=Very useless to 2=Very useful). For the subjective norm (direct construct), three items were used: «People that are the most important to me think that it is appropriate to discuss nutrigenetics with my patients/clients», «Most people who are important to me think that I should discuss nutrigenetics with my patients/clients» and «I feel a social pressure which pushes me to discuss nutrigenetics with my patients/clients» (five-point scale: -2=strongly disagree; 2=strongly agree). Perceived behavioral control (direct construct) was assessed with four items (five-point scale: -2=strongly disagree; 2=strongly agree): «With the resources that I have, I feel capable to discuss nutrigenetics with my patients/clients», «If I wanted I could easily discuss nutrigenetics with my patients/clients», «I feel confident to be able to discuss nutrigenetics with my patients/clients» and «Many obstacles out of my control could prevent me to discuss nutrigenetics with my patients/clients». Behavioral beliefs (indirect construct) were measured by means of a sentence starting with «To discuss nutrigenetics with my patients/client would…» followed by the five following items: «…be hard to understand for my patients/clients», «…allow me to further personalize dietary recommendations», «…increase the efficiency of the dietary interventions», «…create a sense of powerlessness among patients/clients» and «…be hard to explain to the patients/clients». Normative beliefs (indirect construct) were measured with four items assessing whether researchers, other dietitians, employers and fellow workers would approve or disapprove that they discuss nutrigenetics with their patients/clients (five-point scale: -2=strongly disapprove to 2=strongly approve). Control beliefs (indirect construct) were assessed by means of four items asking the participants to answer on a five-point scale (-2=very unlikely to 2=very likely) with the following items: «If I had enough knowledge/training, I could discuss nutrigenetics with my patients/clients», «If my patient/client would be interested, I could discuss nutrigenetics with him», «If I had clinical tools about nutrigenetics, it would allow me to discuss nutrigenetics with my patients/clients» and «Even if wanted, I would not have enough time to discuss nutrigenetics during my nutritional consultations».

The questionnaire was in French and validated by six individuals including two researchers of the Institute of Nutrition and Functional Foods (INAF) and four dietitians for the clarity of the questions. Questions were then modified according to the comments received.

Psychometric qualities

A five-point Likert-type scale was used for this study. Nutrigenetics may be considered as an emerging field in the practice of dietitians and is still not well understood among these professionals (Cormier *et al*., 2014), thus a smaller scale (5 instead of 7) was preferred. Moreover, five-point scales or five possible answers have been used in other surveys conducted among dietitians focusing on genetics and nutritional genomics (McCarthy *et al*., 2008). The Alpha Cronbach's coefficient was used to assess internal consistency (fidelity).

Statistical analyses

Descriptive analyses were performed to better describe the variables related to the participants (means, standard deviation, variable distribution). Scores for negative items were reversed. Pearson correlations were computed between the variables related to the TPB. The determinants of intention were assessed by a multiple linear regression including attitude, subjective norm and perceived behavioral control. Potential confounding factors (descriptive variables) were identified with ANOVAs predicting the intention and were included into the multiple linear regression only if p value was <0.10. Potential covariates which were associated with the intention at p-value <0.10 were also included into the multiple linear regression analyses. Because this study is a first step towards the development of continuing education for dietitians, beliefs related to the direct constructs found significant in the previous regression model were further examined in relation to the intention in a separate multiple linear regression model. Beliefs included into the regression model were chosen according to Mallow's C_p statistic (Mallows, 1973). A third multiple linear regression predicting the behavior was performed including intention and perceived behavioral control. P-values <0.05 were considered significant. All analyses were performed using SAS software, Version 9.3 (SAS Institute, Cary, NC, USA).

Results

The questionnaire was sent by e-mail to the 2823 members of OPDQ. A total of 164 dietitians completed the survey online and gave written consent to participate in this study. The response rate was 5.8%. Participants with >10% of unanswered TPB specific questions were excluded. Thus, 141 participants were included into the analyses.

Descriptive characteristics of the study population are presented in Table 10.1. Briefly, age was almost equally distributed across age groups with a slightly higher proportion of the participants aged between 26 and 30 years old (28.4%) and fewer being 50 years old or higher (11.4%). Approximately ~16% of the dietitians had a Master degree in Nutrition, only ~2% of the dietitians had a PhD in Nutrition, whereas ~15% had completed graduate studies in other fields or programs. Most of the participants (~51%) had 5 years or less of experience as dietitians. The majority of dietitians reported practicing in the area of clinical nutrition in public health settings (~59%). Participants were mostly from urban centres such as Montreal (~28%) and Capitale-Nationale (Quebec City) (~25%). Most dietitians had never interpreted the results from a nutrigenetic test (n=134, 96.4%). The majority of dietitians never recommended a nutrigenetic test (n=133, 96.4%). When asked if they were ever approached by companies offering nutrigenetic tests, most dietitians responded negatively (n=130, 64.2%).

As presented in Table 10.2, means of Cronbach's alpha coefficient were all between 0.65 and 0.87, which is considered adequate (Gagne & Godin, 1999). Thus, all items were kept for the analyses. Table 10.3 presents the descriptive statistics related to TPB direct constructs and salient beliefs. Briefly, the intention to discuss nutrigenetics with patients/clients was neutral (neither agree nor disagree) with a mean of -0.07±0.92. The attitude towards the behavior was slightly positive with a mean of 0.41±0.65, the subjective norm and the perceived behavioral control were negative, with means of -0.22±0.65 and -0.53±0.79, respectively.

The correlation matrix between the TPB direct constructs and intention are presented in Table 10.4. All direct constructs of the TPB were correlated with an r value ranging from 0.52 to 0.74. The attitude construct was the most strongly correlated with intention ($r=0.74$, $p<0.0001$).

The regression model including the variables of TPB explained 63% (R^2) of the variance in the intention of dietitians to discuss nutrigenetics with their patients/clients (Figure 10.1). The three TPB constructs contributed to the model as follows: attitude ($\beta=0.66$, $p<0.0001$), subjective norm ($\beta=0.21$, $p=0.03$) and perceived behavioral control ($\beta=0.33$, $p<0.0001$). When observing the impact of potential confounding variables, age ($p=0.67$), sex ($p=0.78$), years of practice as a dietitian ($p=0.11$) and the number of patient/client per week ($p=0.10$) did not contribute to explain the intention to discuss nutrigenetics with the patient/client. However, education levels ($p=0.09$) and area of practice ($p=0.04$) were associated with intention ($p<0.10$). To take into account their impact on intention, these two confounding variables were then included into the regression model with the direct TPB constructs. Neither of the confounding variables contributed significantly to explain the intention (education levels, $p=0.52$ and area of practice, $p=0.44$) of dietitians to discuss nutrigenetics with their patients/clients.

The second regression model included the salient beliefs related to attitude, subjective norm and perceived behavioral control. According to Mallow's C_p statistic, only six salient beliefs were included into the multiple linear regression model. Of these six salient beliefs, four were associated with intention (Table 10.5). The influence of employers was important ($\beta=0.31$, $p=0.0005$) followed by the behavioral belief that discussing nutrigenetics with their patients/clients would increase the efficacy of dietary interventions ($\beta=0.26$, $p<0.0001$). Two control beliefs also contributed to the model, «If my patient/client would be interested» and «If I had enough knowledge/training» ($\beta=0.18$, $p=0.0001$ and $\beta=0.17$, $p=0.008$, respectively). Approximately, 30% of dietitians mentioned that their employers would approve the fact that they discuss nutrigenetics with their patients/client whereas most dietitians (~54%) thought that employers would not approve or disapprove. Approximately, 66% of dietitians agreed or strongly agreed that discussing nutrigenetics

with their patients/clients would increase the efficacy of dietary interventions. Most dietitians (~71%) agreed or strongly agreed that if their patients/clients would be interested they would discuss nutrigenetics with them. Finally, the majority also agreed or strongly agreed (~79%) that with enough knowledge/training they would discuss nutrigenetics with their patients/clients.

Finally, 13 out of 141 dietitians (~9%) reported practicing the behavior which was to discuss nutrigenetics with their patients/clients in the last three months. To better understand the importance of intention and perceived behavioral control on the behavior, a third multiple linear regression model was performed. As shown in Figure 10.1, only perceived behavioral control contributed to explain the behavior ($\beta=0.17$, $p<0.0001$). This regression model explained 29% (R^2) of the variance in the behavior.

Discussion

To our knowledge, factors affecting dietitians' intention of discussing nutrigenetics with their patients/clients have never been studied using a theoretical framework such as the TPB. The results from this study indicate that all three direct constructs (attitude, subjective norm and perceived behavioral control) of the TPB affect dietitians' intention to discuss nutrigenetics with their patients/clients. The attitude towards the behavior was the most influent TPB construct in predicting intention, followed by perceived behavioral control and subjective norm. Most dietitians had a slightly positive attitude towards discussing nutrigenetics with their patients/clients.

Among indirect constructs of attitude, the behavioral belief that discussing nutrigenetics with the patient/client would increase the efficacy of dietary interventions was the most important determinant of intention. Most dietitians agreed or strongly agreed that discussing nutrigenetics with their patients/clients would increase the efficacy of dietary interventions. These results are consistent with the work of Rosen *et al*. (Rosen *et al*., 2006) which have observed that most dietitians (75%) agreed that applying nutrigenomics would result in nutritional interventions which could help to better manage/prevent certain diseases. A few studies have observed that dietary recommendations provided by dietitians based on genetics may further motivate patients/clients to follow the recommendations provided (Nielson & El-Sohemy, 2012; Arkadianos *et al*., 2007). Arkadianos *et al*. (Arkadianos *et al*., 2007) also observed an increase in the efficacy of a weight loss program among individuals receiving nutrigenetic tailored advices.

Two of the four control beliefs predicted the intention to discuss nutrigenetics with their patients/clients. Having enough knowledge/training was important in predicting the intention. A lack of knowledge in genetics and/or nutritional genomic among dietitians has been reported frequently (Collins *et al*., 2013; McCarthy *et al*., 2008; Whelan *et al*., 2008; Cormier *et al*., 2014). Associations between knowledge levels and confidence levels in discussing genetics and/or nutritional genomics have been reported among dietitians from the UK (Whelan *et al*., 2008). Increased knowledge levels are also associated with

increased professional activities related to genetics and/or nutritional genomics such as discussing with patients of the genetic basis of a disease, providing training or education to students/health professionals related to genetics and/or nutritional genomics and discussing with patients how diet may interact with genes to affect the risk of disease (Whelan *et al*., 2008). In this study, most dietitians reported that they would discuss nutrigenetics with their patients/clients if they had enough knowledge/training. However, levels of knowledge are not always associated with greater confidence levels and the degree of involvement in genetics and/or nutritional genomics activities (Collins *et al*., 2013). This may indicate that other barriers and factors may affect the confidence and involvement levels of dietitians in activities related to genetics and/or nutritional genomics. In our study, another important control belief in predicting the intention was related to the interest of the patient/client in nutrigenetics, indicating that if patients/clients showed interest, dietitians would have a greater intention to discuss nutrigenetics with them. It is likely that the interest of the public in nutrigenetics/nutrigenomics will increase in the future. The availability of direct-to-consumer (DTC) personalized genomic tests and DTC nutrigenetic tests has increased during the years 2000 and in 2010 more than 30 DTC companies offered approximately 400 health related tests (Camp & Trujillo, 2014; Bloss *et al*., 2011). Most of the consumers were interested in DTC personalized genomic tests out of curiosity and to increase their knowledge about their genetic profile (Bloss *et al*., 2011). Nielsen *et al*. (Nielson & El-Sohemy, 2012) also reported that dietary recommendations based on nutrigenetic tests were perceived by individuals as more understandable and useful than general dietary recommendations.

In this study, only one of the normative beliefs, the employers' approval/disapproval, influenced the intention of dietitians to discuss of nutrigenetics with their patients/clients. However, most dietitians mentioned that their employers would currently not approve or disapprove that they discuss nutrigenetics with their patients/clients. Thus, even if their employer's approval/disapproval is important, this may currently not have an important impact on the intention of dietitians to discuss nutrigenetics with their patients/clients.

Few dietitians did actually discuss nutrigenetics with their patients/clients in the last three months (~9%). Only perceived behavioral control contributed to explain part of the variance in dietitians' already discussing nutrigenetics with their patients/clients. Thus, it seems that perceived behavioral control is more important than intention to predict this behavior among this sample of dietitians. Most dietitians had a slightly low perceived behavioral control score (-0.53±0.79). The notion of perceived behavioral control, as described by Ajzen (Ajzen, 2002), *refers to the perceived ease or difficulty of performing the behavior*. The fact that only perceived behavioral control is associated with the behavior may indicate that this behavior is under partial volitional control and that external factors may have an impact on this behavior (Ajzen, 2002). For example, nutrigenetics is quite a new concept for dietitians and they may feel that they need «external» aid in obtaining more education to develop the skills necessary to discuss nutrigenetics with their patients/clients (Camp & Trujillo, 2014). For example, the lack of professional expertise for continuing education may be an important external barrier (Rosen *et al*., 2006). Moreover, this eventual field of practice in nutrigenetics is still in its infancy. There are many obstacles to overcome such as ethical considerations and validation of the findings, before the implementation of nutrigenetics into dietitians' practice (Camp & Trujillo, 2014; Gorman *et al*., 2013).

Limits

The low response rate (5.8%) of this study may limit its generalisation among all dietitians of the province of Quebec. A previous survey conducted a year earlier by our research group among dietitians members of OPDQ focusing on more general aspects of nutrigenomics had a response rate of 13.5% Cormier *et al*., 2014). It is possible that our response rate was lower because dietitians thought that this survey was the same or found a lack of relevance. As examined by Godin *et al*. (Godin *et al*., 2008) studies with 150 participants or more had better prediction capabilities of intention or behavior when using TPB. Thus, 141 participants were deemed acceptable. Response rates obtained by online surveys are usually lower than response rates obtained by postal surveys (Shih & Fan, 2009), however they can reach easily with lower costs a wide range of participants. Moreover, we cannot rule out the possibility of a positive response bias among the

responders. However, the descriptive characteristics of dietitians who completed this survey were quite similar to those who had responded our previous survey conducted a year earlier (Cormier *et al*., 2014). Still, small differences were noticed, dietitians from this study had generally less years of experience as dietitians, a greater proportion worked in clinical nutrition in private settings and a smaller proportion from academic/research area.

Conclusion

To our knowledge this study is the first to use a theoretical framework such as the TPB to investigate determinants of dietitians' intention to discuss nutrigenetics with their patients/clients and the actual behavior. The intention of the dietitians to discuss of nutrigenetics with their patients/clients was mostly influenced by their attitude towards the behavior. Interestingly, only perceived behavioral control had an influence on the behavior of discussing nutrigenetics with their patients/clients. Dietitians need to overcome many barriers before they can implement nutrigenetics into their professional practice. The lack of knowledge and/or training and the interest of the patient/client may be two important barriers for dietitians to discuss nutrigenetics with the patient/client. Thus, the development of educational content about nutrigenetics specific for dietitians is needed.

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References

Ajzen, I. (2006) Constructing a TPB Questionnaire : Conceptual and Methodological Considerations. Available at: http://people.umass.edu/aizen/pdf/tpb.measurement.pdf (accessed on 20 June 2014).

Ajzen, I. (2002) Perceived behavioral control, self-efficacy, locus of control, and the theory of planned behavior. Journal of Applied Social Psychology 32, 665-683.

Arkadianos, I., Valdes, A.M., Marinos, E., Florou, A., Gill, R.D. & Grimaldi, K.A. (2007) Improved weight management using genetic information to personalize a calorie controlled diet. Nutr J 6, 29.

Beaulieu, D. & Godin, G. (2012) Development of an intervention programme to encourage high school students to stay in school for lunch instead of eating at nearby fast-food restaurants. Eval Program Plann 35, 382-389.

Bloss, C.S., Darst, B.F., Topol, E.J. & Schork, N.J. (2011) Direct-to-consumer personalized genomic testing. Hum Mol Genet 20, R132-R141.

Bouchard, C. & Ordovas, J.M. (2012) Fundamentals of nutrigenetics and nutrigenomics. Prog Mol Biol Transl Sci 108, 1-15.

Camp, K.M. & Trujillo, E. (2014) Position of the Academy of Nutrition and Dietetics: nutritional genomics. J Acad Nutr Diet 114, 299-312.

Casper, E.S. (2007) The theory of planned behavior applied to continuing education for mental health professionals. Psychiatr Serv 58, 1324-1329.

Chase, K., Reicks, M. & Jones, J.M. (2003) Applying the theory of planned behavior to promotion of whole-grain foods by dietitians. J Am Diet Assoc 103, 1639-1642.

Collins, J., Bertrand, B., Hayes, V., Li, S.X., Thomas, J., Truby, H. & Whelan, K. (2013) The application of genetics and nutritional genomics in practice: an international survey of knowledge, involvement and confidence among dietitians in the US, Australia and the UK. Genes Nutr 8, 523-533.

Corella, D. & Ordovas, J.M. (2012) Interactions between dietary n-3 fatty acids and genetic variants and risk of disease. Br J Nutr 107 Suppl 2, S271-S283.

Cormier, H., Tremblay, B.L., Paradis, A.M., Garneau, V., Desroches, S., Robitaille, J. & Vohl, M.C. (2014) Nutrigenomics - perspectives from registered dietitians: a report from the Quebec-wide e-consultation on nutrigenomics among registered dietitians. J Hum Nutr Diet.

de Roos, B. (2013) Personalised nutrition: ready for practice? Proc Nutr Soc 72, 48-52.

Edwards, H., Walsh, A., Courtney, M., Monaghan, S., Wilson, J. & Young, J. (2007) Promoting evidence-based childhood fever management through a peer education programme based on the theory of planned behaviour. J Clin Nurs 16, 1966-1979.

Fenech, M., El-Sohemy, A., Cahill, L., et al. (2011) Nutrigenetics and nutrigenomics: viewpoints on the current status and applications in nutrition research and practice. J Nutrigenet Nutrigenomics 4, 69-89.

Gagne, C. & Godin, G. (1999) Les théories sociales cognitives: guide pour la mesure des variables et le développement de questionnaire. Available at: http://www.godin.fsi.ulaval.ca/Fichiers/Rapp/Guide_mesure_variables.pdf (accessed on 12 May 2014).

Godin, G., Belanger-Gravel, A., Eccles, M. & Grimshaw, J. (2008) Healthcare professionals' intentions and behaviours: a systematic review of studies based on social cognitive theories. Implement Sci 3, 36.

Gorman, U., Mathers, J.C., Grimaldi, K.A., Ahlgren, J. & Nordstrom, K. (2013) Do we know enough? A scientific and ethical analysis of the basis for genetic-based personalized nutrition. Genes Nutr 8, 373-381.

Guttmacher, A.E., Jenkins, J. & Uhlmann, W.R. (2001) Genomic medicine: who will practice it? A call to open arms. Am J Med Genet 106, 216-222.

Liu, J.X., Liu, J., Li, P.Q., Xie, X.D., Guo, Q., Tian, L.M., Ma, X.Q., Zhang, J.P., Liu, J. & Gao, J.Y. (2008) Association of sterol regulatory element-binding protein-1c gene polymorphism with type 2 diabetes mellitus, insulin resistance and blood lipid levels in Chinese population. Diabetes Res Clin Pract 82, 42-47.

Mallows, C.L. (1973) Some Comments on Cp. pp. 661-675: Technometrics.

McCarthy, S., Pufulete, M. & Whelan, K. (2008) Factors associated with knowledge of genetics and nutritional genomics among dietitians. J Hum Nutr Diet 21, 547-554.

Nielsen, D.E. & El-Sohemy, A. (2012) A randomized trial of genetic information for personalized nutrition. Genes Nutr 7, 559-566.

Patterson, R.E., Eaton, D.L. & Potter, J.D. (1999) The genetic revolution: change and challenge for the dietetics profession. J Am Diet Assoc 99, 1412-1420.

Perkins, M.B., Jensen, P.S., Jaccard, J., Gollwitzer, P., Oettingen, G., Pappadopulos, E. & Hoagwood, K.E. (2007) Applying theory-driven approaches to understanding and modifying clinicians' behavior: what do we know? Psychiatr Serv 58, 342-348.

Phillips, C.M. (2013) Nutrigenetics and metabolic disease: current status and implications for personalised nutrition. Nutrients 5, 32-57.

Ries, N.M. & Castle, D. (2008) Nutrigenomics and ethics interface: direct-to-consumer services and commercial aspects. OMICS 12, 245-250.

Rosen, R., Earthman, C., Marquart, L. & Reicks, M. (2006) Continuing education needs of registered dietitians regarding nutrigenomics. J Am Diet Assoc 106, 1242-1245.

Sarwar, N., Danesh, J., Eiriksdottir, G., Sigurdsson, G., Wareham, N., Bingham, S., Boekholdt, S.M., Khaw, K.T. & Gudnason. V. (2007) Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. Circulation 115, 450-458.

Shih, T.H. & Fan, X. (2009) Comparing response rates in e-mail and paper surveys: A meta-analysis. Educational Research Review.

Whelan, K., McCarthy, S. & Pufulete, M. (2008) Genetics and diet--gene interactions: involvement, confidence and knowledge of dietitians. Br J Nutr 99, 23-28.

Woolf, L.I., Griffiths, R. & Moncrieff, A. (1955) Treatment of phenylketonuria with a diet low in phenylalanine. Br Med J 1, 57-64.

Table 10.1 Descriptive characteristics of the study population.

Variables	**n=141** **n (%)**
Sex (women/men)	134/7
Age	
≤25	30 (21.3)
26-30	40 (28.4)
31-40	29 (20.6)
41-50	26 (18.4)
≥51	16 (11.4)
Level of education	
Undergraduate degree in nutrition	92 (66.2)
Master in nutrition	22 (15.8)
Ph.D. in nutrition	2 (1.4)
Other graduate studies	18 (12.8)
Years of experience as dietitians	
≤5	70 (50.7)
6-10	19 (13.8)
11-20	23 (16.7)
≥21	26 (18.8)
Area of practice	
Clinical nutrition in public health settings	82 (59.4)
Clinical nutrition in private practice	21 (15.2)
Food service management	4 (2.9)
Public health nutrition/community nutrition	17 (12.3)
Academic	2 (1.5)
Communications/public relations/journalism	2 (1.5)
Research	8 (5.8)
Representation	-
Industrial	-
Other	2 (1.5)
Urban centres	
Capitale-Nationale (Quebec City)	35 (24.8)
Montreal	39 (27.7)
Monteregie	20 (14.2)
Elsewhere in the province of Quebec	54 (38.3)

Table 10.2 Internal consistency.

Variables	Number of items	Alpha coefficients (Cronbach)
Intention (n=140)	3	.87
Attitude (n=138)	4	.85
Subjective norm (n=141)	3	.65
Perceived behavioural control (n=138)	4	.70

Table 10.3 Descriptive statistics of the TPB direct constructs and salient beliefs.

Construct	Mean	SD
Intention (n=140)	-0.07	0.92
Attitude (n=138)	0.41	0.65
Subjective norm (n=141)	-0.22	0.65
Perceived behavioral control (n=138)	-0.53	0.79
Behavioral beliefs		
Hard to understand for my patients/clients (n=141)	0.79	1.03
Could help to further personalise dietary recommendations (n=141)	0.92	0.95
Would increase the efficacy of the dietary interventions (n=140)	0.57	1.07
Would create a feeling of powerlessness among the patients/clients (n=141)	0.16	1.05
Would be hard to explain to the patients/clients (n=140)	0.65	1.05
Normative beliefs		
Researchers (n=141)	0.50	0.74
Other dietitians (n=141)	0.43	0.64
Employers (n=141)	0.31	0.78
Fellow workers (n=140)	0.30	0.72
Control beliefs		
If I had enough knowledge/training (n=141)	0.90	1.02
If my patient/client would be interested (n=141)	0.65	1.23
If I had clinical tools about nutrigenetics (n=141)	1.05	0.90
I would lack time to discuss nutrigenetics (n=141)	0.28	1.15

Means ± SD.

Table 10.4 Correlation matrix of the variables.

	Intention (n=140)	**Attitude (n=138)**	**Subjective norm (n=141)**	**Behavioral control (n=138)**
Mean	**-0.07**	**0.41**	**-0.22**	**-0.53**
St. Dev.	**0.92**	**0.65**	**0.65**	**0.79**
Intention	-			
Attitude	0.74 (<0.0001[1]) (n=137)	-		
Subjective norm	0.62 (<0.0001[1]) (n=140)	0.65 (<0.0001[1]) (n=138)	-	
Behavioral control	0.64 (<0.0001[1]) (n=138)	0.58 (<0.0001[1]) (n=136)	0.52 (<0.0001[1]) (n=138)	-

[1]P-values were determined using Pearson correlations.

Table 10.5 Factors influencing the intention to discuss nutrigenetics with the patient/client.

Beliefs	Coefficient β[1]	P-value[1]
Behavioral beliefs		
Hard to understand for my patients/clients	0.05	0.37
Could help to further personalise dietary recommendations	-	-
Would increase the efficacy of the dietary interventions	0.26	<0.0001
Would create a feeling of powerlessness among the patients/clients	-	-
Would be hard to explain to the patients/clients	-	-
Normative beliefs		
Researchers	-	-
Other dietitians	-	-
Employers	0.31	0.0005
Fellow workers	0.02	0.82
Control beliefs		
If I had enough knowledge/training	0.17	0.008
If my patient/client would be interested	0.18	0.0001
If I had clinical tools about nutrigenetics	-	-
I would lack time to discuss nutrigenetics	-	-

$R^2 = 0.61$

[1]β coefficients and P-values were determined using a multiple linear regression model.

Figure 10.1 Specified TPB framework for predicting intention and behavior of dietitians to discuss nutrigenetics with the patient/client.

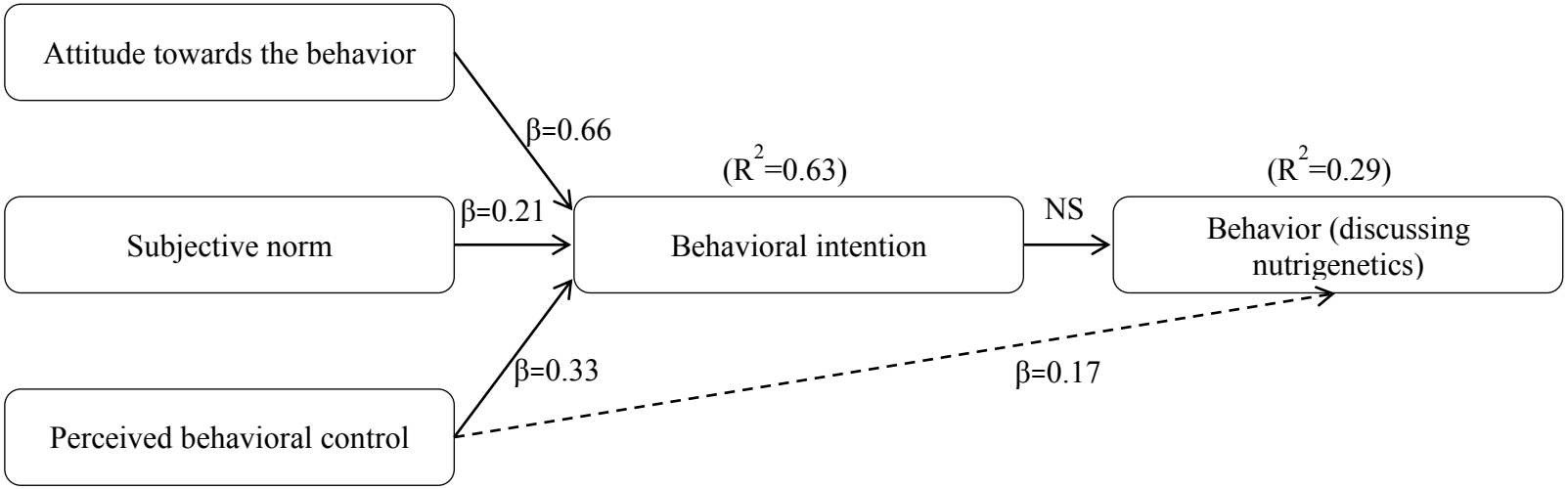

Figure legend. β coefficients were determined using a multiple linear regression

Chapitre 11 :
Conclusion

Ce projet de doctorat avait pour objectif global d'employer la science de la génomique nutritionnelle afin de mieux comprendre l'impact de profils alimentaires sur la santé métabolique et la variabilité de la réponse des facteurs de risque de MCV à une supplémentation en huile de poisson. La combinaison de plusieurs approches à différents niveaux d'intégration telles que l'étude entre autres, de l'expression génique, des protéines et des métabolites permet de mieux comprendre les systèmes biologiques [238].

Dans ce projet de doctorat, nous avons utilisé l'approche de la biologie des systèmes en observant l'effet des profils alimentaires à deux niveaux, soit sur les profils d'expression génique (transcriptomique) et sur les profils de métabolites (métabolomique). Autant les profils d'expression génique que les profils de métabolites étaient différents en fonction des scores aux profils alimentaires Prudent et Western. Très peu d'études ont jusqu'à maintenant observé l'impact de profils alimentaires sur les profils d'expression ou les profils de métabolites. Jusqu'à maintenant, la diète Méditerranéenne a été le type d'alimentation le plus étudié en lien avec l'expression génique. Les gènes ou les profils d'expression génique modifiés en lien avec la diète Méditerranéenne sont reliés à des voies métaboliques telles que la réponse inflammatoire et l'athérosclérose [83-85]. L'inflammation est un point commun aux MCV, au diabète et au cancer [239]. Divers composés alimentaires ont été démontrés comme affectant les concentrations de différents marqueurs de l'inflammation tels que les protéines encodées par les gènes *C-reactive protein* (*CRP*), *tumor necrosis factor alpha* (*TNFA*) et *interleukin 6* (*IL6*) [240,241]. Ces protéines pro-inflammatoire seraient augmentées entre autres lorsque l'alimentation est élevée en sucres ajoutés et/ou en gras saturés [240,241], deux composantes retrouvées dans le profil alimentaire Western [38]. D'autre part, une alimentation riche en légumes et en fruits telle que le profil alimentaire Prudent, serait inversement associée aux concentrations de CRP [240]. Dans le cadre de ce projet de doctorat, les résultats observés corroborent ceux de la littérature. Nous avons observé que l'expression de gènes dans des voies métaboliques reliées à la réponse inflammatoire, à l'immunité, au cancer et à l'athérosclérose était différente en fonction des scores aux profils alimentaires Prudent et Western. Des associations entre les scores au profil alimentaire Prudent et les concentrations de CRP et IL6 ont d'ailleurs été remarquées dans la cohorte FAS (n=210).

Les individus ayant les scores les plus élevés au profil alimentaire Prudent avaient des concentrations de CRP et IL6 plus basses que les individus ayant des scores bas au profil alimentaire Prudent (p=0,04 et p=0,05, respectivement) indépendamment de l'effet de l'âge, du sexe et de l'IMC (données non publiées). Conséquemment, il est possible que les profils d'expression génique associés aux profils alimentaires aient affecté les concentrations des protéines inflammatoires (CRP et IL6). Il a été observé que lorsque des adipocytes sont exposés à l'acide palmitique, un acide gras saturé, l'expression du gène *IL6* et la production de la protéine IL6 est augmentée, possiblement sous l'influence du facteur de transcription NFKB [242]. Dans nos résultats, la voie de signalisation de NFKB était significativement différente chez les femmes en fonction du score au profil alimentaire Western. De plus, les scores au profil alimentaire Western étaient associés avec un profil de métabolites caractérisé par des concentrations plus élevées de certains acides aminés, tels que les acides aminés aromatiques et ramifiés, et de certains acylcarnitines à courtes chaînes. Lorsque l'on examinait les associations isolées des macronutriments avec les profils de métabolites, ce sont les acides gras saturés qui étaient les plus associés avec le profil de métabolite caractérisé par la présence d'acides aminés aromatiques et ramifiés de même que de certains acylcarnitines à courtes chaînes. Dans la littérature, ce type de signature métabolique tout comme le profil alimentaire Western sont associés à des conditions telles que l'obésité, le diabète de type 2 de même qu'à un métabolisme altéré, c'est-à-dire la présence de glucose à jeun élevé, d'hypertension artérielle, d'hypertriglycéridémie, de C-HDL bas et/ou de résistance à l'insuline [59,111-113,117-120,243,244]. Ces conditions sont également associées à la présence d'inflammation chronique [239]. Conséquemment, un profil de métabolites caractérisé par des concentrations plus élevées d'acides aminés aromatiques et ramifiés et d'acylcarnitines à courtes chaînes pourrait découler de l'impact du profil alimentaire Western sur l'expression de gènes impliqués dans les voies métaboliques de l'inflammation, de l'immunité, de l'athérosclérose et du cancer de même que sur le métabolisme subséquent des protéines.

Outre l'impact de l'alimentation sur les profils d'expression et de métabolites, la présence de variations génétiques telles que les SNPs affecte la réponse des individus à divers composés alimentaires. L'étude de ces phénomènes se nomme nutrigénétique. Dans le

cadre de ce projet de doctorat, une supplémentation en huile de poisson a été réalisée afin d'étudier la variabilité interindividuelle de la réponse des facteurs de risque des MCV, plus particulièrement, la réponse des concentrations de TG. L'effet hypotriglycéridémiant de l'huile de poisson est bien connu [245]. Notre équipe de recherche s'est penchée sur différentes voies métaboliques afin de mieux comprendre la variabilité interindividuelle observée dans la réponse des concentrations de TG suite à la supplémentation en huile de poisson. L'huile de poisson, dont les principaux constituants sont l'AEP et l'ADH, des AGPI n-3 à très longues chaînes, a des impacts à de nombreux niveaux. Entre autres, les AGPI n-3 à très longues chaînes, s'incorporent dans les parois des membranes cellulaires affectant la fluidité des membranes cellulaires [212]. Ces types d'acides gras sont également des précurseurs des eicosanoïdes, des dérivés ayant des propriétés anti-inflammatoires [212]. Les AGPI n-3 de même que leurs dérivés sont des ligands des facteurs de transcriptions nommés PPARs et affectent l'expression d'autres facteurs de transcriptions tels que le gène *SREBF1* [211].

Les résultats de ce projet de doctorat démontrent que la variabilité génétique présente dans certains gènes impliqués dans la voie de la lipogénèse de novo, spécifiquement *ACLY* et *ACACA*, affecterait la réponse des concentrations de TG à la supplémentation en huile de poisson. Il a été observé que l'expression de ces deux gènes est diminuée chez des rats recevant une alimentation contenant des AGPI n-3 sous forme de phospholipides [246]. De plus, l'AEP et l'ADH ont tous deux pour effet de diminuer l'expression du gène *SREBF1*, le facteur de transcription régulant la lipogénèse de novo [128]. D'ailleurs, une diminution de la lipogénèse de novo a été observée chez des rats suite à une supplémentation en huile de poisson en calculant la proportion de nouveaux lipides produits par le foie et ne provenant pas de la diète [247]. Dans le cadre de ce projet, nous n'avons toutefois pas observé de changements dans les niveaux d'expression du gène *SREBF1*. Il est possible que la mesure de l'expression du gène *SREBF1* dans les PBMCs comme substitut à la mesure directe de l'expression dans les cellules hépatiques puisse expliquer cette absence de différence. Effectivement, cet effet des AGPI sur l'expression du gène *SREBF1* a été observé dans les cellules hépatiques [25,248]. Toutefois, une étude observant l'effet de l'atorvastatine, un médicament hypocholestérolémiant, sur l'expression du gène *SREBF1* a

observé une diminution des concentrations d'ARMm dans les PBMCs suite à la prise de 10mg/jour durant quatre semaines d'atorvastatine [249]. Liu et collaborateurs [250] ont observé que 83% des gènes exprimés dans le foie l'étaient également dans les PBMCs, ce qui en fait un substitut intéressant et moins invasif que la mesure directe de l'expression dans les cellules du foie. De plus, l'état de santé métabolique pourrait affecter les changements observés dans les niveaux d'expression génique. Il a été observé que la mesure des niveaux d'expression du gène *SREBF1* dans les PBMCs reflétait adéquatement les changements alimentaires chez des rats d'un groupe contrôle et que ces différences n'étaient pas observables chez des rats obèses [251]. Schmidt et collaborateurs [252] ont plutôt observé que les différences dans l'expression génique mesurée dans les cellules sanguines entières (*whole blood*) des gènes reliés au métabolisme des lipides étaient plus marquées chez des hommes dyslipidémiques comparativement aux hommes normo-lipidémiques suite à une supplémentation de 6 semaines de 2,7g/jour d'AEP+ADH. La mesure de l'expression génique dans les cellules sanguines entières et dans les PBMCs serait comparable pour les gènes reliés aux facteurs de risque des MCV [253]. Ces résultats indiqueraient que la mesure de l'expression du gène *SREBF1* dans les PBMCs constituerait un substitut valide à la mesure de l'expression directe dans les cellules hépatiques. Conséquemment, il est possible que tel que dans l'étude de Schmidt et collaborateurs [252], les changements d'expression génique chez des individus normo-lipidémiques suite à une supplémentation en huile de poisson soient trop infimes pour être observables et qu'en étudiant des participants dyslipidémiques, une différence dans les niveaux d'expression génique aurait été observée.

Le facteur de transcription SREBP1 régule également la voie métabolique de la glycolyse [254]. Par contre, contrairement à ce qui avait été observé il y a de cela quelques années [255], SREBP1 ne semble pas nécessaire à l'activation de GCK, une enzyme limitante qui convertit le glucose en glucose-6-phosphate [254]. Malgré tout, étant donné la corrélation positive entre l'activité de la lipogénèse de novo et l'activité de GCK, le gène *GCK* était un candidat potentiellement intéressant à étudier en lien avec la réponse des concentrations de TG suite à une supplémentation en huile de poisson. De plus, dans le foie, les gènes *GCK* et *SREBF1* sont tous deux activés par l'action de l'insuline [256]. Dans le cadre de ce projet

de doctorat, nous avons observé un effet d'interaction gène-diète, entre le gène *GCK* (rs741038) et les apports alimentaires en glucides, affectant la réponse des concentrations de TG suite à la supplémentation en huile de poisson. Étant donné les corrélations positives existant entre les concentrations d'ARNm du gène *GCK*, la lipogénèse de novo et les concentrations de TG hépatiques, nos résultats indiquent que la variabilité génétique présente dans le gène *GCK* en fonction des apports alimentaires en glucides affecterait la réponse des TG suite à une supplémentation en huile de poisson [257]. Le mécanisme exact expliquant cette association n'est pas connu. Il est possible que l'impact de la variabilité génétique du gène *GCK* dans la réponse des TG soit en lien avec l'effet de l'huile de poisson sur la sensibilité à l'insuline. Toutefois, l'effet de l'huile de poisson sur les concentrations d'insuline, la sensibilité à l'insuline et le diabète de type 2 demeure controversé [258]. Dans le cadre de ce projet de recherche, les concentrations d'insuline de même que la sensibilité à l'insuline n'ont pas été modifiées tandis que les concentrations de glucose ont légèrement augmenté suite à la supplémentation en huile de poisson. Tel que mentionné précédemment, bien que les niveaux d'expression du gène *SREBF1* sont demeurés inchangés suite à la supplémentation en huile de poisson, la variabilité génétique présente dans le gène *SREBF1* était associée avec la réponse des concentrations d'insuline à jeun et la sensibilité à l'insuline. Conséquemment, il est possible que la présence de différents SNPs dans le gène *SREBF1* module l'impact de l'huile de poisson et ainsi modifie l'activation du gène *SREBF1* et la voie de la lipogénèse de novo. Ces changements moduleraient à leur tour la sensibilité à l'insuline hépatique et également la sécrétion pancréatique d'insuline selon une théorie nommée lipotoxicité [259,260]. La lipotoxicité découle d'une accumulation d'acides gras et leurs intermédiaires dans les tissus non-adipeux [261-263]. Par exemple, l'augmentation de la présence d'acides gras dans le foie pourrait augmenter le stress oxydatif et le stress au niveau du réticulum endoplasmique [263]. Au niveau du pancréas, l'exposition des cellules bêta aux acides gras pourrait engendrer un stress oxydatif au niveau du réticulum endoplasmique et éventuellement conduire à l'apoptose [262].

Outre la voie métabolique de la lipogénèse de novo, nous avons également étudié la voie de la bêta-oxydation des acides gras qui serait augmentée suite à la prise d'huile de poisson, ce

qui aurait pour effet de diminuer la disponibilité des acides gras pour la synthèse des TG et leur sécrétion subséquente sous la forme de particules VLDL [165]. Contrairement à notre hypothèse de départ, aucune association entre des SNPs présents dans des gènes impliqués dans la voie de la bêta-oxydation des acides gras et la réponse des TG plasmatiques suite à la supplémentation en huile de poisson n'a été observée. Toutefois, lorsque les apports alimentaires en gras totaux, saturés et polyinsaturés ont été considérés, des effets d'interaction gène-diète ont pu être constatés, principalement pour le gène *RXRA*. Ce gène encode une protéine qui forme un hétérodimère avec le facteur de transcription PPARA et ensuite régule l'activité de nombreux gènes impliqués dans le transport des acides gras à travers la membrane cellulaire, l'activation des acides gras, l'oxydation des acides gras, la cétogénèse, l'entreposage des TG et la lipolyse [264]. Conséquemment, bien que les effets d'interaction gène-diète observés sur la réponse des TG suite à la supplémentation en huile de poisson soient possiblement dus à une augmentation des voies mitochondriale et peroxisomale de la bêta-oxydation des acides gras, il n'est pas exclut que ces effets soient également dus à l'impact de l'hétérodimère PPARA-RXRA sur d'autres voies métaboliques. Par exemple, plusieurs gènes cibles du facteur de transcription PPARA sont impliqués dans la synthèse des TG tels que les gènes *glycerol-3-phosphate acyltransferase, mitochondrial* (*GPAM*) et *diacylglycerol O-acyltransferase 1* (*DGAT1*) [264]. D'ailleurs, des résultats de notre équipe de recherche, démontrent un effet d'interaction gène-diète entre la supplémentation en huile de poisson et le gène *GPAM* affectant les concentrations de TG [265]. De plus, le facteur de transcription PPARA module également la clairance des lipoprotéines riches en TG. Entre autres, PPARA diminue l'expression du gène *apolipoprotein C-III* (*APOC3*), un inhibiteur majeur de la lipoprotéine lipase (LPL) et augmente l'expression du gène *apolipoprotein A-V* (*APOA5*) qui régule positivement la LPL [264].

Ce projet de doctorat a examiné entre autres l'impact de la variabilité génétique de gènes modulés par les facteurs de transcriptions *SREBF1* et *PPARA*. Toutefois, d'autres facteurs de transcription pourraient être intéressants à étudier en lien avec la réponse des concentrations de TG suite à une supplémentation en huile de poisson. Par exemple, le facteur de transcription *MLX interacting protein-like* (*MLXIPL*), plus communément appelé

carbohydrate responsive element-binding protein (*ChREBP*), se fixe aux éléments de réponse nommés *carbohydrate response element* et module l'expression de gènes impliqués dans le transport du glucose, la glycolyse, la lipogénèse de novo, l'élongation et la désaturation des acides gras de même que la synthèse des TG [211,266]. Les facteurs de transcription liver X receptors (LXRs) encodés par les gènes *NR1H3* et *NR1H2*, respectivement LXRA et LXRB, sont régulés par les oxystérols et des dérivés du métabolisme du cholestérol mais également par les acides gras [267]. Ces derniers régulent l'activité de nombreux gènes qui sont impliqués dans les métabolismes du cholestérol, des lipides de même que de l'homéostasie du glucose [267]. D'autres facteurs de transcription potentiellement impliqués dans la réponse des concentrations de TG à l'huile de poisson seraient *forkhead box O1* (*FOXO1*) et *forkhead box O3* (*FOXO3*). Il a été observé que l'expression de ces deux facteurs de transcription était diminuée par l'ADH [268]. Le facteur de transcription FOXO1 entre autres régule l'expression du gène de l'*APOC3* [268]. L'effet des AGPI n-3 sur le métabolisme des lipides pourrait également être partiellement médié par leurs impacts sur les microARNs, qui sont de courts fragments d'ARN non-codante régulant l'expression génique [269]. Il a été observé que l'ADH régulait l'expression de microARNs modulant l'expression de nombreux gènes impliqués dans le métabolisme des lipides et du cancer [269].

Mis à part l'approche des gènes candidats, nous avons également étudié certains SNPs identifiés préalablement lors d'un GWAS [270]. Les GWAS permettent d'étudier de nouvelles associations entre des SNPs et un trait en particulier, dans le cas présent, le statut de répondeur ou non-répondeur à une supplémentation en huile de poisson. Brièvement, les répondeurs et les non-répondeurs avaient été classifiés en fonction de la réponse relative des concentrations de TG suite à la supplémentation en huile de poisson (répondeur<0% et non-répondeur≥0%). Treize SNPs avaient été identifiés dans l'étude GWAS menée dans la cohorte FAS [270]. De ces treize SNPs, quelques associations ont été observées avec les concentrations de TG et d'AGPI n-3 total de même que l'AEP et l'ADH dans les phospholipides du plasma dans la cohorte INFOGENE. INFOGENE est une cohorte où aucune supplémentation en huile de poisson n'a été fournie aux participants. Conséquemment, la présence d'associations dans cette cohorte avec les SNPs identifiés

précédemment renforce le fait que des SNPs près ou à l'intérieur des gènes *NELL1*, *NXPH1*, *SLIT2*, *JADE1* et/ou *IQCJ-SCHIP1* peuvent affecter les concentrations de TG soit directement ou par le biais d'effets d'interaction gène-diète de même que les concentrations d'AGPI n-3 dans les phospholipides du plasma. Les fonctions de ces gènes en lien avec le métabolisme des AGPI n-3 et des lipides sanguins sont encore très peu connues, conséquemment davantage d'études devront être réalisées pour avoir une meilleure vision d'ensemble.

Bien que les travaux décrits précédemment fussent essentiels, ce projet de doctorat n'aurait pas été complet sans un volet d'application des connaissances. L'application des connaissances permet entre autres le transfert et l'utilisation optimale de l'information issue des recherches scientifiques vers les professionnels de la santé ou encore la population. Dans le cadre de ce projet, nos travaux se sont concentrés sur l'étude des déterminants de l'intention et du fait de réaliser le comportement pour les diététistes du Québec de discuter de nutrigénétique avec leurs patients/clients. Nos résultats ont démontré que l'attitude des diététistes était déterminante dans leur intention de discuter de nutrigénétique avec le patient/client. Toutefois, la perception de contrôle et la norme subjective s'avèrent également des déterminants significatifs de l'intention des diététistes de discuter de nutrigénétique avec leurs patients/clients. Un autre aspect intéressant était que seule la perception de contrôle affectait le comportement de discuter de nutrigénétique avec le patient/client. Conséquemment, la perception de contrôle serait un point à clé à travailler éventuellement afin de permettre aux diététistes de discuter adéquatement de nutrigénétique avec leurs patients/clients. La création d'un outil tel qu'une boîte à décision [271] spécifique pour les diététistes pourrait contribuer à améliorer la perception de contrôle des diététistes. La boîte à décision, telle que décrite par Giguère et collaborateurs [271], est un outil intéressant qui permettrait de donner des lignes de conduite aux diététistes afin de pouvoir discuter de manière éclairée de nutrigénétique avec le patient/client. Giguère et collaborateurs [271], utilisent d'ailleurs cet outil afin de faciliter la prise de décision partagée des médecins avec les patients ce qui pourrait aussi s'avérer intéressant pour le fait de discuter de nutrigénétique. Ruth DeBusk, une diététiste et chercheure des États-Unis, a récemment publié quelques considérations pour les cliniciens reliées à l'utilisation des tests

de nutrigénétique pour leurs patients [272]. De plus, des formations devront être disponibles pour les diététistes puisqu'un élément clé ressortant de ce projet était tout comme dans la littérature, le manque de connaissances et de formation des diététistes en lien avec la nutrigénétique [29,30,32,217]. Des connaissances de base en génétique, des exemples concrets en nutrigénétique de même qu'un état des avancées en génomique nutritionnelle pourraient être inclus dans ces formations. Dans les prochaines années, les diététistes entendront parler davantage de génomique nutritionnelle, nutrigénomique et de nutrigénétique. Par exemple, le test de nutrigénétique de la compagnie *Nutrigenomix* est déjà offert via des diététistes à travers 19 cliniques de la province de Québec et également à travers le Canada, les États-Unis, en Amérique du Sud, en Europe, en Asie et en Australie [231].

Il est important de considérer les limites de ce projet de doctorat. Un des aspects relevés par les réviseurs de certains articles scientifiques présentés dans cette thèse était l'absence d'un groupe contrôle ne recevant pas la supplémentation en huile de poisson. Toutefois, dans le cadre de ce projet bien qu'un groupe contrôle aurait permis de créer un modèle statistique intégrant l'effet d'interaction du groupe (avec ou sans supplémentation), l'effet hypotriglycéridémiant de l'huile de poisson est selon nous suffisamment bien documenté afin de présumer que la diminution des concentrations de TG observée dans le cadre de ce projet était due à la prise de l'huile de poisson et non un effet dû à un autre changement par exemple, dans les habitudes alimentaires ou d'activité physique. Conséquemment, nous avons pu étudier les impacts de la présence de différents SNPs sur la réponse à la supplémentation en huile de poisson. L'absence d'un groupe contrôle a également permis d'obtenir une cohorte contenant un nombre plus élevé de participants sans toutefois élever les coûts pour le volet clinique de l'étude. Une autre limite de l'étude à mon avis était le fait que les participants de ce projet avaient des concentrations de TG de départ en moyenne de 1,23±0,64mmol/L ce qui est considéré comme étant des valeurs normales [273]. Étant donné que l'effet hypotriglycéridémiant de l'huile de poisson est fortement dépendant des valeurs de départ [165], il est possible qu'en étudiant des individus ayant un profil plus fortement détérioré, davantage d'associations auraient pu être observées. Dans le cadre de ce projet de recherche, des participants ayant un IMC entre 25 kg/m^2 et 40 kg/m^2 ont été

recrutés et l'IMC moyen était de 27,88 ±3,78 kg/m^2. Il est possible que les participants de ce projet ayant un IMC moyen les classant dans la catégorie de surplus de poids, n'avaient pas un profil métabolique suffisamment altéré. Par exemple, très peu de changements dans les niveaux d'expression des gènes habituellement relatés dans la littérature comme étant affectés par la prise d'huile de poisson ont été observés.

Globalement, ce projet de recherche a permis de mettre en lumière que selon les profils alimentaires, les profils d'expression génique de même que de métabolites sont différents. À mon avis, l'alimentation habituelle et le profil génétique d'un individu a un impact majeur sur son métabolisme. Le fait de s'alimenter selon un certain profil alimentaire affecte le métabolisme bien avant que l'on voit des répercussions sur les marqueurs traditionnels du risque de MCV ou d'autres maladies chroniques. Le fait que le métabolisme soit plus ou moins altéré affectera également le niveau de réponse à l'huile de poisson. L'alimentation est d'ailleurs, un déterminant majeur en ce qui a trait à la prévention des risques de MCV et de cancer. Un des résultats intéressants fut l'impact de la présence de SNPs dans des gènes de la voie de la lipogénèse de novo sur la réponse des concentrations de TG suite à la supplémentation en huile de poisson. Cette voie métabolique, bien que ne contribuant pas directement aux TG qui sont incorporés dans les particules VLDL, est importante et a un impact crucial tant au niveau hépatique que du pancréas. Ce projet de doctorat a mis en lumière que des effets d'interaction gène-diète affectent la réponse à un autre nutriment, dans ce cas-ci l'huile de poisson. Conséquemment, l'approche nutritionnelle optimale pour un individu hypertriglycéridémique serait potentiellement la prise d'huile de poisson avec en plus la modification de ses habitudes alimentaires. Du côté nutrigénétique, bien que ce projet démontre des associations entre des SNPs et la réponse à une supplémentation en huile de poisson, le pourcentage de variance expliqué par chacun des SNPs est faible et n'aurait potentiellement pas une réelle utilité clinique. Toutefois, l'équipe de recherche poursuit ces travaux afin de construire un modèle de prédiction qui expliquerait avec plusieurs SNPs un maximum de variance dans la réponse des concentrations de TG à la supplémentation en huile de poisson. Conséquemment, suite à l'élaboration de ce modèle prédictif, il sera fort intéressant de tenter de le répliquer dans une nouvelle cohorte de participants recevant une

supplémentation en huile de poisson. Cette cohorte pourrait être constituée d'individu ayant un IMC légèrement plus élevé, par exemple 30 kg/m^2 à 40 kg/m^2 et les participants pourraient être recrutés sur la base du tour de taille qui est fortement associé aux concentrations de TG [274]. Ces travaux de recherche combinés à des travaux d'application des connaissances contribueront à éventuellement intégrer la génomique nutritionnelle à la pratique des diététistes.

En conclusion, l'alimentation affecte notre métabolisme dès l'expression des gènes et cela se reflète dans les profils de métabolites. Toutes ces modifications sur le métabolisme de même que le profil génétique des individus affectent la réponse à une supplémentation en huile de poisson. La génomique nutritionnelle permet et permettra d'approfondir nos connaissances du métabolisme de base et des déterminants du développement des maladies chroniques reliées à l'alimentation. Les diététistes devront être adéquatement préparées à intégrer les connaissances issues de cette science à leur pratique professionnelle.

Bibliographie

1. Organisation mondiale de la santé. **Maladies chroniques.** [http://www.who.int/topics/chronic_diseases/fr/] en ligne, consulté le 19 juin 2014.

2. Anderson TJ, Gregoire J, Hegele RA, Couture P, Mancini GB, McPherson R *et al*.: **2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult.** *Can J Cardiol* 2013, **29:** 151-167.

3. Organisation modiale de la santé. **Maladies cardio-vasculaires.** [http://www.who.int/mediacentre/factsheets/fs317/fr/] en ligne, consulté le 20 juin 2014.

4. Organisation mondiale de la santé. **Cancer.** [http://www.who.int/mediacentre/factsheets/fs297/fr/] en ligne, consulté le 20 juin 2014.

5. Mathers CD, Loncar D: **Projections of global mortality and burden of disease from 2002 to 2030.** *PLoS Med* 2006, **3:** e442.

6. Organisation mondiale de la santé. **Maladies non transmissibles.** [http://www.who.int/mediacentre/factsheets/fs355/fr/] en ligne, consulté le 20 juin 2014.

7. Statistique Canada. **Les principales causes de décès au Canada, 2009.** [http://www.statcan.gc.ca/pub/84-215-x/2012001/hl-fs-fra.htm] en ligne, consulté le 22 juin 2014.

8. Statistique Canada. **Le quotidien, Les principales causes de décès au Canada, 2009.** [http://www.statcan.gc.ca/daily-quotidien/120725/dq120725b-fra.htm] en ligne, consulté le 22 juin 2014.

9. Agence canadienne de santé publique du Canada. **Facteurs de risque des maladies chroniques.** [http://www.phac-aspc.gc.ca/cd-mc/facteurs_risque-risk_factors-fra.php] en ligne, consulté le 23 juin 2014.

10. World Heart Federation. **Cardiovascular disease risk factors.** [http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/] en ligne, consulté le 23 juin 2014.

11. Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A *et al*.: **Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire.** *Am J Clin Nutr* 1999, **69:** 243-249.

12. Paradis AM, Godin G, Perusse L, Vohl MC: **Associations between dietary patterns and obesity phenotypes.** *Int J Obes (Lond)* 2009, **33:** 1419-1426.

13. Sadakane A, Tsutsumi A, Gotoh T, Ishikawa S, Ojima T, Kario K *et al*.: **Dietary patterns and levels of blood pressure and serum lipids in a Japanese population.** *J Epidemiol* 2008, **18:** 58-67.

14. Mikkila V, Rasanen L, Raitakari OT, Marniemi J, Pietinen P, Ronnemaa T *et al*.: **Major dietary patterns and cardiovascular risk factors from childhood to adulthood. The Cardiovascular Risk in Young Finns Study.** *Br J Nutr* 2007, **98:** 218-225.

15. Meyer J, Doring A, Herder C, Roden M, Koenig W, Thorand B: **Dietary patterns, subclinical inflammation, incident coronary heart disease and mortality in middle-aged men from the MONICA/KORA Augsburg cohort study.** *Eur J Clin Nutr* 2011, **65:** 800-807.

16. Heidemann C, Schulze MB, Franco OH, van Dam RM, Mantzoros CS, Hu FB: **Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women.** *Circulation* 2008, **118:** 230-237.

17. Yusof AS, Isa ZM, Shah SA: **Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies (2000-2011).** *Asian Pac J Cancer Prev* 2012, **13:** 4713-4717.

18. Meyerhardt JA, Niedzwiecki D, Hollis D, Saltz LB, Hu FB, Mayer RJ *et al*.: **Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer.** *JAMA* 2007, **298:** 754-764.

19. Nazki FH, Sameer AS, Ganaie BA: **Folate: metabolism, genes, polymorphisms and the associated diseases.** *Gene* 2014, **533:** 11-20.

20. Guertin KA, Moore SC, Sampson JN, Huang WY, Xiao Q, Stolzenberg-Solomon RZ *et al*.: **Metabolomics in nutritional epidemiology: identifying metabolites associated with diet and quantifying their potential to uncover diet-disease relations in populations.** *Am J Clin Nutr* 2014.

21. Zheng Y, Yu B, Alexander D, Steffen LM, Boerwinkle E: **Human metabolome associates with dietary intake habits among african americans in the atherosclerosis risk in communities study.** *Am J Epidemiol* 2014, **179:** 1424-1433.

22. Caron-Dorval D, Paquet P, Paradis AM, Rudkowska I, Lemieux S, Couture P *et al*.: **Effect of the PPAR-Alpha L162V polymorphism on the cardiovascular disease risk factor in response to n-3 polyunsaturated fatty acids.** *J Nutrigenet Nutrigenomics* 2008, **1:** 205-212.

23. Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK *et al*.: **Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study.** *Am J Clin Nutr* 2008, **88:** 618-629.

24. Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H *et al*.: **The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes.** *Annu Rev Nutr* 2011, **31:** 203-234.

25. Hannah VC, Ou J, Luong A, Goldstein JL, Brown MS: **Unsaturated fatty acids down-regulate srebp isoforms 1a and 1c by two mechanisms in HEK-293 cells.** *J Biol Chem* 2001, **276:** 4365-4372.

26. Kato T, Shimano H, Yamamoto T, Ishikawa M, Kumadaki S, Matsuzaka T *et al*.: **Palmitate impairs and eicosapentaenoate restores insulin secretion through regulation of SREBP-1c in pancreatic islets.** *Diabetes* 2008, **57:** 2382-2392.

27. Tanaka N, Zhang X, Sugiyama E, Kono H, Horiuchi A, Nakajima T *et al*.: **Eicosapentaenoic acid improves hepatic steatosis independent of PPARalpha activation through inhibition of SREBP-1 maturation in mice.** *Biochem Pharmacol* 2010, **80:** 1601-1612.

28. American Heart Association. **Fish 101.** [http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/Fish-101_UCM_305986_Article.jsp] en ligne, consulté le 2 juillet 2014.

29. Cormier H, Tremblay BL, Paradis AM, Garneau V, Desroches S, Robitaille J *et al*.: **Nutrigenomics - perspectives from registered dietitians: a report from the Quebec-wide e-consultation on nutrigenomics among registered dietitians.** *J Hum Nutr Diet* 2014.

30. McCarthy S, Pufulete M, Whelan K: **Factors associated with knowledge of genetics and nutritional genomics among dietitians.** *J Hum Nutr Diet* 2008, **21:** 547-554.

31. Rosen R, Earthman C, Marquart L, Reicks M: **Continuing education needs of registered dietitians regarding nutrigenomics.** *J Am Diet Assoc* 2006, **106:** 1242-1245.

32. Whelan K, McCarthy S, Pufulete M: **Genetics and diet--gene interactions: involvement, confidence and knowledge of dietitians.** *Br J Nutr* 2008, **99:** 23-28.

33. Godin G, Belanger-Gravel A, Eccles M, Grimshaw J: **Healthcare professionals' intentions and behaviours: a systematic review of studies based on social cognitive theories.** *Implement Sci* 2008, **3:** 36.

34. Panico S, Mattiello A, Panico C, Chiodini P: **Mediterranean Dietary Pattern and Chronic Diseases.** *Cancer Treat Res* 2014, **159:** 69-81.

35. Randall E, Marshall JR, Graham S, Brasure J: **Patterns in food use and their associations with nutrient intakes.** *Am J Clin Nutr* 1990, **52:** 739-745.

36. Ursin G, Ziegler RG, Subar AF, Graubard BI, Haile RW, Hoover R: **Dietary patterns associated with a low-fat diet in the national health examination follow-up study: identification of potential confounders for epidemiologic analyses.** *Am J Epidemiol* 1993, **137:** 916-927.

37. Jacques PF, Tucker KL: **Are dietary patterns useful for understanding the role of diet in chronic disease?** *Am J Clin Nutr* 2001, **73:** 1-2.

38. Newby PK, Tucker KL: **Empirically derived eating patterns using factor or cluster analysis: a review.** *Nutr Rev* 2004, **62:** 177-203.

39. Schwerin HS, Stanton JL, Smith JL, Riley AM, Jr., Brett BE: **Food, eating habits, and health: a further examination of the relationship between food eating patterns and nutritional health.** *Am J Clin Nutr* 1982, **35:** 1319-1325.

40. Sacks FM, Obarzanek E, Windhauser MM, Svetkey LP, Vollmer WM, McCullough M *et al*.: **Rationale and design of the Dietary Approaches to Stop Hypertension trial (DASH). A multicenter controlled-feeding study of dietary patterns to lower blood pressure.** *Ann Epidemiol* 1995, **5:** 108-118.

41. Ocke MC: **Evaluation of methodologies for assessing the overall diet: dietary quality scores and dietary pattern analysis.** *Proc Nutr Soc* 2013, **72:** 191-199.

42. Kennedy ET, Ohls J, Carlson S, Fleming K: **The Healthy Eating Index: design and applications.** *J Am Diet Assoc* 1995, **95:** 1103-1108.

43. Hu FB: **Dietary pattern analysis: a new direction in nutritional epidemiology.** *Curr Opin Lipidol* 2002, **13:** 3-9.

44. Reedy J, Wirfalt E, Flood A, Mitrou PN, Krebs-Smith SM, Kipnis V *et al*.: **Comparing 3 dietary pattern methods--cluster analysis, factor analysis, and index analysis--With colorectal cancer risk: The NIH-AARP Diet and Health Study.** *Am J Epidemiol* 2010, **171:** 479-487.

45. Kant AK: **Dietary patterns and health outcomes.** *J Am Diet Assoc* 2004, **104:** 615-635.

46. Gorst-Rasmussen A, Dahm CC, Dethlefsen C, Scheike T, Overvad K: **Exploring dietary patterns by using the treelet transform.** *Am J Epidemiol* 2011, **173:** 1097-1104.

47. Schulze MB, Hoffmann K: **Methodological approaches to study dietary patterns in relation to risk of coronary heart disease and stroke.** *Br J Nutr* 2006, **95:** 860-869.

48. Kroke A: **Re: "Application of a new statistical method to derive dietary patterns in nutritional epidemiology".** *Am J Epidemiol* 2004, **160:** 1132-1133.

49. Lee AB Nadler B WL: *Ann Appl Stat*, in press.

50. Schoenaker DA, Dobson AJ, Soedamah-Muthu SS, Mishra GD: **Factor analysis is more appropriate to identify overall dietary patterns associated with diabetes when compared with Treelet transform analysis.** *J Nutr* 2013, **143:** 392-398.

51. Sherzai A, Heim LT, Boothby C, Sherzai AD: **Stroke, food groups, and dietary patterns: a systematic review.** *Nutr Rev* 2012, **70:** 423-435.

52. Martinez-Gonzalez MA, Sanchez-Tainta A, Corella D, Salas-Salvado J, Ros E, Aros F *et al*.: **A provegetarian food pattern and reduction in total mortality in the Prevencion con Dieta Mediterranea (PREDIMED) study.** *Am J Clin Nutr* 2014.

53. Kant AK: **Dietary patterns: biomarkers and chronic disease risk.** *Appl Physiol Nutr Metab* 2010, **35:** 199-206.

54. Williams CM, Lovegrove JA, Griffin BA: **Dietary patterns and cardiovascular disease.** *Proc Nutr Soc* 2013, 1-5.

55. Judd SE, Gutierrez OM, Newby PK, Howard G, Howard VJ, Locher JL *et al*.: **Dietary patterns are associated with incident stroke and contribute to excess risk of stroke in black Americans.** *Stroke* 2013, **44:** 3305-3311.

56. Zazpe I, Sanchez-Tainta A, Toledo E, Sanchez-Villegas A, Martinez-Gonzalez MA: **Dietary Patterns and Total Mortality in a Mediterranean Cohort: The SUN Project.** *J Acad Nutr Diet* 2013.

57. Alhazmi A, Stojanovski E, McEvoy M, Garg ML: **The association between dietary patterns and type 2 diabetes: a systematic review and meta-analysis of cohort studies.** *J Hum Nutr Diet* 2013.

58. Buscemi S, Nicolucci A, Mattina A, Rosafio G, Massenti FM, Lucisano G *et al*.: **Association of dietary patterns with insulin resistance and clinically silent carotid atherosclerosis in apparently healthy people.** *Eur J Clin Nutr* 2013.

59. Sun J, Buys NJ, Hills AP: **Dietary pattern and its association with the prevalence of obesity, hypertension and other cardiovascular risk factors among Chinese older adults.** *Int J Environ Res Public Health* 2014, **11:** 3956-3971.

60. Mu M, Wang SF, Sheng J, Zhao Y, Wang GX, Liu KY *et al*.: **Dietary patterns are associated with body mass index and bone mineral density in Chinese freshmen.** *J Am Coll Nutr* 2014, **33:** 120-128.

61. Vilela AA, Sichieri R, Pereira RA, Cunha DB, Rodrigues PR, Goncalves-Silva RM *et al*.: **Dietary patterns associated with anthropometric indicators of abdominal fat in adults.** *Cad Saude Publica* 2014, **30:** 502-510.

62. Tucker LA, Tucker JM, Bailey B, Lecheminant JD: **Dietary Patterns as Predictors of Body Fat and BMI in Women: A Factor Analytic Study.** *Am J Health Promot* 2014.

63. Yang J, Farioli A, Korre M, Kales SN: **Modified mediterranean diet score and cardiovascular risk in a North American working population.** *PLoS One* 2014, **9:** e87539.

64. Koch M, Borggrefe J, Barbaresko J, Groth G, Jacobs G, Siegert S *et al*.: **Dietary patterns associated with magnetic resonance imaging-determined liver fat content in a general population study.** *Am J Clin Nutr* 2014, **99:** 369-377.

65. Liu X, Wang X, Lin S, Yuan J, Yu IT: **Dietary patterns and oesophageal squamous cell carcinoma: a systematic review and meta-analysis.** *Br J Cancer* 2014, **110:** 2785-2795.

66. Albuquerque RC, Baltar VT, Marchioni DM: **Breast cancer and dietary patterns: a systematic review.** *Nutr Rev* 2014, **72:** 1-17.

67. Shu L, Wang XQ, Wang SF, Wang S, Mu M, Zhao Y *et al*.: **Dietary patterns and stomach cancer: a meta-analysis.** *Nutr Cancer* 2013, **65:** 1105-1115.

68. Bertuccio P, Rosato V, Andreano A, Ferraroni M, Decarli A, Edefonti V *et al*.: **Dietary patterns and gastric cancer risk: a systematic review and meta-analysis.** *Ann Oncol* 2013, **24:** 1450-1458.

69. Yusof AS, Isa ZM, Shah SA: **Dietary patterns and risk of colorectal cancer: a systematic review of cohort studies (2000-2011).** *Asian Pac J Cancer Prev* 2012, **13:** 4713-4717.

70. Edefonti V, Hashibe M, Ambrogi F, Parpinel M, Bravi F, Talamini R *et al*.: **Nutrient-based dietary patterns and the risk of head and neck cancer: a pooled analysis in the International Head and Neck Cancer Epidemiology consortium.** *Ann Oncol* 2012, **23:** 1869-1880.

71. Brennan SF, Cantwell MM, Cardwell CR, Velentzis LS, Woodside JV: **Dietary patterns and breast cancer risk: a systematic review and meta-analysis.** *Am J Clin Nutr* 2010, **91:** 1294-1302.

72. Mourouti N, Kontogianni MD, Papavagelis C, Plytzanopoulou P, Vassilakou T, Malamos N *et al*.: **Adherence to the Mediterranean Diet is Associated With Lower Likelihood of Breast Cancer: A Case-Control Study.** *Nutr Cancer* 2014, 1-8.

73. Grosso G, Biondi A, Galvano F, Mistretta A, Marventano S, Buscemi S *et al*.: **Factors associated with colorectal cancer in the context of the mediterranean diet: a case-control study.** *Nutr Cancer* 2014, **66:** 558-565.

74. Askari F, Parizi MK, Jessri M, Rashidkhani B: **Dietary patterns in relation to prostate cancer in Iranian men: a case-control study.** *Asian Pac J Cancer Prev* 2014, **15:** 2159-2163.

75. Kumagai Y, Chou WT, Tomata Y, Sugawara Y, Kakizaki M, Nishino Y *et al*.: **Dietary patterns and colorectal cancer risk in Japan: the Ohsaki Cohort Study.** *Cancer Causes Control* 2014, **25:** 727-736.

76. Denova-Gutierrez E, Hernandez-Ramirez RU, Lopez-Carrillo L: **Dietary patterns and gastric cancer risk in Mexico.** *Nutr Cancer* 2014, **66:** 369-376.

77. Nimptsch K, Malik VS, Fung TT, Pischon T, Hu FB, Willett WC *et al*.: **Dietary patterns during high school and risk of colorectal adenoma in a cohort of middle-aged women.** *Int J Cancer* 2014, **134:** 2458-2467.

78. Ax E, Garmo H, Grundmark B, Bill-Axelson A, Holmberg L, Becker W *et al*.: **Dietary patterns and prostate cancer risk: report from the population based ULSAM cohort study of swedish men.** *Nutr Cancer* 2014, **66:** 77-87.

79. Contreras AV, Torres N, Tovar AR: **PPAR-alpha as a key nutritional and environmental sensor for metabolic adaptation.** *Adv Nutr* 2013, **4:** 439-452.

80. Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W: **Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers.** *Proc Natl Acad Sci U S A* 1993, **90:** 2160-2164.

81. Coll T, Barroso E, Alvarez-Guardia D, Serrano L, Salvado L, Merlos M *et al*.: **The Role of Peroxisome Proliferator-Activated Receptor beta/delta on the Inflammatory Basis of Metabolic Disease.** *PPAR Res* 2010, **2010**.

82. Supic G, Jagodic M, Magic Z: **Epigenetics: a new link between nutrition and cancer.** *Nutr Cancer* 2013, **65:** 781-792.

83. Konstantinidou V, Covas MI, Sola R, Fito M: **Up-to date knowledge on the in vivo transcriptomic effect of the Mediterranean diet in humans.** *Mol Nutr Food Res* 2013, **57:** 772-783.

84. Castaner O, Corella D, Covas MI, Sorli JV, Subirana I, Flores-Mateo G *et al*.: **In vivo transcriptomic profile after a Mediterranean diet in high-cardiovascular risk patients: a randomized controlled trial.** *Am J Clin Nutr* 2013, **98:** 845-853.

85. Ravasco P, Aranha MM, Borralho PM, Moreira da Silva IB, Correia L, Fernandes A *et al*.: **Colorectal cancer: can nutrients modulate NF-kappaB and apoptosis?** *Clin Nutr* 2010, **29:** 42-46.

86. Renaud HJ, Cui JY, Lu H, Klaassen CD: **Effect of diet on expression of genes involved in lipid metabolism, oxidative stress, and inflammation in mouse liver-insights into mechanisms of hepatic steatosis.** *PLoS One* 2014, **9:** e88584.

87. Ong KR, Sims AH, Harvie M, Chapman M, Dunn WB, Broadhurst D *et al*.: **Biomarkers of dietary energy restriction in women at increased risk of breast cancer.** *Cancer Prev Res (Phila)* 2009, **2:** 720-731.

88. Franck N, Gummesson A, Jernas M, Glad C, Svensson PA, Guillot G *et al*.: **Identification of adipocyte genes regulated by caloric intake.** *J Clin Endocrinol Metab* 2011, **96:** E413-E418.

89. Capel F, Klimcakova E, Viguerie N, Roussel B, Vitkova M, Kovacikova M *et al*.: **Macrophages and adipocytes in human obesity: adipose tissue gene expression and insulin sensitivity during calorie restriction and weight stabilization.** *Diabetes* 2009, **58:** 1558-1567.

90. Mutch DM, Pers TH, Temanni MR, Pelloux V, Marquez-Quinones A, Holst C *et al*.: **A distinct adipose tissue gene expression response to caloric restriction predicts 6-mo weight maintenance in obese subjects.** *Am J Clin Nutr* 2011, **94:** 1399-1409.

91. van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG *et al*.: **A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome.** *Am J Clin Nutr* 2009, **90:** 1656-1664.

92. Kien CL, Bunn JY, Stevens R, Bain J, Ikayeva O, Crain K *et al*.: **Dietary intake of palmitate and oleate has broad impact on systemic and tissue lipid profiles in humans.** *Am J Clin Nutr* 2014, **99:** 436-445.

93. Bergouignan A, Gozansky WS, Barry DW, Leitner W, MacLean PS, Hill JO *et al*.: **Increasing dietary fat elicits similar changes in fat oxidation and markers of muscle oxidative capacity in lean and obese humans.** *PLoS One* 2012, **7:** e30164.

94. Engeli S, Lehmann AC, Kaminski J, Haas V, Janke J, Zoerner AA *et al*.: **Influence of dietary fat intake on the endocannabinoid system in lean and obese subjects.** *Obesity (Silver Spring)* 2014, **22:** E70-E76.

95. Guebre-Egziabher F, Debard C, Drai J, Denis L, Pesenti S, Bienvenu J *et al*.: **Differential dose effect of fish oil on inflammation and adipose tissue gene expression in chronic kidney disease patients.** *Nutrition* 2013, **29:** 730-736.

96. Morine MJ, Tierney AC, van OB, Daniel H, Toomey S, Gjelstad IM *et al*.: **Transcriptomic coordination in the human metabolic network reveals links between n-3 fat intake, adipose tissue gene expression and metabolic health.** *PLoS Comput Biol* 2011, **7:** e1002223.

97. Myhrstad MC, Narverud I, Telle-Hansen VH, Karhu T, Lund DB, Herzig KH *et al*.: **Effect of the fat composition of a single high-fat meal on inflammatory markers in healthy young women.** *Br J Nutr* 2011, **106:** 1826-1835.

98. Rudkowska I, Paradis AM, Thifault E, Julien P, Tchernof A, Couture P *et al*.: **Transcriptomic and metabolomic signatures of an n-3 polyunsaturated fatty acids supplementation in a normolipidemic/normocholesterolemic Caucasian population.** *J Nutr Biochem* 2013, **24:** 54-61.

99. Meneses ME, Camargo A, Perez-Martinez P, Delgado-Lista J, Cruz-Teno C, Jimenez-Gomez Y *et al*.: **Postprandial inflammatory response in adipose tissue of patients with metabolic syndrome after the intake of different dietary models.** *Mol Nutr Food Res* 2011, **55:** 1759-1770.

100. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L *et al*.: **Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial.** *Am J Clin Nutr* 2012, **95:** 1003-1012.

101. Scalbert A, Brennan L, Manach C, Andres-Lacueva C, Dragsted LO, Draper J *et al*.: **The food metabolome: a window over dietary exposure.** *Am J Clin Nutr* 2014, **99:** 1286-1308.

102. Fardet A, Llorach R, Orsoni A, Martin JF, Pujos-Guillot E, Lapierre C *et al*.: **Metabolomics provide new insight on the metabolism of dietary phytochemicals in rats.** *J Nutr* 2008, **138:** 1282-1287.

103. O'Sullivan A, Gibney MJ, Brennan L: **Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies.** *Am J Clin Nutr* 2011, **93:** 314-321.

104. May DH, Navarro SL, Ruczinski I, Hogan J, Ogata Y, Schwarz Y *et al*.: **Metabolomic profiling of urine: response to a randomised, controlled feeding study of select fruits and vegetables, and application to an observational study.** *Br J Nutr* 2013, **110:** 1760-1770.

105. Armah CN, Traka MH, Dainty JR, Defernez M, Janssens A, Leung W *et al*.: **A diet rich in high-glucoraphanin broccoli interacts with genotype to reduce discordance in plasma metabolite profiles by modulating mitochondrial function.** *Am J Clin Nutr* 2013, **98:** 712-722.

106. Xu J, Yang S, Cai S, Dong J, Li X, Chen Z: **Identification of biochemical changes in lactovegetarian urine using 1H NMR spectroscopy and pattern recognition.** *Anal Bioanal Chem* 2010, **396:** 1451-1463.

107. Garcia-Aloy M, Llorach R, Urpi-Sarda M, Tulipani S, Estruch R, Martinez-Gonzalez MA *et al*.: **Novel multi-metabolite prediction of walnut consumption by a urinary biomarker model in a free-living population.** *J Proteome Res* 2014.

108. Floegel A, von RA, Drogan D, Schulze MB, Prehn C, Adamski J *et al*.: **Variation of serum metabolites related to habitual diet: a targeted metabolomic approach in EPIC-Potsdam.** *Eur J Clin Nutr* 2013, **67:** 1100-1108.

109. Floegel A, Stefan N, Yu Z, Muhlenbruch K, Drogan D, Joost HG *et al*.: **Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach.** *Diabetes* 2013, **62:** 639-648.

110. Floegel A, Wientzek A, Bachlechner U, Jacobs S, Drogan D, Prehn C *et al*.: **Linking diet, physical activity, cardiorespiratory fitness and obesity to serum metabolite networks: findings from a population-based study.** *Int J Obes (Lond)* 2014.

111. Khoo CM, Muehlbauer MJ, Stevens RD, Pamuklar Z, Chen J, Newgard CB *et al*.: **Postprandial metabolite profiles reveal differential nutrient handling after bariatric surgery compared with matched caloric restriction.** *Ann Surg* 2014, **259:** 687-693.

112. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF *et al*.: **A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance.** *Cell Metab* 2009, **9:** 311-326.

113. Dumas ME, Kinross J, Nicholson JK: **Metabolic phenotyping and systems biology approaches to understanding metabolic syndrome and fatty liver disease.** *Gastroenterology* 2014, **146:** 46-62.

114. Laferrere B, Reilly D, Arias S, Swerdlow N, Gorroochurn P, Bawa B *et al*.: **Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss.** *Sci Transl Med* 2011, **3:** 80re2.

115. Mutch DM, Fuhrmann JC, Rein D, Wiemer JC, Bouillot JL, Poitou C *et al*.: **Metabolite profiling identifies candidate markers reflecting the clinical adaptations associated with Roux-en-Y gastric bypass surgery.** *PLoS One* 2009, **4:** e7905.

116. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E *et al*.: **Metabolite profiles and the risk of developing diabetes.** *Nat Med* 2011, **17:** 448-453.

117. McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, Gerszten RE *et al*.: **Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents.** *Pediatr Obes* 2013, **8:** 52-61.

118. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL *et al*.: **Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men.** *Diabetologia* 2010, **53:** 757-767.

119. Lu J, Xie G, Jia W, Jia W: **Insulin resistance and the metabolism of branched-chain amino acids.** *Front Med* 2013, **7:** 53-59.

120. Batch BC, Shah SH, Newgard CB, Turer CB, Haynes C, Bain JR *et al*.: **Branched chain amino acids are novel biomarkers for discrimination of metabolic wellness.** *Metabolism* 2013, **62:** 961-969.

121. Rolfes, Pinna, Whitney: **Understanding Normal and Clinical Nutrition Seventh Edition,** Edited by Thomson Learning I edn. United States of America: 2006, 1152pp.

122. De CR: **n-3 fatty acids in cardiovascular disease.** *N Engl J Med* 2011, **364:** 2439-2450.

123. Burdge GC, Calder PC: **Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults.** *Reprod Nutr Dev* 2005, **45:** 581-597.

124. Meyer BJ: **Are we consuming enough long chain omega-3 polyunsaturated fatty acids for optimal health?** *Prostaglandins Leukot Essent Fatty Acids* 2011, **85:** 275-280.

125. Santé Canada. **Tableaux des ANREF.** [http://www.hc-sc.gc.ca/fn-an/nutrition/reference/table/index-fra.php] en ligne, consulté le 4 juillet 2014.

126. Santé Canada. **Lignes directrices sur la nutrition pendant la grossess à l'intention des professionnels de la santé - Le poisson et les acides gras oméga-3**. [http://www.hc-sc.gc.ca/fn-an/pubs/nutrition/omega3-fra.php] en ligne, consulté le 4 juillet 2014.

127. Mozaffarian D, Wu JH: **Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events.** *J Am Coll Cardiol* 2011, **58:** 2047-2067.

128. Davidson MH: **Omega-3 fatty acids: new insights into the pharmacology and biology of docosahexaenoic acid, docosapentaenoic acid, and eicosapentaenoic acid.** *Curr Opin Lipidol* 2013, **24:** 467-474.

129. Raphael W, Sordillo LM: **Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid biosynthesis.** *Int J Mol Sci* 2013, **14:** 21167-21188.

130. Glaser C, Heinrich J, Koletzko B: **Role of FADS1 and FADS2 polymorphisms in polyunsaturated fatty acid metabolism.** *Metabolism* 2010, **59:** 993-999.

131. Serra-Majem L, Nissensohn M, Overby NC, Fekete K: **Dietary methods and biomarkers of omega 3 fatty acids: a systematic review.** *Br J Nutr* 2012, **107 Suppl 2:** S64-S76.

132. Harris WS, Pottala JV, Sands SA, Jones PG: **Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids.** *Am J Clin Nutr* 2007, **86:** 1621-1625.

133. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M: **Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study.** *J Lipid Res* 1997, **38:** 2012-2022.

134. Walker CG, Browning LM, Mander AP, Madden J, West AL, Calder PC *et al*.: **Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans.** *Br J Nutr* 2014, **111:** 679-689.

135. Browning LM, Walker CG, Mander AP, West AL, Gambell J, Madden J *et al*.: **Compared with daily, weekly n-3 PUFA intake affects the incorporation of eicosapentaenoic acid and docosahexaenoic acid into platelets and mononuclear cells in humans.** *J Nutr* 2014, **144:** 667-672.

136. Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM: **Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized controlled trial.** *J Am Heart Assoc* 2013, **2:** e000513.

137. Dyerberg J, Schmidt EB: **n-3 fatty acids and cardiovascular disease--observations generated by studies in Greenland Eskimos.** *Wien Klin Wochenschr* 1989, **101:** 277-282.

138. Harris WS: **Are n-3 fatty acids still cardioprotective?** *Curr Opin Clin Nutr Metab Care* 2013, **16:** 141-149.

139. Harris WS: **n-3 fatty acids and serum lipoproteins: human studies.** *Am J Clin Nutr* 1997, **65:** 1645S-1654S.

140. Kromhout D, Giltay EJ, Geleijnse JM: **n-3 fatty acids and cardiovascular events after myocardial infarction.** *N Engl J Med* 2010, **363:** 2015-2026.

141. Marchioli R, Barzi F, Bomba E, Chieffo C, Di GD, Di MR *et al*.: **Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione.** *Circulation* 2002, **105:** 1897-1903.

142. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y *et al*.: **Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis.** *Lancet* 2007, **369:** 1090-1098.

143. Kromhout D, Geleijnse JM, de GJ, Oude Griep LM, Mulder BJ, de Boer MJ *et al*.: **n-3 fatty acids, ventricular arrhythmia-related events, and fatal myocardial infarction in postmyocardial infarction patients with diabetes.** *Diabetes Care* 2011, **34:** 2515-2520.

144. Eussen SR, Geleijnse JM, Giltay EJ, Rompelberg CJ, Klungel OH, Kromhout D: **Effects of n-3 fatty acids on major cardiovascular events in statin users and non-users with a history of myocardial infarction.** *Eur Heart J* 2012, **33:** 1582-1588.

145. Rauch B, Schiele R, Schneider S, Diller F, Victor N, Gohlke H *et al*.: **OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction.** *Circulation* 2010, **122:** 2152-2159.

146. Papageorgiou N, Tousoulis D, Stefanadis C: **Letter by Papageorgiou et al regarding article, "OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction".** *Circulation* 2011, **124:** e22-e25.

147. Bosch J, Gerstein HC, Dagenais GR, Diaz R, Dyal L, Jung H *et al*.: **n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia.** *N Engl J Med* 2012, **367:** 309-318.

148. von SC: **Omega-3 fatty Acids in cardiovascular disease - An uphill battle.** *Prostaglandins Leukot Essent Fatty Acids* 2014.

149. Rodriguez-Leyva D, Dupasquier CM, McCullough R, Pierce GN: **The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid.** *Can J Cardiol* 2010, **26:** 489-496.

150. Claude Gagné, Daniel Gaudet: **Les dyslipoprotéinémies : L'approche clinique.** 3e édition. Edition Lipimed Communications; 2007. 305pp.

151. Berglund L, Brunzell JD, Goldberg AC, Goldberg IJ, Stalenhoef A: **Treatment options for hypertriglyceridemia: From risk reduction to pancreatitis.** *Best Pract Res Clin Endocrinol Metab* 2014, **28:** 423-437.

152. Austin MA, Hokanson JE, Edwards KL: **Hypertriglyceridemia as a cardiovascular risk factor.** *Am J Cardiol* 1998, **81:** 7B-12B.

153. Assmann G, Schulte H, Funke H, von EA: **The emergence of triglycerides as a significant independent risk factor in coronary artery disease.** *Eur Heart J* 1998, **19 Suppl M:** M8-14.

154. Morrison A, Hokanson JE: **The independent relationship between triglycerides and coronary heart disease.** *Vasc Health Risk Manag* 2009, **5:** 89-95.

155. Chen AH, Tseng CH: **The role of triglyceride in cardiovascular disease in asian patients with type 2 diabetes--a systematic review.** *Rev Diabet Stud* 2013, **10:** 101-109.

156. Di AE, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A *et al.*: **Major lipids, apolipoproteins, and risk of vascular disease.** *JAMA* 2009, **302:** 1993-2000.

157. Fédération Internationale du Diabète. **Le syndrome métabolique.** [http://www.idf.org/sites/default/files/attachments/issue_43_fr.pdf] en ligne, consulté le 5 juillet 2014.

158. **An International Atherosclerosis Society Position Paper: global recommendations for the management of dyslipidemia--full report.** *J Clin Lipidol* 2014, **8:** 29-60.

159. Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS: **Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey.** *Circulation* 2004, **109:** 42-46.

160. Havel PJ: **Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism.** *Nutr Rev* 2005, **63:** 133-157.

161. Kris-Etherton PM, Harris WS, Appel LJ: **Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association.** *Arterioscler Thromb Vasc Biol* 2003, **23:** 151-152.

162. Jacobson TA: **Role of n-3 fatty acids in the treatment of hypertriglyceridemia and cardiovascular disease.** *Am J Clin Nutr* 2008, **87:** 1981S-1990S.

163. Balogun KA, Randunu RS, Cheema SK: **The effect of dietary omega-3 polyunsaturated fatty acids on plasma lipids and lipoproteins of C57BL/6 mice is age and sex specific.** *Prostaglandins Leukot Essent Fatty Acids* 2014, **91:** 39-47.

164. St-Pierre AC, Ruel IL, Cantin B, Dagenais GR, Bernard PM, Despres JP *et al.*: **Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease.** *Circulation* 2001, **104:** 2295-2299.

165. Harris WS, Bulchandani D: **Why do omega-3 fatty acids lower serum triglycerides?** *Curr Opin Lipidol* 2006, **17:** 387-393.

166. Davidson MH: **Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids.** *Am J Cardiol* 2006, **98:** 27i-33i.

167. Salakhutdinov NF, Laev SS: **Triglyceride-lowering agents.** *Bioorg Med Chem* 2014, **22:** 3551-3564.

168. Oliver E, McGillicuddy F, Phillips C, Toomey S, Roche HM: **The role of inflammation and macrophage accumulation in the development of obesity-induced type 2 diabetes mellitus and the possible therapeutic effects of long-chain n-3 PUFA.** *Proc Nutr Soc* 2010, **69:** 232-243.

169. Gibbons GF, Bartlett SM, Sparks CE, Sparks JD: **Extracellular fatty acids are not utilized directly for the synthesis of very-low-density lipoprotein in primary cultures of rat hepatocytes.** *Biochem J* 1992, **287 (Pt 3):** 749-753.

170. Gibbons GF, Wiggins D, Brown AM, Hebbachi AM: **Synthesis and function of hepatic very-low-density lipoprotein.** *Biochem Soc Trans* 2004, **32:** 59-64.

171. Xu J, Teran-Garcia M, Park JH, Nakamura MT, Clarke SD: **Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay.** *J Biol Chem* 2001, **276:** 9800-9807.

172. SABiosciences **aQc. EpiTect ChiP qPCR Primers.** [http://www.sabiosciences.com/chipqpcrsearch.php?app=TFBS] en ligne, consulté le 5 juillet 2014.

173. Schoonjans K, Staels B, Auwerx J: **The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation.** *Biochim Biophys Acta* 1996, **1302:** 93-109.

174. Kersten S: **Integrated physiology and systems biology of PPARalpha.** *Mol Metab* 2014, **3:** 354-371.

175. Issemann I, Prince RA, Tugwood JD, Green S: **The peroxisome proliferator-activated receptor:retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs.** *J Mol Endocrinol* 1993, **11:** 37-47.

176. Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG *et al.*: **Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay.** *Mol Endocrinol* 1997, **11:** 779-791.

177. Cormier H, Rudkowska I, Paradis AM, Thifault E, Garneau V, Lemieux S *et al.*: **Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an n-3 PUFA supplementation.** *Nutrients* 2012, **4:** 1026-1041.

178. Lindi V, Schwab U, Louheranta A, Laakso M, Vessby B, Hermansen K *et al.*: **Impact of the Pro12Ala polymorphism of the PPAR-gamma2 gene on serum triacylglycerol response to n-3 fatty acid supplementation.** *Mol Genet Metab* 2003, **79:** 52-60.

179. Madden J, Carrero JJ, Brunner A, Dastur N, Shearman CP, Calder PC *et al.*: **Polymorphisms in the CD36 gene modulate the ability of fish oil supplements**

to lower fasting plasma triacyl glycerol and raise HDL cholesterol concentrations in healthy middle-aged men. *Prostaglandins Leukot Essent Fatty Acids* 2008, **78:** 327-335.

180. Phillips CM, Goumidi L, Bertrais S, Ferguson JF, Field MR, Kelly ED *et al.*: **Complement component 3 polymorphisms interact with polyunsaturated fatty acids to modulate risk of metabolic syndrome.** *Am J Clin Nutr* 2009, **90:** 1665-1673.

181. Rudkowska I, Caron-Dorval D, Verreault M, Couture P, Deshaies Y, Barbier O *et al.*: **PPARalpha L162V polymorphism alters the potential of n-3 fatty acids to increase lipoprotein lipase activity.** *Mol Nutr Food Res* 2010, **54:** 543-550.

182. Shin JA, Lee JH, Lim SY, Ha HS, Kwon HS, Park YM *et al.*: **Metabolic syndrome as a predictor of type 2 diabetes, and its clinical interpretations and usefulness.** *J Diabetes Investig* 2013, **4:** 334-343.

183. Abeywardena MY, Patten GS: **Role of omega3 long-chain polyunsaturated fatty acids in reducing cardio-metabolic risk factors.** *Endocr Metab Immune Disord Drug Targets* 2011, **11:** 232-246.

184. Pinel A, Morio-Liondore B, Capel F: **n-3 Polyunsaturated fatty acids modulate metabolism of insulin-sensitive tissues: implication for the prevention of type 2 diabetes.** *J Physiol Biochem* 2014, **70:** 647-658.

185. Li J, Li FR, Wei D, Jia W, Kang JX, Stefanovic-Racic M *et al.*: **Endogenous omega-3 PUFAs Production Confers Resistance to Obesity, Dyslipidemia, and Diabetes in Mice.** *Mol Endocrinol* 2014, me20141011.

186. Bremer AA, Stanhope KL, Graham JL, Cummings BP, Ampah SB, Saville BR *et al.*: **Fish oil supplementation ameliorates fructose-induced hypertriglyceridemia and insulin resistance in adult male rhesus macaques.** *J Nutr* 2014, **144:** 5-11.

187. Lionetti L, Mollica MP, Sica R, Donizzetti I, Gifuni G, Pignalosa A *et al.*: **Differential effects of high-fish oil and high-lard diets on cells and cytokines involved in the inflammatory process in rat insulin-sensitive tissues.** *Int J Mol Sci* 2014, **15:** 3040-3063.

188. Haag M, Dippenaar NG: **Dietary fats, fatty acids and insulin resistance: short review of a multifaceted connection.** *Med Sci Monit* 2005, **11:** RA359-RA367.

189. Wu JH, Micha R, Imamura F, Pan A, Biggs ML, Ajaz O *et al.*: **Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis.** *Br J Nutr* 2012, **107 Suppl 2:** S214-S227.

190. Wallin A, Di GD, Orsini N, Patel PS, Forouhi NG, Wolk A: **Fish consumption, dietary long-chain n-3 fatty acids, and risk of type 2 diabetes: systematic**

review and meta-analysis of prospective studies. *Diabetes Care* 2012, **35:** 918-929.

191. Hartweg J, Perera R, Montori V, Dinneen S, Neil HA, Farmer A: **Omega-3 polyunsaturated fatty acids (PUFA) for type 2 diabetes mellitus.** *Cochrane Database Syst Rev* 2008, CD003205.

192. Farsi PF, Djazayery A, Eshraghian MR, Koohdani F, Saboor-Yaraghi AA, Derakhshanian H *et al*.: **Effects of supplementation with omega-3 on insulin sensitivity and non-esterified free fatty acid (NEFA) in type 2 diabetic patients.** *Arq Bras Endocrinol Metabol* 2014, **58:** 335-340.

193. Rajkumar H, Mahmood N, Kumar M, Varikuti SR, Challa HR, Myakala SP: **Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial.** *Mediators Inflamm* 2014, **2014:** 348959.

194. Rafraf M, Mohammadi E, Asghari-Jafarabadi M, Farzadi L: **Omega-3 fatty acids improve glucose metabolism without effects on obesity values and serum visfatin levels in women with polycystic ovary syndrome.** *J Am Coll Nutr* 2012, **31:** 361-368.

195. Juarez-Lopez C, Klunder-Klunder M, Madrigal-Azcarate A, Flores-Huerta S: **Omega-3 polyunsaturated fatty acids reduce insulin resistance and triglycerides in obese children and adolescents.** *Pediatr Diabetes* 2013, **14:** 377-383.

196. Dasarathy S, Dasarathy J, Khiyami A, Yerian L, Hawkins C, Sargent R *et al*.: **Double-blind Randomized Placebo-controlled Clinical Trial of Omega 3 Fatty Acids for the Treatment of Diabetic Patients With Nonalcoholic Steatohepatitis.** *J Clin Gastroenterol* 2014.

197. Woodman RJ, Mori TA, Burke V, Puddey IB, Watts GF, Beilin LJ: **Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension.** *Am J Clin Nutr* 2002, **76:** 1007-1015.

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

199. Montori VM, Farmer A, Wollan PC, Dinneen SF: **Fish oil supplementation in type 2 diabetes: a quantitative systematic review.** *Diabetes Care* 2000, **23:** 1407-1415.

200. Friedberg CE, Janssen MJ, Heine RJ, Grobbee DE: **Fish oil and glycemic control in diabetes. A meta-analysis.** *Diabetes Care* 1998, **21:** 494-500.

201. Lou DJ, Zhu QQ, Si XW, Guan LL, You QY, Yu ZM *et al*.: **Serum phospholipid omega-3 polyunsaturated fatty acids and insulin resistance in type 2 diabetes mellitus and non-alcoholic fatty liver disease.** *J Diabetes Complications* 2014.

202. Mahendran Y, Agren J, Uusitupa M, Cederberg H, Vangipurapu J, Stancakova A *et al*.: **Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes.** *Am J Clin Nutr* 2014, **99:** 79-85.

203. Muniyappa R, Lee S, Chen H, Quon MJ: **Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage.** *Am J Physiol Endocrinol Metab* 2008, **294:** E15-E26.

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

205. Antuna-Puente B, Faraj M, Karelis AD, Garrel D, Prud'homme D, Rabasa-Lhoret R *et al*.: **HOMA or QUICKI: is it useful to test the reproducibility of formulas?** *Diabetes Metab* 2008, **34:** 294-296.

206. Bellia A, Giardina E, Lauro D, Tesauro M, Di FG, Cusumano G *et al*.: **"The Linosa Study": epidemiological and heritability data of the metabolic syndrome in a Caucasian genetic isolate.** *Nutr Metab Cardiovasc Dis* 2009, **19:** 455-461.

207. Li S, Duan H, Pang Z, Zhang D, Duan H, Hjelmborg JV *et al*.: **Heritability of eleven metabolic phenotypes in Danish and Chinese twins: a cross-population comparison.** *Obesity (Silver Spring)* 2013, **21:** 1908-1914.

208. van DJ, Willemsen G, Chen WM, de Geus EJ, Boomsma DI: **Heritability of metabolic syndrome traits in a large population-based sample.** *J Lipid Res* 2013, **54:** 2914-2923.

209. Rasmussen-Torvik LJ, Pankow JS, Jacobs DR, Steffen LM, Moran AM, Steinberger J *et al*.: **Heritability and genetic correlations of insulin sensitivity measured by the euglycaemic clamp.** *Diabet Med* 2007, **24:** 1286-1289.

210. Clarke SD: **Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance.** *Br J Nutr* 2000, **83 Suppl 1:** S59-S66.

211. Jump DB: **N-3 polyunsaturated fatty acid regulation of hepatic gene transcription.** *Curr Opin Lipidol* 2008, **19:** 242-247.

212. Calder PC: **Fatty acids and inflammation: the cutting edge between food and pharma.** *Eur J Pharmacol* 2011, **668 Suppl 1:** S50-S58.

213. Omics-Ethics Group. **Qu'est-ce que le transfert des connaissances?** [http://www.omics-ethics.org/fr/definition-transfert-de-connaissances] en ligne, consulté le 7 juillet 2014.

214. Instituts de recherche en santé du Canada. **À propos de l'application des connaissances et de la commercialisation.** [http://www.cihr-irsc.gc.ca/f/29418.html] en ligne, consulté le 7 juillet 2014.

215. Graham ID, Tetroe J, Gagnon M: **Lost in translation: just lost or beginning to find our way?** *Ann Emerg Med* 2009, **54:** 313-314.

216. Camp KM, Trujillo E: **Position of the Academy of Nutrition and Dietetics: nutritional genomics.** *J Acad Nutr Diet* 2014, **114:** 299-312.

217. Collins J, Bertrand B, Hayes V, Li SX, Thomas J, Truby H *et al*.: **The application of genetics and nutritional genomics in practice: an international survey of knowledge, involvement and confidence among dietitians in the US, Australia and the UK.** *Genes Nutr* 2013, **8:** 523-533.

218. Patterson RE, Eaton DL, Potter JD: **The genetic revolution: change and challenge for the dietetics profession.** *J Am Diet Assoc* 1999, **99:** 1412-1420.

219. Ries NM, Castle D: **Nutrigenomics and ethics interface: direct-to-consumer services and commercial aspects.** *OMICS* 2008, **12:** 245-250.

220. Berger A, Mutch DM, German JB, Roberts MA: **Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression.** *Lipids Health Dis* 2002, **1:** 2.

221. Saukko P: **State of play in direct-to-consumer genetic testing for lifestyle-related diseases: market, marketing content, user experiences and regulation.** *Proc Nutr Soc* 2013, **72:** 53-60.

222. Zeisel SH, Waterland RA, Ordovas JM, Muoio DM, Jia W, Fodor A: **Highlights of the 2012 Research Workshop: Using nutrigenomics and metabolomics in clinical nutrition research.** *JPEN J Parenter Enteral Nutr* 2013, **37:** 190-200.

223. de RB: **Personalised nutrition: ready for practice?** *Proc Nutr Soc* 2013, **72:** 48-52.

224. Borecki IB: **Contemporary approaches to gene discovery: progress toward personalized medicine?** *Circ Cardiovasc Genet* 2009, **2:** 1-2.

225. Ioannidis JP: **Prediction of cardiovascular disease outcomes and established cardiovascular risk factors by genome-wide association markers.** *Circ Cardiovasc Genet* 2009, **2:** 7-15.

226. Becker F, van El CG, Ibarreta D, Zika E, Hogarth S, Borry P *et al*.: **Genetic testing and common disorders in a public health framework: how to assess relevance and possibilities. Background Document to the ESHG recommendations on genetic testing and common disorders.** *Eur J Hum Genet* 2011, **19 Suppl 1:** S6-44.

227. Janssens AC, Wilde AA, van Langen IM: **The sense and nonsense of direct-to-consumer genetic testing for cardiovascular disease.** *Neth Heart J* 2011, **19:** 85-88.

228. Corella D, Ordovas JM: **Interactions between dietary n-3 fatty acids and genetic variants and risk of disease.** *Br J Nutr* 2012, **107 Suppl 2:** S271-S283.

229. Minihane AM: **Fatty acid-genotype interactions and cardiovascular risk.** *Prostaglandins Leukot Essent Fatty Acids* 2010, **82:** 259-264.

230. Weir M, Morin K, Ries N, Castle D: **Canadian health care professionals' knowledge, attitudes and perceptions of nutritional genomics.** *Br J Nutr* 2010, **104:** 1112-1119.

231. Nutrigenomix. [https://www.nutrigenomix.com/fr] en ligne, consulté le 7 juillet 2014.

232. Scott SD, Albrecht L, O'Leary K, Ball GD, Hartling L, Hofmeyer A *et al*.: **Systematic review of knowledge translation strategies in the allied health professions.** *Implement Sci* 2012, **7:** 70.

233. Lafreniere D, Menuz V, Hurlimann T, Godard B. **Knowledge Dissemination Interventions: A Literature Review.** *SAGE Open* 2013,3(3).

234. Grimshaw JM, Eccles MP, Walker AE, Thomas RE: **Changing physicians' behavior: what works and thoughts on getting more things to work.** *J Contin Educ Health Prof* 2002, **22:** 237-243.

235. Eccles MP, Hrisos S, Francis J, Kaner EF, Dickinson HO, Beyer F *et al*.: **Do self-reported intentions predict clinicians' behaviour: a systematic review.** *Implement Sci* 2006, **1:** 28.

236. Paschal Sheeran. **Intention—Behavior Relations: A Conceptual and Empirical Review.** *European Review of Social Psychology* 2002, **12:**4-15.

237. Ajzen I. **The Theory of Planned Behavior**. *Organizational Behavior and Human Decision Processes*1991, **50:**179-211.

238. Zak DE, Aderem A: **Systems biology of innate immunity.** *Immunol Rev* 2009, **227:** 264-282.

239. Hardman WE: **Diet components can suppress inflammation and reduce cancer risk.** *Nutr Res Pract* 2014, **8:** 233-240.

240. Lee H, Lee IS, Choue R: **Obesity, inflammation and diet.** *Pediatr Gastroenterol Hepatol Nutr* 2013, **16:** 143-152.

241. Myles IA: **Fast food fever: reviewing the impacts of the Western diet on immunity.** *Nutr J* 2014, **13:** 61.

242. Joffe YT, Collins M, Goedecke JH: **The relationship between dietary fatty acids and inflammatory genes on the obese phenotype and serum lipids.** *Nutrients* 2013, **5:** 1672-1705.

243. Shadman Z, Akhoundan M, Poorsoltan N, Larijani B, Qorbani M, Nikoo MK: **New challenges in dietary pattern analysis: combined dietary patterns and calorie adjusted factor analysis in type 2 diabetic patients.** *J Diabetes Metab Disord* 2014, **13:** 71.

244. McEvoy CT, Cardwell CR, Woodside JV, Young IS, Hunter SJ, McKinley MC: **A Posteriori Dietary Patterns Are Related to Risk of Type 2 Diabetes: Findings from a Systematic Review and Meta-Analysis.** *J Acad Nutr Diet* 2014.

245. Bays HE, Tighe AP, Sadovsky R, Davidson MH: **Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications.** *Expert Rev Cardiovasc Ther* 2008, **6:** 391-409.

246. Rossmeisl M, Medrikova D, van Schothorst EM, Pavlisova J, Kuda O, Hensler M *et al.*: **Omega-3 phospholipids from fish suppress hepatic steatosis by integrated inhibition of biosynthetic pathways in dietary obese mice.** *Biochim Biophys Acta* 2014, **1841:** 267-278.

247. Lamaziere A, Wolf C, Barbe U, Bausero P, Visioli F: **Lipidomics of hepatic lipogenesis inhibition by omega 3 fatty acids.** *Prostaglandins Leukot Essent Fatty Acids* 2013, **88:** 149-154.

248. Devarshi PP, Jangale NM, Ghule AE, Bodhankar SL, Harsulkar AM: **Beneficial effects of flaxseed oil and fish oil diet are through modulation of different hepatic genes involved in lipid metabolism in streptozotocin-nicotinamide induced diabetic rats.** *Genes Nutr* 2013, **8:** 329-342.

249. Arazi SS, Genvigir FD, Willrich MA, Hirata MH, Dorea EL, Bernik M *et al.*: **Atorvastatin effects on SREBF1a and SCAP gene expression in mononuclear cells and its relation with lowering-lipids response.** *Clin Chim Acta* 2008, **393:** 119-124.

250. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA: **The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool.** *J Lab Clin Med* 2006, **147:** 126-132.

251. Oliver P, Reynes B, Caimari A, Palou A: **Peripheral blood mononuclear cells: a potential source of homeostatic imbalance markers associated with obesity development.** *Pflugers Arch* 2013, **465:** 459-468.

252. Schmidt S, Willers J, Stahl F, Mutz KO, Scheper T, Hahn A *et al*.: **Regulation of lipid metabolism-related gene expression in whole blood cells of normo- and dyslipidemic men after fish oil supplementation.** *Lipids Health Dis* 2012, **11:** 172.

253. Joehanes R, Johnson AD, Barb JJ, Raghavachari N, Liu P, Woodhouse KA *et al*.: **Gene expression analysis of whole blood, peripheral blood mononuclear cells, and lymphoblastoid cell lines from the Framingham Heart Study.** *Physiol Genomics* 2012, **44:** 59-75.

254. Ruiz R, Jideonwo V, Ahn M, Surendran S, Tagliabracci VS, Hou Y *et al*.: **Sterol regulatory element-binding protein-1 (SREBP-1) is required to regulate glycogen synthesis and gluconeogenic gene expression in mouse liver.** *J Biol Chem* 2014, **289:** 5510-5517.

255. Becard D, Hainault I, Azzout-Marniche D, Bertry-Coussot L, Ferre P, Foufelle F: **Adenovirus-mediated overexpression of sterol regulatory element binding protein-1c mimics insulin effects on hepatic gene expression and glucose homeostasis in diabetic mice.** *Diabetes* 2001, **50:** 2425-2430.

256. Guan HP, Chen G: **Factors affecting insulin-regulated hepatic gene expression.** *Prog Mol Biol Transl Sci* 2014, **121:** 165-215.

257. Peter A, Stefan N, Cegan A, Walenta M, Wagner S, Konigsrainer A *et al*.: **Hepatic glucokinase expression is associated with lipogenesis and fatty liver in humans.** *J Clin Endocrinol Metab* 2011, **96:** E1126-E1130.

258. Jafari T, Fallah AA, Azadbakht L: **Role of dietary n-3 polyunsaturated fatty acids in type 2 diabetes: a review of epidemiological and clinical studies.** *Maturitas* 2013, **74:** 303-308.

259. Shimano H, Amemiya-Kudo M, Takahashi A, Kato T, Ishikawa M, Yamada N: **Sterol regulatory element-binding protein-1c and pancreatic beta-cell dysfunction.** *Diabetes Obes Metab* 2007, **9 Suppl 2:** 133-139.

260. Takahashi A, Motomura K, Kato T, Yoshikawa T, Nakagawa Y, Yahagi N *et al*.: **Transgenic mice overexpressing nuclear SREBP-1c in pancreatic beta-cells.** *Diabetes* 2005, **54:** 492-499.

261. Ide T, Shimano H, Yahagi N, Matsuzaka T, Nakakuki M, Yamamoto T *et al*.: **SREBPs suppress IRS-2-mediated insulin signalling in the liver.** *Nat Cell Biol* 2004, **6:** 351-357.

262. Biden TJ, Boslem E, Chu KY, Sue N: **Lipotoxic endoplasmic reticulum stress, beta cell failure, and type 2 diabetes mellitus.** *Trends Endocrinol Metab* 2014.

263. Zambo V, Simon-Szabo L, Szelenyi P, Kereszturi E, Banhegyi G, Csala M: **Lipotoxicity in the liver.** *World J Hepatol* 2013, **5:** 550-557.

264. Rakhshandehroo M, Knoch B, Muller M, Kersten S: **Peroxisome proliferator-activated receptor alpha target genes.** *PPAR Res* 2010, **2010**.

265. Ouellette C, Cormier H, Rudkowska I, Guenard F, Lemieux S, Couture P *et al.*: **Polymorphisms in genes involved in the triglyceride synthesis pathway and marine omega-3 polyunsaturated fatty acid supplementation modulate plasma triglyceride levels.** *J Nutrigenet Nutrigenomics* 2013, **6:** 268-280.

266. Iizuka K, Horikawa Y: **ChREBP: a glucose-activated transcription factor involved in the development of metabolic syndrome.** *Endocr J* 2008, **55:** 617-624.

267. Masi LN, Rodrigues AC, Curi R: **Fatty acids regulation of inflammatory and metabolic genes.** *Curr Opin Clin Nutr Metab Care* 2013, **16:** 418-424.

268. Chen YJ, Chen CC, Li TK, Wang PH, Liu LR, Chang FY *et al.*: **Docosahexaenoic acid suppresses the expression of FoxO and its target genes.** *J Nutr Biochem* 2012, **23:** 1609-1616.

269. Gil-Zamorano J, Martin R, Daimiel L, Richardson K, Giordano E, Nicod N *et al.*: **Docosahexaenoic acid modulates the enterocyte Caco-2 cell expression of microRNAs involved in lipid metabolism.** *J Nutr* 2014, **144:** 575-585.

270. Rudkowska I, Guenard F, Julien P, Couture P, Lemieux S, Barbier O *et al.*: **Genome-wide association study of the plasma triglyceride response to an n-3 polyunsaturated fatty acid supplementation.** *J Lipid Res* 2014, **55:** 1245-1253.

271. Giguere A, Legare F, Grad R, Pluye P, Rousseau F, Haynes RB *et al.*: **Developing and user-testing Decision boxes to facilitate shared decision making in primary care--a study protocol.** *BMC Med Inform Decis Mak* 2011, **11:** 17.

272. DeBusk R: **Nutrigenetic Testing: Considerations for Clinicians.** *Alternative and Complentary Therapies*, in press.

273. **Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report.** *Circulation* 2002, **106:** 3143-3421.

274. Blackburn P, Lemieux I, Almeras N, Bergeron J, Cote M, Tremblay A *et al.*: **The hypertriglyceridemic waist phenotype versus the National Cholesterol Education Program-Adult Treatment Panel III and International Diabetes**

Federation clinical criteria to identify high-risk men with an altered cardiometabolic risk profile. *Metabolism* 2009, **58:** 1123-1130.