Alimentation et inflammation : considérations épidémiologiques, cliniques et métaboliques

Thèse

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RÉSUMÉ

L'inflammation systémique et chronique dite « de faible intensité » est un élément clé du développement et de la progression de plusieurs désordres métaboliques tels que l'athérosclérose, le diabète de type 2 et les maladies cardiovasculaires. De plus en plus d'évidences suggèrent que l'alimentation jouerait un rôle de premier plan dans la modulation du profil inflammatoire, mais plusieurs questions demeurent non résolues à ce jour. Ainsi, l'**objectif global** du présent projet de doctorat était d'étudier l'impact de l'alimentation sur l'inflammation et ses mécanismes sous-jacents en utilisant trois approches expérimentales, soit 1) une approche épidémiologique, 2) une approche clinique et 3) une approche métabolique. L'alimentation a elle-même été étudiée sous différents angles incluant de simples nutriments (acides gras, dont les oméga-3), des aliments (produits laitiers) et des profils alimentaires reflétant l'alimentation dans sa globalité. **En premier lieu**, nous avons évalué les associations entre divers facteurs nutritionnels (oméga-3 et profils alimentaires) et le profil inflammatoire chez deux nations autochtones du Nord-du-Québec. Ces populations ont été choisies étant donné la forte et récente augmentation de la prévalence de plusieurs désordres métaboliques chez celles-ci parallèlement à un important phénomène de transition nutritionnelle. De façon globale, nos travaux démontrent que l'alimentation des Cris de la Baie-James et des Inuits du Nunavik semble exercer une influence non significative sur leur profil inflammatoire. **En second lieu**, nous avons réalisé une étude d'intervention nutritionnelle randomisée contrôlée portant sur l'impact de la consommation de produits laitiers sur l'inflammation ainsi qu'une revue systématique de la littérature sur le sujet. Il ressort de ces travaux que la consommation de produits laitiers dans le cadre d'une alimentation saine n'exerce aucun effet défavorable sur le profil inflammatoire. **En troisième lieu**, nous avons réalisé deux études d'intervention nutritionnelle randomisées contrôlées, conçues selon un devis en chassé-croisé, qui suggèrent que la consommation de divers acides gras, dont des acides gras oméga-3 d'origine marine, influence peu ou pas l'expression de gènes inflammatoires dans le sang de sujets avec obésité abdominale ou dans le duodénum d'hommes obèses et atteints du diabète de type 2. Bref, d'après l'ensemble des présents travaux, l'alimentation influencerait peu l'inflammation.

ABSTRACT

Low-grade systemic inflammation is a key etiological factor in the development and progression of several multifactorial disorders including atherosclerosis, type 2 diabetes and cardiovascular diseases. Increasing evidence suggests that diet significantly modulates the inflammatory profile. However, several questions about this topic remain unanswered at this time. The **major aim** of the present PhD project was to study the impact of diet on inflammation and its underlying mechanisms by using three different experimental approaches, namely 1) an epidemiological approach, 2) a clinical approach and 3) a metabolic approach. Diet also has been studied from various angles including nutrients (dietary fatty acids, such as omega-3), foods (dairy products) and dietary patterns reflecting diet as a whole. **First**, we assessed the associations between different nutritional factors (omega-3 and dietary patterns) and circulating inflammatory biomarkers in two Aboriginal nations from Northern Quebec. These nations were selected based on the considerable and recent increase in the prevalence of several metabolic disorders in these populations in conjunction with an important nutrition transition. Overall, our work indicates that the diet of the James Bay Cree and Nunavik Inuit populations appears to exert only a trivial influence on their inflammatory profile. **Second**, we conducted a randomized crossover controlled nutrition intervention study assessing the impact of dairy product consumption on inflammation as well as a systematic review of the literature on this topic. Our work suggests that consumption of dairy products as part of a healthy diet has no adverse effect on the inflammatory profile. **Third**, we conducted two randomized crossover controlled nutrition intervention studies which showed that the consumption of different dietary fatty acids, including omega-3 fatty acids from marine sources, exerts little or no influence on the expression of inflammatory genes in whole blood cells of individuals with abdominal obesity or in duodenal cells of obese men with type 2 diabetes. Taken together, our various works presented here suggest that diet has a minor influence on inflammation.

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AA : Acide arachidonique (*Arachidonic acid* ; C20:4 n-6)
AG : Acide gras
AGMI : Acide gras monoinsaturé
AGPI : Acide gras polyinsaturé
AGS : Acide gras saturé
AGT : Acide gras *trans*
ALA : Acide alpha-linolénique (*Alpha-linolenic acid* ; C18:3 n-3)
ARN : Acide ribonucléique
ARNm : ARN messager
AVC : Accident vasculaire cérébral
C : Cholestérol
CANTOS : *Canakinumab Anti-Inflammatory Thrombosis Outcomes Study*
CCL2 : *Chemokine (C-C motif) ligand 2* (autre nom donné à MCP-1)
CHU : Centre hospitalier universitaire
CIRT : *Cardiovascular Inflammation Reduction Trial*
COMIT : *Canola Oil Multicenter Intervention Trial*
CRP : Protéine C-réactive (*C-reactive protein*)
DHA : Acide docosahexaénoïque (*Docosahexaenoic acid* ; C22:6 n-3)
DPAn-3 : Acide docosapentaénoïque (*Docosapentaenoic acid* ; C22:5 n-3)
EPA : Acide eicosapentaénoïque (*Eicosapentaenoic acid* ; C20:5 n-3)
FADS2 : Enzyme Δ-6 désaturase ou *Fatty acid desaturase 2*
FFQ : Questionnaire de fréquence alimentaire
HDL : Lipoprotéine de haute densité (*High density lipoprotein*)
hs-CRP : Protéine C-réactive mesurée par dosage ultra-sensible *(high-sensitivity C-reactive protein)*
IC 95% : Intervalle de confiance à 95%
IL : Interleukine
IFN-γ : Interféron-gamma
IMC : Indice de masse corporelle
INAF : Institut sur la nutrition et les aliments fonctionnels
JNK : *c-Jun N-terminal kinase* (voie de signalisation inflammatoire)
JUPITER : *Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin*
LA : Acide linoléique (*Linoleic acid* ; C18:2 n-6)
LDL : Lipoprotéine de faible densité (*Low density lipoprotein*)
MCP-1 : *Monocyte chemoattractant protein-1* (facteur chimiotactique)
MCV : Maladies cardiovasculaires
M.G. : Matières grasses

MIP-1α : Protéine inflammatoire des macrophages 1α (*Macrophage inflammatory protein 1α*)

NF-κB : Facteur de transcription nucléaire kappa-B (*Nuclear transcription factor kappa B*)

OA : Acide oléique (*Oleic acid* ; C18:1 n-9)

PAI-1 : Inhibiteur de l'activateur du plasminogène 1

PBMC : Cellules mononucléaires du sang périphérique (*Peripheral blood mononuclear cells)*

PennState : *Pennsylvania State University*

PLI : Projet « Produits Laitiers et Inflammation »

PPAR : *Peroxisome proliferator-activated receptor* (récepteur nucléaire)

RCFFN : *Richardson Centre for Functional Foods and Nutraceuticals*

SAA : Protéine amyloïde A sérique (S*erum amyloid A*)

SP-POS : Axe Santé des Populations et Pratiques Optimales en Santé

STAT3 : *Signal transducer and activator of transcription 3* (facteur de transcription)

TLR : Récepteurs de type *Toll* (*Toll-like receptors)*

TNF-α : Facteur de nécrose tumorale (*Tumor necrosis factor alpha*)

À l'amour de ma vie, Simon, mon plus grand supporteur.
Merci pour ta précieuse présence à mes côtés depuis le
tout début ainsi que pour les années à venir…La Ville
Reine nous attend avec de nouveaux défis!

« Ce qu'on obtient en atteignant nos objectifs n'est pas aussi
important que ce que l'on devient en les atteignant. »
– Zig Ziglar

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Au cours des quatre années et demie de mes études au doctorat en nutrition à l'Institut sur la nutrition et les aliments fonctionnels (INAF) de l'Université Laval, je me considère privilégiée d'avoir eu l'opportunité de contribuer à huit projets de recherche différents ayant fait l'objet de neuf articles scientifiques dont j'ai le statut de principale auteure, sans compter ma contribution à quelques autres projets ayant fait l'objet d'articles dont j'ai le statut de coauteure. Mon implication dans ces projets de recherche fort diversifiés aura été une expérience extrêmement enrichissante!

Bien entendu, la réalisation de ces divers projets n'aurait guère été possible sans la contribution de NOMBREUSES personnes. Ainsi, j'ai décidé de réserver la présente section à mes remerciements de nature plus personnelle. Mes remerciements envers les toutes les personnes (investigateurs, collaborateurs, coordonnateurs, personnel de laboratoire, personnel infirmier, étudiants, participants, etc.) impliquées dans chacun des projets qui font partie de cette thèse seront mentionnés en **avant-propos**.

Je désire d'abord remercier LA personne sans qui le présent projet de doctorat n'aurait pas vu le jour, mon directeur de recherche, le Dr Benoît Lamarche. Benoît, j'espère que tu réalises à quel point j'ai adoré travailler avec toi au cours de mon doctorat, de ma maîtrise et de mon baccalauréat. Au moment où j'effectuerai le dépôt final de cette thèse, cela fera près de 9 ans (déjà!) que nous faisons équipe. En toute honnêteté, je n'ai vraiment pas vu le temps passer! Je désire te remercier pour m'avoir lancé ce beau et grand défi qui est celui d'avoir intégré des aspects épidémiologiques, cliniques et métaboliques dans un seul et même projet de doctorat. Merci pour ta disponibilité et ton écoute, particulièrement lors des derniers mois, lorsque fut le temps de décider de mon avenir « postdoctoral » ;-) Merci pour tes conseils et tes encouragements qui ont sans aucun doute contribué à mon évolution sur le plan professionnel et personnel. Ça paraît que tu aimes la recherche et tu sais très bien transmettre ton enthousiasme à quiconque a la chance de te côtoyer. Maintenant que je vais commencer à voler de mes propres ailes, tout ce que je souhaite c'est que nous ayons à nouveau l'opportunité de travailler/collaborer ensemble dans le futur.

Je désire maintenant remercier mon codirecteur, le Dr Patrick Couture, pour avoir poussé mes réflexions vers un point de vue davantage clinique. J'ai bien aimé découvrir ton petit côté « pince-sans-rire » lors des congrès de l'ATVB et je me rappellerai d'ailleurs pendant longtemps de tes anecdotes hilarantes.

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Merci aux étudiant(e)s et professionnel(le)s de recherche en nutrition que j'ai eu l'occasion de côtoyer et qui font de l'INAF un endroit tellement agréable où travailler. Un merci spécial à Amélie Charest : même si nous avons moins collaboré ensemble pendant mon doctorat que lors de mes études précédentes, je tiens à te remercier pour ta générosité et ta disponibilité lorsque tu as répondu à mes nombreuses et diverses questions de nature « terrain ». Merci à Johanne Marin qui m'a très bien « *coaché* » lors de mon introduction au monde du labo à l'hiver 2013. J'ai grandement apprécié les « après-midis d'extraction » passés en ta compagnie. Je remercie aussi spécialement mes collègues et amies doctorantes, « Câro », « Vickaï » et Alex, pour leur gentillesse, leur bonne humeur et pour avoir partagé quotidiennement ce bout de vie avec moi. Je vous aime pas mal!

Je désire enfin remercier tous mes proches qui me permettent de garder un équilibre dans ma vie et de persévérer dans les moments plus difficiles. Ma chère « gang de *chummys* », peu importe si vous vous trouvez en Australie (clin d'œil pour Anick), à Montréal (clin d'œil pour Francis), à Jonquière (clin d'œil pour Kim et Vincent) ou dans la grande région de Québec (clin d'œil pour tous les autres, vous vous reconnaissez), merci de votre présence! Chaque occasion que j'ai de vous parler ou de vous voir me permet de décrocher, que ce soit l'instant d'un souper, d'une partie de jeu de société, d'une partie de football ou d'une journée de ski. Merci à ma famille et à ma belle-famille, qui m'encouragent dans mes

projets et qui croient en moi depuis le tout début. Les nombreux soupers du dimanche passés en votre bonne compagnie m'ont tellement fait du bien. Puis, je remercie de tout cœur mon amoureux Simon, à qui j'ai dédié cette thèse. Merci pour ton amour, pour ton support inconditionnel, pour ta joie de vivre et pour ton côté farceur qui embellissent chacune de mes journées!

AVANT-PROPOS

Je m'excuse à l'avance, chers lecteurs, pour la longueur de cet avant-propos! Comme je l'ai mentionné précédemment, beaucoup de personnes ont contribué à la réalisation des projets de recherche constituant « mon projet de doctorat ». C'est également dans cet avant-propos que je dois décrire ma contribution à chacun de mes articles scientifiques de même que celle de mes coauteurs. À ce sujet, six des neuf articles que j'ai eu l'opportunité de rédiger en tant que principale auteure au cours de mon doctorat font partie intégrante de cette thèse, aux **chapitres 4, 5, 8, 9, 12 et 13**. Comme le dévoile le titre de ma thèse, ces six articles, bien que résultant parfois de projets avec des méthodologies très différentes, ont toutes une même thématique en commun : l'alimentation et l'inflammation. Un septième article, touchant à la question de l'inflammation, mais pas de l'alimentation, se trouve également en **annexe B**. Je ferai précisément référence à cet article au cours du chapitre 3.

Il est à noter que j'ai divisé les prochains paragraphes en fonction des numéros de chapitre incluant mes articles scientifiques, qui sont tous publiés dans des revues scientifiques avec comité de lecture. Dans chaque cas, je décrirai d'abord ma contribution à l'article/projet de recherche en question. Dans un deuxième temps, j'en profiterai pour remercier et souligner la contribution de mes coauteurs et des autres personnes impliquées dans la réalisation de chacun des projets à la base de mes travaux.

Tout d'abord, je voudrais remercier sincèrement **tous les participants aux différents projets de recherche**. Sans leur contribution, aucun de ces projets et publications n'aurait vu le jour. Merci aussi une fois de plus au **Dr Benoît Lamarche**, professeur titulaire à l'École de nutrition de l'Université Laval, et au **Dr Patrick Couture**, médecin clinicien et professeur titulaire au Département de médecine de l'Université Laval. Respectivement en tant que mon directeur et mon co-directeur, le Dr Lamarche et le Dr Couture ont été de fidèles coauteurs sur chacun de mes articles. Le Dr Lamarche était également responsable de l'approbation finale de chacun des articles. La contribution plus spécifique du Dr Lamarche et du Dr Couture à chaque manuscrit/projet de recherche est décrite plus bas. Puis, je suis sincèrement désolée si jamais j'oublie de souligner la contribution de certaines personnes. Un tel oubli ne s'avèrerait aucunement volontaire!

Chapitre 4

Labonté ME, Dewailly E, Lucas M, Couture P, Lamarche B. Association of red blood cell n-3 polyunsaturated fatty acids with plasma inflammatory biomarkers among the Quebec Cree population. *Eur J Clin Nutr*. 2014. 68(9):1042-7.

Chapitre 5

Labonté ME, Dewailly E, Lucas M, Chateau-Degat ML, Couture P, Lamarche B. Traditional dietary pattern is associated with elevated cholesterol among the Inuit of Nunavik. *J Acad Nutr Diet*. 2014. 114(8):1208-1215.e3.

Les données nécessaires à la réalisation de chacune de ces deux publications ayant été recueillies il y a quelques années dans le cadre de grandes enquêtes de santé chez les Cris de la Baie-James et les Inuits du Nunavik (des détails à ce sujet seront fournis plus tard), mes tâches auront principalement consisté en l'analyse et l'interprétation des données, puis la rédaction des articles scientifiques en tant que principale auteure. J'ai apporté des modifications aux articles suite à leur révision par les coauteurs, avant leur soumission. J'ai également effectué les révisions requises par les réviseurs des journaux scientifiques avant que les articles soient acceptés pour publication.

La réalisation des grandes enquêtes de santé à la base de ces publications n'aurait aucunement été possible sans la contribution du **Dr Éric Dewailly**, qui était professeur au Département de médecine sociale et préventive de l'Université Laval et chercheur de l'axe Santé des Populations et Pratiques Optimales en Santé (SP-POS) du Centre de recherche du Centre hospitalier universitaire (CHU) de Québec. Le Dr Dewailly a entre autres participé à la conception de ces projets ainsi qu'à la collecte de données sur le terrain. Le **Dr Benoît Lamarche** et le **Dr Patrick Couture** ont collaboré à l'analyse et l'interprétation des données. Le **Dr Michel Lucas**, professeur au Département de médecine sociale et préventive de l'Université Laval et chercheur de l'axe SP-POS du Centre de recherche du CHU de Québec, a également contribué à l'analyse et l'interprétation des données sur la base de son expertise en épidémiologie nutritionnelle. La **Dre Marie-Ludivine Chateau-Degat**, professeure associée au Département de médecine familiale et de médecine

d'urgence de l'Université Laval et chercheuse de l'axe SP-POS du Centre de recherche du CHU de Québec, m'est grandement venue en aide lors de mes débuts dans le domaine de l'épidémiologie et de l'analyse de grandes banques de données. Je désire ensuite remercier **Elhadji A.-Laouan-Sidi**, statisticien de l'axe SP-POS du Centre de recherche du CHU de Québec, qui m'a fourni une aide précieuse en lien avec les analyses statistiques ayant mené à la réalisation des présentes publications. Dans le cadre du projet chez les Cris, je veux également remercier **Suzanne Côté**, professionnelle de recherche au Centre de recherche du CHU de Québec, qui a toujours fourni rapidement réponse à mes diverses questions. Je remercie aussi tous les **coordonnateurs**, le **personnel infirmier** et les **interviewers** qui ont grandement contribué à chaque étape de la collecte des données dans chacune des enquêtes de santé. Dans le cadre du projet chez les Inuits, mes remerciements vont également à l'**équipage du bateau de la garde-côtière Amundsen**, sur lequel la collecte de données a été effectuée.

Chapitre 8

Labonté ME, Couture P, Richard C, Desroches S, Lamarche B. Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults. *Am J Clin Nutr*. 2013;97(4):706-17.

Cette publication est une revue systématique de la littérature à laquelle j'ai participé à toutes les étapes, incluant la planification de la stratégie de recherche de la littérature scientifique, la réalisation de cette recherche, l'extraction des données de chaque étude retenue, l'analyse du risque de biais à l'intérieur de chaque étude et la rédaction de l'article en entier. J'ai apporté des modifications à l'article suite à sa révision par les coauteurs, avant sa soumission. J'ai également effectué les révisions requises par les réviseurs de la revue scientifique avant que l'article soit accepté pour publication.

Le **Dr Benoît Lamarche** et le **Dr Patrick Couture** ont participé à la conception de cette recherche systématique de la littérature et supervisé sa réalisation. La **Dre Sophie Desroches**, professeure agrégée à l'École de nutrition de l'Université Laval, a également contribué à la mise en œuvre et à la réussite de ce projet sur la base de son expertise reconnue dans le domaine des revues systématiques. La **Dre Caroline Richard**, qui était

étudiante au doctorat en nutrition sous la direction du Dr Lamarche au moment de la réalisation de ce projet, a participé à la recherche systématique de la littérature et à l'analyse du risque de biais à l'intérieur de chaque étude.

Chapitre 9

Labonté ME, Cyr A, Abdullah MM, Lépine MC, Vohl MC, Jones P, Couture P, Lamarche B. Dairy product consumption has no impact on biomarkers of inflammation among men and women with low-grade systemic inflammation. *J Nutr*. 2014;144:1760-7.

Il est important de souligner que je partage le statut de premier auteur de cette publication avec une ancienne étudiante à la maîtrise dans l'équipe Lamarche, **Audrey Cyr**. En effet, Audrey a grandement participé au volet clinique de cette étude d'intervention. Elle a aussi réalisé le premier jet de l'article à partir des résultats relatifs à la comparaison des concentrations des biomarqueurs inflammatoires à la fin de chacune des diètes (diète riche en produits laitiers vs diète témoin). Ce premier jet a fait l'objet de son mémoire de maîtrise. Ma contribution à ce projet aura d'abord été de fournir de l'aide à Audrey pour la réalisation des analyses préliminaires. Une fois la maîtrise d'Audrey terminée, j'ai pris en charge le reste des analyses du projet. J'ai ré-analysé les données concernant les concentrations des biomarqueurs inflammatoires à la fin de chacune des diètes avec le nombre final de participants. J'ai effectué les analyses concernant la variation des concentrations des biomarqueurs inflammatoires à l'intérieur de chacune des diètes (variations « post- moins pré-diète » ou « *within-diet variations* »). Au laboratoire, j'ai participé à l'extraction de l'acide ribonucléique (ARN) provenant d'échantillons sanguins d'un sous-groupe de participants en vue de la mesure de l'expression de gènes inflammatoires dans les cellules sanguines complètes (« *whole blood* ») à la fin de chacune des diètes, puis j'ai analysé ces données. J'ai finalement réalisé la dernière ébauche de l'article en intégrant l'ensemble des résultats obtenus, ce qui a nécessité des ajouts, modifications et mises à jour dans toutes les sections allant de l'introduction à la conclusion. J'ai apporté d'autres modifications à l'article suite à sa révision par les coauteurs, avant sa soumission, puis j'ai effectué les révisions requises par les réviseurs de la revue scientifique avant qu'il soit accepté pour publication.

Le **Dr Benoît Lamarche**, le **Dr Patrick Couture** et le **Dr Peter J Jones**, directeur du *Richardson Centre for Functional Foods and Nutraceuticals* (RCFFN) de l'Université du Manitoba, à Winnipeg, ont participé à la conception de ce projet d'intervention nutritionnelle multicentrique. Le Dr Couture était également responsable du volet médical du projet. La **Dre Marie-Claude Vohl**, professeure titulaire à l'École de nutrition de l'Université Laval, a contribué à ce projet sur la base de son expertise dans le domaine de la génomique. **Marie-Claude Lépine**, professionnelle de recherche à l'INAF, ainsi que **Mohammad M. Abdullah**, étudiant au doctorat sous la direction du Dr Jones au RCFFN, ont coordonné le volet clinique de l'étude en collaboration avec Audrey Cyr. Je remercie le personnel infirmier de l'Unité d'investigation clinique de l'INAF, **Steeve Larouche**, **Danielle Aubin** et **Myriam Bouchard**, de même que le personnel infirmier au RCFFN, pour leurs soins précieux envers les participants. Je remercie aussi **Johanne Marin**, professionnelle de recherche à l'INAF, pour les analyses de laboratoire. Finalement, merci aux **stagiaires** et **étudiant(e)s** qui ont contribué entre autres à la saisie de données et à la préparation des produits fournis aux participants.

Chapitre 12

Baril-Gravel L, **Labonté ME**, Couture P, Vohl MC, Charest A, Guay V, Jenkins DA, Connelly PW, West S, Kris-Etherton PM, Jones PJ, Fleming JA, Lamarche B. Docosahexaenoic acid-enriched canola oil increases adiponectin concentrations: A randomized crossover controlled intervention trial. *Nutr Metab Cardiovasc Dis*. 2015;25:52-59.

Il est important de souligner que je partage ici aussi le statut de premier auteur de la publication avec une ancienne étudiante à la maîtrise dans l'équipe Lamarche, **Lisa Baril-Gravel**. En effet, Lisa a grandement participé au volet clinique de cette étude. Elle a aussi réalisé le premier jet de l'article à partir des résultats relatifs à la comparaison des concentrations des biomarqueurs inflammatoires à la fin de chacune des 5 diètes expérimentales (ces diètes seront décrites plus tard). Ce premier jet a fait l'objet de son mémoire de maîtrise. Mon implication aura d'abord été de contribuer à l'occasion à la réalisation du volet clinique de l'étude et de fournir de l'aide à Lisa pour la réalisation des analyses préliminaires. Une fois la maîtrise de Lisa terminée, j'ai pris en charge le reste des

analyses du projet. J'ai ré-analysé les données concernant les concentrations des biomarqueurs inflammatoires à la fin de chacune des diètes expérimentales avec le nombre final de participants. Au laboratoire, j'ai participé à l'extraction de l'ARN provenant d'échantillons sanguins d'un sous-groupe de participants en vue de la mesure de l'expression de gènes inflammatoires dans les cellules sanguines complètes à la fin de chacune des diètes, puis j'ai analysé ces données. J'ai finalement réalisé la dernière ébauche de l'article en intégrant l'ensemble des résultats obtenus, ce qui a nécessité des ajouts, modifications et mises à jour dans toutes les sections allant de l'introduction à la conclusion. J'ai apporté d'autres modifications à l'article suite à sa révision par les co-auteurs, avant sa soumission, puis j'ai effectué les révisions requises par la revue scientifique avant qu'il soit accepté pour publication.

Le **Dr Peter J Jones,** le **Dr Patrick Couture**, le **Dr Benoît Lamarche**, le **Dr David JA Jenkins** de la St-Michael's Hospital de l'Université de Toronto, le **Dr Philip W Connelly** également de la St-Michael's Hospital de l'Université de Toronto, la **Dre Sheila West** de *Pennsylvania State University* (PennState) de même que la **Dre Penny Kris-Etherton** de PennState ont participé à la conception de ce vaste projet d'intervention nutritionnelle multicentrique. Le Dr Couture était également responsable du volet médical du projet du côté de l'INAF. La **Dre Marie-Claude Vohl** a contribué à ce projet sur la base de son expertise dans le domaine de la génomique. **Amélie Charest** et **Valérie Guay**, professionnelles de recherche, ont coordonné le volet clinique de l'étude du côté de l'INAF en collaboration avec Lisa Baril-Gravel. **Jennifer A Fleming** a quant à elle coordonné le volet clinique du projet du côté de PennState. Je remercie le personnel infirmier de l'Unité d'investigation clinique de l'INAF, **Steeve Larouche**, **Danielle Aubin** et **Myriam Bouchard**, pour leurs soins précieux envers les participants. Je remercie aussi **Johanne Marin** pour les analyses de laboratoire. Un énorme merci aux techniciennes de la cuisine métabolique de l'INAF, **Sandra Gagnon**, **Stéphanie Ouellet**, **Marie-Pier Devost**, **Émilie Martel**, **Catherine Blouin**, qui ont préparé les aliments fournis aux participants. Merci aussi aux **coordonnateurs**, **personnel infirmier**, **personnel de laboratoire** et **personnel de la cuisine métabolique des autres centres** qui ont pris part à ce projet. Finalement, merci à tous les **stagiaires** et **étudiant(e)s** qui ont contribué entre autres à la saisie de données et à la préparation des repas fournis aux participants.

Chapitre 13

Labonté ME, Couture P, Tremblay AJ, Hogue JC, Lemelin V, Lamarche B. Eicosapentaenoic and docosahexaenoic acid supplementation and inflammatory gene expression in the duodenum of obese patients with type 2 diabetes. *Nutr J*. 2013;12(1):98.

Le volet clinique étant déjà terminé au moment où mon implication dans ce projet a débuté, mes tâches auront principalement consisté en l'analyse et l'interprétation des données, puis la rédaction de l'article scientifique en tant que principale auteure. J'ai également apporté des modifications à l'article suite à sa révision par les coauteurs, avant sa soumission, puis j'ai effectué les révisions requises par les réviseurs de la revue scientifique avant qu'il soit accepté pour publication.

Le **Dr Patrick Couture**, le **Dr Benoît Lamarche** ainsi que la **Dre Valéry Lemelin**, gastro-entérologue au Centre hospitalier de l'Université Laval, ont conçu le projet à la base de ces travaux. La Dre Lemelin a réalisé les biopsies nécessaires à la mesure de l'expression de gènes dans le duodénum. Le Dr Lamarche a quant à lui également contribué à l'analyse et l'interprétation des données. Le **Dr André Tremblay**, professionnel de recherche à l'INAF, et le **Dr Jean-Charles Hogue**, professionnel de recherche à l'INAF au moment de la réalisation de cette étude, ont coordonné le volet clinique et participé à la collecte de données. Je remercie enfin les infirmières de recherche **Marjolaine Lapointe** et **Danielle Aubin** pour leurs soins précieux envers les participants.

Annexe B

Labonté ME, Dewailly E, Chateau-Degat ML, Couture P, Lamarche B. Population-based study of high plasma C-reactive protein concentrations among the Inuit of Nunavik. *Int J Circumpolar Health*. 2012;71:19066. doi: 10.3402/ijch.v71i0.19066.

Ma contribution à cet article de même que celle de mes coauteurs est essentiellement la même que dans la description fournie plus haut en lien avec les articles constituant les chapitres 4 et 5 de la présente thèse.

Note concernant les publications non incluses dans ma thèse

Je tiens enfin à souligner ma participation, au tout début de mon doctorat, à la rédaction de deux articles scientifiques en tant que principale auteure qui ne font toutefois pas partie de ma thèse. Premièrement, j'ai rédigé un article intitulé « *Validity and reproducibility of a web-based, self-administered food frequency questionnaire* » qui est publié dans la revue *European Journal of Clinical Nutrition* (Labonté *et al.* 2012;66(2):166-73). J'ai eu l'opportunité de coordonner toutes les étapes de ce projet, ce qui fait que je le considère en quelque sorte comme « mon bébé ». Au cours de mon baccalauréat, j'ai entre autres participé au développement du questionnaire de fréquence alimentaire web (choix des questions à inclure, création d'une base de données nutritionnelles) en partenariat avec des programmeurs en informatique. J'ai aussi pris en charge l'étude de validation elle-même (approbation à l'éthique, recrutement des participants, collecte de données). Au cours de ma maîtrise, en « *sideline* » de mon projet officiel, j'ai réalisé l'analyse et l'interprétation des données issues de l'étude de validation. Au début de mon doctorat, j'ai terminé les analyses, rédigé l'article de validation et pris en charge les révisions demandées par les réviseurs de la revue scientifique. Je suis fière d'avoir contribué à la conception de cet outil d'évaluation alimentaire bilingue et automatisé maintenant utilisé par de nombreux chercheurs à l'INAF ainsi que dans d'autres institutions de recherche à travers le Canada.

Le deuxième article qui ne fait pas partie de ma thèse s'intitule « *Adding MUFA to a dietary portfolio of cholesterol-lowering foods reduces apoAI fractional catabolic rate in subjects with dyslipidaemia* ». Cet article est publié dans la revue *British Journal of Nutrition* (Labonté *et al.* 2013;110(3):426-36). Ma contribution aura principalement consisté en l'analyse et l'interprétation des données sur la cinétique des apolipoprotéines A1, B100 et B48 en réponse à la consommation de diètes portfolio composées d'aliments hypocholestérolémiants avec un contenu soit faible ou élevé en acides gras monoinsaturés. J'ai également rédigé l'article en entier, puis j'ai pris en charge les révisions requises par les coauteurs et par les réviseurs de la revue scientifique. Cette étude aura permis de mieux comprendre les mécanismes par lesquels l'ajout d'acides gras monoinsaturés à une diète reconnue pour abaisser les concentrations plasmatiques de cholestérol peut potentialiser davantage ses effets hypocholestérolémiants.

INTRODUCTION GÉNÉRALE

Les maladies chroniques d'origine multifactorielle telles que le diabète de type 2, le cancer et les maladies cardiovasculaires (MCV) représentent un lourd fardeau économique pour les pays industrialisés et le Québec n'échappe pas à cette situation. Selon le *Conference Board* du Canada, en 2013, les coûts totaux de six maladies chroniques (cardiopathies ischémiques, maladies vasculaires cérébrales, maladie pulmonaire obstructive chronique, cancer du poumon, hypertension artérielle et diabète) ont été évalués à 8,1 G$ dans notre « Belle Province » (1). On reconnaît de plus en plus l'existence d'un état pathologique sous-jacent commun à l'ensemble des désordres métaboliques multifactoriels : l'inflammation systémique et chronique dite « de faible intensité » (2) (**Figure I**). Le fait que l'inflammation systémique soit associée au développement et à la progression de plusieurs désordres métaboliques multifactoriels dénote l'importance de s'attarder à cette condition dans le but ultime de viser une amélioration de l'état de santé des individus. On reconnaît aussi de plus en plus, comme j'aurai l'occasion d'en discuter au prochain chapitre, qu'un des facteurs clés dans la modulation du profil inflammatoire serait l'« alimentation » au sens large (3). Ainsi, en tant que nutritionniste, il m'apparaissait fort pertinent d'étudier la grande thématique de l'alimentation et de l'inflammation dans le cadre de mon doctorat.

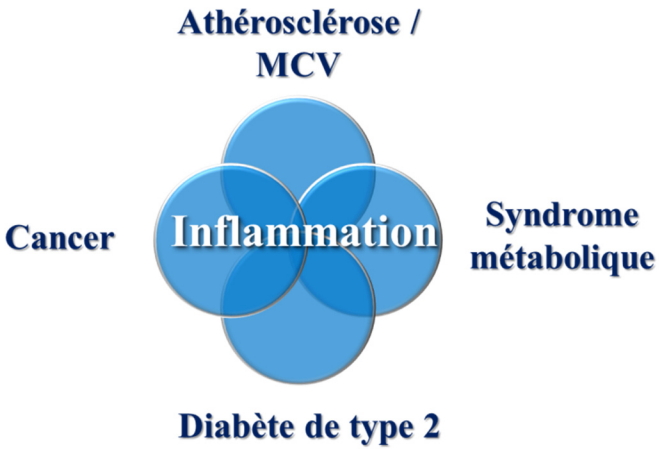

Figure I : L'inflammation : point commun de plusieurs désordres métaboliques d'origine multifactorielle. Création personnelle inspirée de l'article de Scrivo *et al.* (2). Abréviations : MCV, maladies cardiovasculaires.

La présente thèse est divisée en 15 chapitres. Pour le moment, notez simplement que le chapitre 1 présente un portrait global de l'inflammation incluant, entre autres, les différents types d'inflammation et les facteurs influençant le profil inflammatoire. Cette vue d'ensemble m'amène ensuite à vous présenter, au chapitre 2, la problématique générale de mon projet de doctorat. En effet, j'y révèle l'objectif général du projet, l'hypothèse qui s'y rattache et le cadre méthodologique retenu. C'est donc au cours de ce chapitre que j'introduirai les chapitres 3 à 14 qui, comme vous le constaterez, sont divisés en 3 volets. Enfin, le chapitre 15 constitue la conclusion générale de ma thèse.

CHAPITRE 1 : L'INFLAMMATION

1.1 Les différents types d'inflammation

L'inflammation se définit de prime abord comme une réponse physiologique normale de l'organisme contre une agression telle qu'une infection ou une lésion corporelle (blessure, brûlure, irritation) afin de rétablir l'homéostasie aux sites infectés ou endommagés (4). De façon typique, la survenue de l'agression entraîne l'activation de récepteurs du système immunitaire inné tels que les récepteurs de type *Toll* (TLR ; « *Toll-like receptors* »), ce qui mène à la libération de médiateurs chimiques pro-inflammatoires (5, 6). La libération de ces molécules entraîne une augmentation du débit sanguin vers le site de l'agression ainsi qu'une augmentation de la perméabilité capillaire (7). Ce phénomène survient dans le but que les acteurs du système immunitaire arrivent à maîtriser la source de l'agression et le tout se traduit en l'apparition des cinq signes classiques de l'**inflammation aiguë** : la rougeur, la chaleur, la tuméfaction, la douleur et la perte de fonction (6, 8). Une fois l'agresseur « maîtrisé », un processus d'autorégulation nommé **résolution de l'inflammation** se met en branle (8, 9). Ce processus implique la sécrétion de molécules anti-inflammatoires et de médiateurs lipidiques pro-résolutifs (traduction libre de « *pro-resolving lipid mediators* ») dont les rôles sont de protéger les organes de l'hôte contre de possibles dommages « collatéraux », d'activer la réparation des tissus et de promouvoir la clairance des débris cellulaires et/ou microbiens issus de la réaction inflammatoire (8, 9). Bref, il faut retenir que l'inflammation est, à la base, une réaction aiguë, localisée et finement autorégulée, dont la source est généralement exogène et dont les effets sont ultimement protecteurs.

Toutefois, certains états de déséquilibre peuvent mener à l'inflammation chronique, dont les effets sont généralement pathologiques (7, 8). Ces états de déséquilibre incluent la perte d'efficacité du processus d'autorégulation de l'inflammation aiguë, le dysfonctionnement du système immunitaire (réactions auto-immunes) ou le dysfonctionnement de certains tissus et organes induisant alors une libération continue de stimuli inflammatoires endogènes (7, 8). On distingue deux types d'inflammation chronique : un premier type est l'**inflammation chronique dite « franche »**, un terme employé par certains chercheurs

pour désigner les maladies inflammatoires chroniques à composante auto-immune s'accompagnant de manifestations cliniques évidentes ainsi que d'élévations marquées des concentrations de biomarqueurs inflammatoires dans la circulation systémique (ex : arthrite rhumatoïde, maladies inflammatoires de l'intestin, psoriasis) (8). Le deuxième type est l'**inflammation chronique dite « de faible intensité »** (« *low-grade inflammation* »), souvent qualifiée de « silencieuse » puisqu'elle ne s'accompagne pas de manifestations cliniques évidentes. De plus, l'élévation des concentrations de biomarqueurs inflammatoires dans la circulation systémique est modeste comparativement aux conditions associées à l'inflammation chronique « franche » (8). Tel qu'annoncé en introduction, l'inflammation systémique et chronique de faible intensité fait l'objet de la présente thèse.

1.2 Sources de l'inflammation

Le profil inflammatoire se traduit par l'expression d'une panoplie de biomarqueurs provenant de diverses sources. De manière simplifiée, comme l'illustre la **Figure 1.1**, la paroi vasculaire, les cellules immunitaires telles que les macrophages ainsi que le tissu adipeux sécrètent des cytokines pro-inflammatoires, une famille de peptides qui inclut entre autres les interleukines (IL-6, IL-1β) et le facteur de nécrose tumorale (TNF-α ; *« tumor necrosis factor alpha »*) (10). La sécrétion de cytokines stimule la production hépatique de

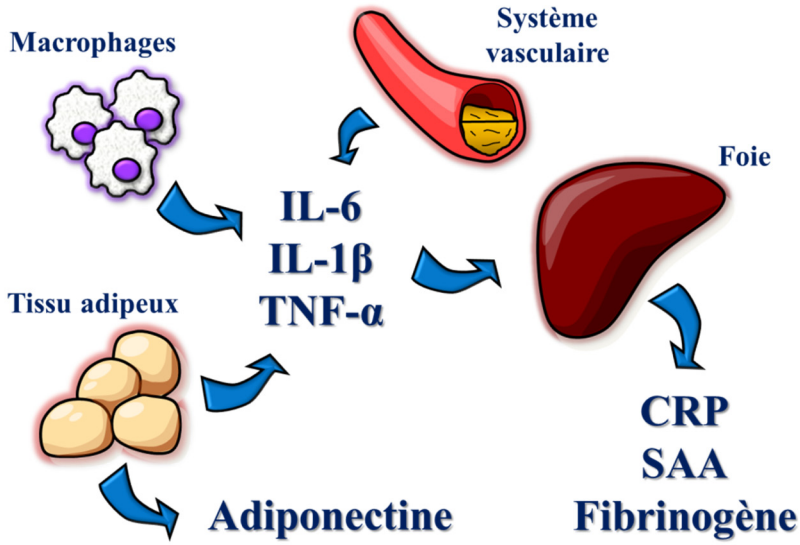

Figure 1.1 : Sources des biomarqueurs inflammatoires. Abréviations : CRP, protéine C-réactive ; IL, interleukine ; SAA, protéine amyloïde A sérique ; TNF-α, facteur de nécrose tumorale alpha.

protéines de phase aiguë incluant le fibrinogène, la protéine amyloïde A sérique (SAA ; « *serum amyloid A* ») et la protéine C-réactive (CRP, « *C-reactive protein* »), cette dernière étant l'un des biomarqueurs inflammatoires les plus étudiés à l'heure actuelle (10). Ensuite, il importe de souligner que le tissu adipeux sécrète non seulement des molécules pro-inflammatoires, mais aussi des molécules anti-inflammatoires, comme l'adiponectine (11, 12).

Le **tissu adipeux**, dans le contexte de l'obésité, jouerait un rôle crucial en ce qui a trait à l'établissement et au maintien d'un état inflammatoire chronique de faible intensité (3). Les concentrations de la CRP et de plusieurs cytokines incluant l'IL-6, l'IL-8, le TNF-α et le facteur chimiotactique MCP-1 (« *monocyte chemoattractant protein-1* ») sont en effet plus élevées chez les individus obèses que chez les individus de poids normal (13-15). À l'inverse, les concentrations de l'adiponectine sont généralement plus faibles chez les individus obèses que chez les individus de poids normal (16). Cette association, qui peut sembler paradoxale à première vue, serait potentiellement attribuable au fait que les adipocytes hypertrophiés auraient une capacité réduite de synthétiser l'adiponectine (17). Ensuite, notons que la perte de poids entraîne une diminution des concentrations de la CRP et des cytokines pro-inflammatoires (14, 18-20) et une augmentation des concentrations d'adiponectine (16, 20).

Outre la quantité totale de tissu adipeux, sa distribution dans le corps humain est un facteur important à considérer en lien avec l'inflammation chronique de faible intensité. L'obésité abdominale serait associée aux concentrations de la CRP indépendamment de l'indice de masse corporelle (IMC) (21). Le tissu adipeux viscéral produirait par ailleurs de 2 à 3 fois plus d'IL-6 que le tissu adipeux sous-cutané (22, 23), possiblement en raison d'une plus grande accumulation de macrophages dans le compartiment viscéral (24). En effet, le tissu adipeux est un tissu « hétérogène » composé non seulement d'adipocytes, mais aussi d'autres cellules telles que les cellules immunitaires (3). Bien que les adipocytes aient eux-mêmes la capacité de produire certaines cytokines comme l'IL-6 et le TNF-α (22, 25), il est important de reconnaître qu'une part considérable des cytokines pro-inflammatoires produites par le tissu adipeux dans le contexte de l'obésité proviendrait de l'infiltration et de l'accumulation d'un grand nombre de macrophages (26, 27). Ce phénomène surviendrait

en réponse à l'hypoxie des adipocytes hypertrophiés (28). Les macrophages s'accumuleraient principalement autour des adipocytes morts par nécrose afin d'éliminer les débris cellulaires (29).

1.3 Description des biomarqueurs inflammatoires

Parmi les nombreux biomarqueurs inflammatoires existants, la présente section vise à fournir davantage de détails sur ceux qui ont été principalement évalués dans le cadre de mes travaux, c'est-à-dire la CRP, l'IL-6, le TNF-α et l'adiponectine.

D'abord, tel que mentionné à la section précédente, la **CRP** est une protéine de phase aiguë principalement synthétisée et éliminée par le foie (30). Les fonctions principales de la CRP semblent reliées à l'immunité innée et à l'élimination des débris cellulaires (31). La CRP a en effet la capacité de se lier à un éventail de ligands incluant des constituants de la paroi cellulaire de microorganismes (phosphocholine à la surface de bactéries ou de mycètes), des lipoprotéines modifiées, des cellules nécrotiques et des cellules apoptotiques (31, 32). La liaison de la CRP à un ligand entraîne alors l'activation de la voie classique du complément (complexe antigène-anticorps), puis l'opsonisation et la phagocytose des microorganismes ou des matériaux cellulaires (31, 32).

Chez les individus en santé, dans des circonstances physiologiques normales, les concentrations sériques de la CRP sont très faibles avec une valeur médiane qui a été estimée à 0,8 mg/L (31). De telles concentrations nécessitent que la CRP soit mesurée à partir de méthodes de dosage dites « ultra-sensibles » (33), d'où l'utilisation répandue du terme hs-CRP (« *high-sensitivity C-reactive protein* »). En présence d'une infection, ses concentrations peuvent toutefois augmenter jusqu'à 1000 fois (31). Mis à part la présence d'une infection bactérienne, bien d'autres stimuli (brûlures, chirurgie, stress et facteurs environnementaux) peuvent entraîner une augmentation des concentrations de la CRP dans la circulation systémique, ce qui en fait un marqueur non spécifique de l'inflammation (31, 34). Malgré tout, la hs-CRP est généralement « LE » biomarqueur utilisé pour catégoriser l'inflammation systémique et chronique de faible intensité. Les concentrations de la hs-CRP sont souvent classifiées sur la base de trois catégories : < 1,0 mg/L ; 1,0 à 3,0 mg/L ; > 3,0 mg/L (35). Ces catégories équivalent respectivement à un risque « faible », « modéré »

ou « élevé » de MCV et représentent les tertiles de distribution approximatifs de la hs-CRP dans la population américaine adulte (35). Ces tertiles furent déterminés en 2003 dans un atelier conjoint des *Centers for Disease Control and Prevention* et de l'*American Heart Association*, à partir de données obtenues dans plus de 15 populations représentant plus de 40 000 individus (35). De plus, des concentrations plus grandes que 10 mg/L sont généralement considérées comme indicatrices d'une inflammation aiguë (35) ou, selon certains chercheurs, comme des concentrations « au-delà d'un stade de faible intensité » (8). Ces éléments expliquent donc que l'inflammation chronique de faible intensité soit souvent définie comme des valeurs de la hs-CRP entre 3 et 10 mg/L. À d'autres occasions, on observe une valeur seuil plus grande ou égale à 2,0 mg/L pour parler d'inflammation chronique de faible intensité associée à un haut risque cardiovasculaire. Ce seuil est basé entre autres sur les résultats de l'étude JUPITER (« *Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin* ») (36). Cette étude a en effet démontré que la prise de rosuvastatine (20 mg/j) avait réduit de 44% le taux d'incidence d'un premier événement cardiovasculaire majeur comparativement à un placebo chez des hommes et des femmes avec de faibles concentrations de cholestérol des lipoprotéines de faible densité (LDL ; « *low density lipoprotein* » ; < 3,4 mmol/L), mais des concentrations de la hs-CRP de 2,0 mg/L ou plus (36). Notez que je reviendrai sur l'association entre la hs-CRP (l'inflammation) et le risque cardiovasculaire à la prochaine section.

L'**IL-6** est quant à elle une cytokine sécrétée par les cellules immunitaires (monocytes/macrophages), les cellules endothéliales, le tissu adipeux et même le tissu musculaire (8, 37). En plus d'activer la synthèse de protéines de phase aiguë, l'IL-6 joue un rôle dans la réaction immunitaire adaptative en stimulant la différentiation des lymphocytes B (37). L'IL-6 jouerait également un rôle dans le métabolisme des lipides en stimulant la libération des triglycérides/acides gras par le tissu adipeux (8, 38) et en régulant à la baisse la lipoprotéine lipase (38). Il a par ailleurs été démontré qu'un polymorphisme dans le gène de l'IL-6, occasionnant une diminution des concentrations circulantes de cette cytokine, serait associé à une amélioration de la sensibilité à l'insuline (39).

De façon similaire à l'IL-6, le **TNF-α** est une cytokine sécrétée par les cellules immunitaires (monocytes/macrophages), les adipocytes, les cellules endothéliales et les

cellules musculaires (8, 37). Le TNF-α contribuerait entre autres à l'apoptose cellulaire (8, 37). Le TNF-α stimulerait également l'expression de molécules d'adhésion cellulaire par les cellules endothéliales (37, 40). Il est aussi suggéré que le TNF-α contribuerait au développement de la résistance à l'insuline en inhibant l'activité tyrosine-kinase du récepteur de l'insuline dans le muscle et dans le tissu adipeux (41, 42).

L'**adiponectine** est une hormone du tissu adipeux dont les concentrations sanguines sont généralement élevées, soit entre 3 et 30 μg/mL (12). Dans le foie et dans le muscle squelettique, l'adiponectine améliore l'utilisation du glucose et la sensibilité à l'insuline et stimule l'oxydation des acides gras (12, 43). L'adiponectine possède aussi plusieurs propriétés anti-inflammatoires (44). Entre autres, elle a la capacité de moduler le phénotype des macrophages en atténuant l'expression de macrophages de type M1 (pro-inflammatoires) en faveur de l'expression de macrophages de type M2 (anti-inflammatoires) (45). Dans le même ordre d'idées, elle stimule la production de l'IL-10 (46), une cytokine anti-inflammatoire préférentiellement produite par les macrophages de type M2 (44). Elle inhibe l'activation du facteur de transcription NF-κB (« *nuclear transcription factor kappa B* ») (47, 48), qui est l'une des principales voies de régulation de la réponse inflammatoire (49, 50). L'adiponectine a par ailleurs la capacité d'inhiber la transformation de macrophages en cellules spumeuses (51), ce qui lui confère des propriétés antiathérogènes.

1.4 Inflammation, athérosclérose et maladies cardiovasculaires

Tel que mentionné en d'autres termes dans l'introduction générale, des concentrations élevées de biomarqueurs pro-inflammatoires comme la hs-CRP, l'IL-6 ou le TNF-α et des concentrations abaissées de biomarqueurs anti-inflammatoires comme l'adiponectine ont été associées au risque de plusieurs désordres métaboliques d'origine multifactorielle incluant le diabète de type 2 (52-55), l'hypertension (56), le cancer (57-59) et les MCV (60-66). Parmi ces désordres métaboliques, je désire aborder un peu plus en profondeur la relation entre l'inflammation et les MCV, en passant aussi par leur principale cause sous-jacente, l'athérosclérose.

Il est maintenant reconnu que l'inflammation joue un rôle crucial à tous les stades de l'athérosclérose, en contribuant à l'initiation de la lésion athérosclérotique, à l'évolution de la plaque athéromateuse, à sa rupture, puis à la formation d'un thrombus pouvant ultimement engendrer un événement cardiovasculaire clinique (67, 68).

En accord avec cette « théorie inflammatoire » de l'athérosclérose, les résultats provenant d'études réalisées dans plus de 30 cohortes différentes au cours des 20 dernières années suggèrent que les biomarqueurs inflammatoires, et plus particulièrement la hs-CRP, **prédiraient de façon indépendante** le risque d'événements cardiovasculaires futurs (69). Une méta-analyse du groupe *Emerging Risk Factors Collaboration* a d'ailleurs démontré qu'une augmentation de 3 fois des concentrations de la hs-CRP (correspondant à un écart-type) serait associée à une augmentation de l'ordre de 27 à 55% du risque de coronaropathie, d'accident vasculaire cérébral (AVC) et de mortalité d'origine vasculaire et non vasculaire (**Tableau 1.1**) (70). La magnitude de l'augmentation du risque de coronaropathie en lien avec la hs-CRP (37%) est autant sinon plus importante que celle calculée pour une augmentation d'un écart-type de la pression sanguine systolique (35%) et du cholestérol non HDL (soustraction entre le cholestérol total et celui provenant des lipoprotéines de haute densité ou « *high density lipoproteins* ») (28%).

Tableau 1.1 : Pourcentages d'augmentation du risque de coronaropathie, d'AVC et de mortalité d'origine vasculaire et non vasculaire associés à une augmentation de 3 fois des concentrations de la hs-CRP (70)

	Méta-analyse		
	Nombre d'études considérées	Nombre de cas considérés	Augmentation du risque associée à une augmentation de 3 fois des concentrations de la hs-CRP [a]
Coronaropathie	31	5373	37%
Accident vasculaire cérébral ischémique	15	1931	27%
Mortalité d'origine vasculaire	23	1544	55%
Mortalité d'origine non vasculaire	26	4599	54%

[a] Calculs ajustés pour l'âge, le sexe, l'étude, la tension artérielle systolique, le tabagisme, l'histoire de diabète de type 2, l'IMC, les triglycérides, le cholestérol sanguin (total, non HDL, HDL) et la consommation d'alcool.

Toutefois, on ne peut passer sous silence l'existence de **certains éléments de controverse** entourant la relation entre la hs-CRP et/ou l'inflammation et les MCV. Entre autres, certains membres de la communauté scientifique s'interrogent sur la valeur ajoutée de la hs-CRP dans la prédiction d'un premier événement cardiovasculaire. Ils basent leur argumentaire sur les résultats de plusieurs études ayant démontré que la hs-CRP améliore peu ou pas la prédiction du risque cardiovasculaire au-delà des facteurs de risque « traditionnels » (ex : âge, tabagisme) (71).

L'implication de la hs-CRP en tant que facteur causal du processus athérothrombotique est par ailleurs remise en question par les résultats de récentes analyses de randomisation mendélienne (71). Des polymorphismes du gène de la CRP ont été associés à des concentrations plus élevées de la protéine et, par le fait même, « théoriquement » associés au risque de coronaropathie (72-74). En réalité, cependant, ces polymorphismes ne montraient aucune association avec le risque de coronaropathie. Selon les auteurs, ces données suggèrent donc l'absence d'une association causale (72-74). Au contraire, d'autres études supportent la présence d'une association causale entre la voie de signalisation du récepteur de l'IL-6 et la coronaropathie (75, 76). Des polymorphismes du récepteur de l'IL-6 ont en effet été associés à de plus faibles concentrations plasmatiques de la hs-CRP ainsi qu'à un risque réduit de maladie coronarienne (75, 76).

Il y a aussi un débat à savoir si la réduction de l'inflammation entraîne réellement une réduction du risque cardiovasculaire indépendamment de la réduction des concentrations de cholestérol LDL. Par exemple, la populaire étude JUPITER dont il fut question précédemment a démontré que de réduire de 37% les concentrations de la hs-CRP à l'aide d'une statine chez des sujets ayant des concentrations initiales de 2,0 mg/L ou plus diminuait le risque cardiovasculaire (36). Par contre, il faut aussi reconnaître dans l'étude JUPITER que la prise de statines a entraîné une réduction des concentrations du cholestérol LDL (-50%) (36), ce qui constitue en fait le rôle principal de cette classe de médicaments. Ainsi, aucune étude sur les statines ne permet de tester formellement la « théorie inflammatoire » de l'athérosclérose (77). Il est intéressant de noter que deux études utilisant des thérapies anti-inflammatoires ont récemment été initiées afin de tenter d'éclaircir la question : l'étude CANTOS (*Canakinumab Anti-Inflammatory Thrombosis Outcomes*

Study) (78) et l'étude CIRT (*Cardiovascular Inflammation Reduction Trial*) (79). Il s'agit de deux études de prévention secondaire qui visent respectivement à évaluer l'impact du canakinumab (un inhibiteur de l'IL-1β) et du méthotrexate (un anti-inflammatoire utilisé dans le traitement de l'arthrite rhumatoïde et du psoriasis) sur le risque d'événements cardiovasculaires chez des sujets présentant un profil pro-inflammatoire.

1.5 Facteurs associés au profil inflammatoire

Il a été mis en évidence à la section 1.2 que le statut pondéral module grandement le profil inflammatoire. D'autres facteurs reliés à l'ethnie, à la physiologie ou bien aux habitudes de vie ont aussi été associés à des variations dans les concentrations de biomarqueurs inflammatoires. Puisque la présente thèse porte sur l'alimentation et l'inflammation, ces facteurs seront brièvement décrits en les classifiant en tant que « non nutritionnels » ou « nutritionnels ». Il importe de souligner que la liste des différents facteurs, qu'ils soient nutritionnels ou non, n'est pas exhaustive.

1.5.1 Facteurs non nutritionnels

D'abord, l'**origine ethnique/la génétique** semble avoir un impact sur le profil inflammatoire. Par exemple, aux États-Unis, les Afro-Américains présenteraient des concentrations plus élevées de la hs-CRP et de l'IL-6 et des concentrations plus faibles d'adiponectine que les Caucasiens, indépendamment du statut pondéral (80-83).

Ensuite, on note des **différences de sexe** dans les concentrations sanguines de biomarqueurs inflammatoires. Les concentrations de la hs-CRP sont généralement plus élevées chez les femmes que chez les hommes (80, 84-88), ce qui serait en partie attribuable aux œstrogènes (85). Cette piste d'explication est d'ailleurs supportée par l'observation de concentrations plus élevées de la hs-CRP chez les femmes post-ménopausées utilisant une thérapie de remplacement hormonal comparativement à celles n'utilisant pas cette thérapie (81, 89-92). D'après Cartier *et al.* (88), une plus grande accumulation de tissu adipeux sous-cutané chez les femmes que chez les hommes pourrait également expliquer la différence sexuelle dans les concentrations de la hs-CRP. Paradoxalement, les concentrations de cytokines pro-inflammatoires (IL-6, TNF-α) seraient

plus faibles chez les femmes que chez les hommes (93), possiblement à cause d'un effet inhibiteur des œstrogènes sur l'expression des gènes de ces cytokines (94, 95). Les concentrations d'adiponectine semblent aussi plus élevées chez les femmes que chez les hommes (12, 96). Dans ce cas, les plus grandes concentrations d'androgènes chez les hommes entraîneraient une diminution de la production d'adiponectine (97).

Plusieurs études suggèrent que le **vieillissement (l'âge)** est positivement associé aux concentrations sanguines de biomarqueurs inflammatoires incluant la hs-CRP (98-100), l'IL-6 (98, 101-103) et le TNF-α (99, 101, 102, 104). On observe parfois que cette association ne semble pas totalement indépendante de la présence d'autres facteurs associés à l'inflammation (ex : tabagisme, sédentarité, consommation d'alcool) ou bien de la présence de certaines maladies chroniques (ex : MCV, diabète, cancer) (98). Une hypothèse a tout de même été émise comme quoi l'état inflammatoire chronique de faible intensité chez les individus âgés serait potentiellement attribuable à une exposition cumulée à divers stimuli inflammatoires tout au long de la vie, entraînant alors une baisse de l'efficacité des mécanismes impliqués dans le processus de résolution de l'inflammation (105).

Le **tabagisme** est un autre facteur positivement associé aux concentrations de biomarqueurs pro-inflammatoires tels que la CRP et l'IL-6 (106-110). Il est suggéré que l'état inflammatoire chronique induit par le tabagisme puisse persister jusqu'à 10 à 20 ans après la cessation tabagique (111). De plus, la nicotine ainsi que les dérivés réactifs de l'oxygène (« *reactive oxygen species* ») formés suite à la consommation de produits du tabac pourraient inhiber l'expression du gène de l'adiponectine et sa sécrétion par les adipocytes (112).

De nombreuses études observationnelles ont rapporté une association inverse entre la pratique d'**activité physique** (évaluée en terme de fréquence et/ou d'intensité) (113-119) ou la **consommation maximale d'oxygène** (VO_2 max) (120-123) et les concentrations sanguines de biomarqueurs pro-inflammatoires, dont principalement la hs-CRP. Par contre, cette association pourrait être dépendante, du moins en partie, du statut pondéral (115, 118). Les conclusions d'une revue de la littérature de Beavers *et al.* (124) qui regroupe les résultats d'études d'intervention randomisées contrôlées vont essentiellement dans le même sens. On y mentionne que l'entraînement aérobie serait potentiellement le plus efficace

pour améliorer le profil inflammatoire, particulièrement chez les individus présentant des concentrations initiales élevées de biomarqueurs inflammatoires ou bien chez les individus dont la pratique d'activité physique s'accompagne d'une légère perte de poids.

1.5.2 Facteurs nutritionnels

Un grand nombre d'études transversales, d'études de cohorte prospectives et d'études d'intervention ont à ce jour permis d'établir que la qualité de l'alimentation est associée au profil inflammatoire. Certaines études se sont attardées à des composantes spécifiques de l'alimentation comme des micronutriments, des macronutriments ou des aliments, alors que d'autres études ont plutôt évalué l'alimentation dans son ensemble. Voici quelques constats qui ressortent actuellement de la littérature scientifique.

Micronutriments

Plusieurs études de nature transversale suggèrent que les apports alimentaires en **magnésium** seraient fortement et inversement associés aux concentrations de biomarqueurs pro-inflammatoires, même après ajustement pour de multiples variables potentiellement confondantes incluant des facteurs nutritionnels (125-128). Par contre, ces études ne permettent pas d'établir de relation de cause à effet entre des apports augmentés en magnésium et une amélioration du profil inflammatoire.

Chez des participants de la *Framingham Offspring Study*, il a été observé que le statut en **vitamine K**, tel que mesuré par les concentrations plasmatiques ainsi que par les apports alimentaires en phylloquinone, était inversement associé à un score inflammatoire regroupant 14 biomarqueurs dont la hs-CRP, l'IL-6, le TNF-α et le MCP-1 (129). En accord avec ces résultats, des études *in vitro* ou dans des modèles animaux ont suggéré que la vitamine K pourrait réduire la production de cytokines pro-inflammatoires (130, 131).

Après avoir mis en commun les résultats de nombreuses études de nature observationnelle et d'intervention, Calder *et al.* (3) ont conclu que la **vitamine C**, la **vitamine E** et les **caroténoïdes** seraient tous des facteurs nutritionnels potentiellement anti-inflammatoires, avec des effets particulièrement notables chez certains sous-groupes d'individus comme les obèses ou les diabétiques.

Macronutriments

Du côté des **glucides**, il semble que l'**index glycémique** et/ou la **charge glycémique** seraient positivement associés aux concentrations de la hs-CRP (132, 133) et inversement associés à l'adiponectine (134). Dans le même ordre d'idées, une récente méta-analyse regroupant des études d'intervention randomisées contrôlées suggère que des diètes avec un index/charge glycémique faible devraient être favorisées par rapport aux diètes avec un index/charge glycémique élevé dans le but de réduire les concentrations de biomarqueurs pro-inflammatoires comme la hs-CRP (135).

Plusieurs études transversales ont rapporté des associations inverses entre la consommation de **fibres** et les concentrations de la hs-CRP (127, 136-138). En accord avec ces observations, une revue de la littérature incluant sept études d'intervention a indiqué, sur la base de six des sept études, que les concentrations de la hs-CRP pouvaient être réduites de l'ordre de 25 à 54% suite à l'augmentation de la consommation de fibres (≥ 3,3 g/MJ) (139). Toutefois, les auteurs soulignent que les réductions de la hs-CRP ont été observées en présence d'une perte de poids dans la plupart des études évaluées. Les auteurs mentionnent également qu'évaluer les effets indépendants de la consommation de fibres sur la hs-CRP n'était pas l'objectif principal des études retenues (sauf peut-être dans un cas) et qu'il n'était pas possible de distinguer les effets des fibres solubles et des fibres insolubles.

Des nutriments particulièrement d'intérêt en lien avec l'inflammation sont les **acides gras alimentaires**, qui se différencient principalement en fonction de leur degré d'insaturation : les acides gras saturés (AGS), les acides gras monoinsaturés (AGMI), les acides gras polyinsaturés (AGPI) et les acides gras insaturés (AGMI ou AGPI) de configuration *trans* (AGT). Les AGPI se subdivisent à leur tour en deux familles, les oméga-3 et les oméga-6, en fonction de la position de la première double liaison (3^e ou 6^e carbone) à partir de l'extrémité méthyle (CH_3) de leur chaîne hydrocarbonée. D'un point de vue mécanistique, il est suggéré que les acides gras ont la capacité d'activer ou d'inhiber certains processus inflammatoires (140-142). Par exemple, les AGS peuvent se lier à des récepteurs du système immunitaire comme les récepteurs TLR4 et ainsi activer la voie de signalisation de NF-κB qui, à son tour, régule à la hausse l'expression de gènes pro-inflammatoires (141-143) (**Figure 1.2**). Toutefois, de la confusion demeure dans la littérature scientifique quant

aux effets de différents acides gras sur les processus pro- et anti-inflammatoires chez l'humain. J'aurai l'occasion d'en discuter en détail plus tard.

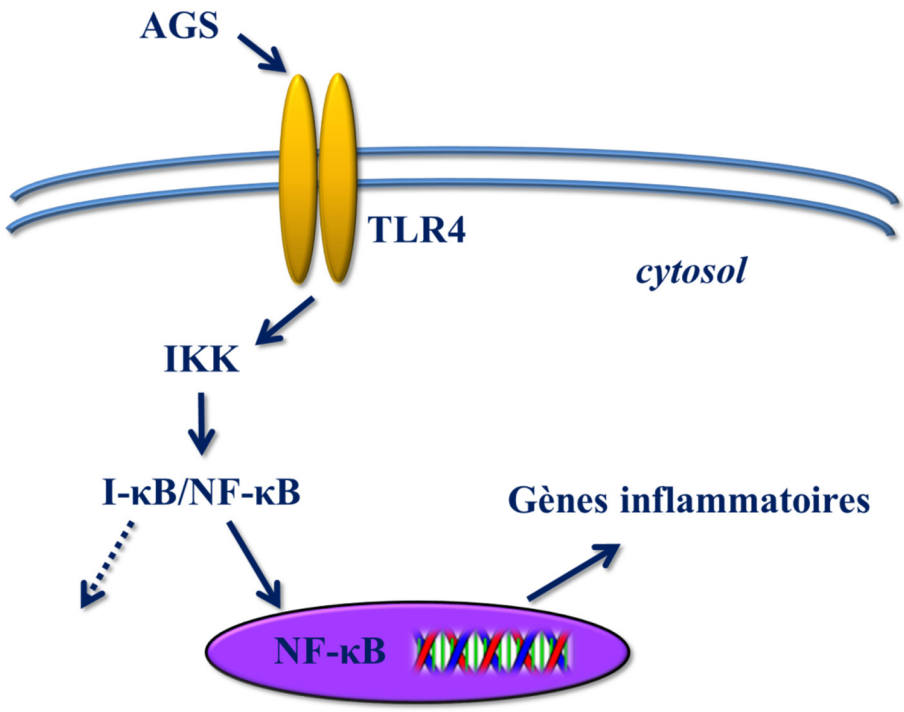

Figure 1.2 : Représentation simplifiée de l'activation de la voie de signalisation de NF-κB et de l'expression de gènes pro-inflammatoires suite à la liaison des acides gras saturés aux récepteurs TLR4. Création personnelle inspirée de l'article de Baker *et al.* (141). Abréviations : AGS, acides gras saturés ; I-κB, protéine inhibitrice de NF-κB ; IKK, complexe enzymatique (I-κB kinase) ; NF-κB, facteur de transcription nucléaire kappa-B ; TLR4, récepteur de type *Toll* 4

Aliments

Une récente revue de la littérature suggère, sur la base d'études épidémiologiques, que chaque portion consommée de **produits céréaliers à grains entiers** serait associée à une diminution de 7% des concentrations de la hs-CRP après ajustement pour d'autres facteurs nutritionnels (144). Toutefois, les études d'intervention nutritionnelle n'arrivent pas à démontrer clairement que la consommation de produits céréaliers à grains entiers exerce des effets bénéfiques sur les concentrations de biomarqueurs pro-inflammatoires (144).

Considérant les effets potentiellement anti-inflammatoires des vitamines C et E, des caroténoïdes et des fibres, il n'est pas surprenant de constater que les **fruits et légumes**

exerceraient eux aussi des effets bénéfiques sur le profil inflammatoire (3). Une étude chez des hommes non-fumeurs a démontré que la consommation de 8 portions/jour de fruits et légumes riches en caroténoïdes pour 4 semaines entraînait une réduction des concentrations de la hs-CRP comparativement à la consommation de 2 portions/jour (145).

Quelques études se sont attardées spécifiquement aux liens entre la consommation de **viande rouge** et l'inflammation. Une étude transversale réalisée chez des femmes iraniennes âgées de 40 à 60 ans a montré que les concentrations de la hs-CRP étaient 38% plus élevées chez celles se situant dans le plus haut quintile de consommation de viande rouge comparativement aux femmes dans le plus bas quintile après ajustement pour de possibles facteurs confondants (146). Une étude randomisée réalisée selon un devis en parallèle dans un contexte isoénergétique a quant à elle démontré que la consommation de 200 g/jour de viande rouge maigre pendant 8 semaines en remplacement d'aliments riches en glucides n'avait aucun effet sur les concentrations de protéines de phase aiguë (hs-CRP, SAA, fibrinogène) chez 60 hommes et femmes non-fumeurs (147).

Il a été démontré que la consommation de **boissons sucrées** (c'est-à-dire avec sucre ajouté, incluant entre autres les boissons gazeuses avec ou sans caféine, les boissons aux fruits et la limonade) était positivement associée aux concentrations de la hs-CRP, de l'IL-6 et des récepteurs TNF de type 1 et 2 chez les participants de la *Health Professionals Follow-Up Study*, sans toutefois être associée à l'adiponectine (148). En accord avec ces résultats, une étude d'intervention nutritionnelle randomisée, en chassé-croisé, réalisée chez 29 hommes en santé a montré que la consommation durant 3 semaines de 600 mL/jour de boissons sucrées comprenant différentes quantités (40 ou 80 g) de fructose, de glucose ou de sucrose augmentait de l'ordre de 60 à 109% les concentrations de la hs-CRP comparativement aux valeurs initiales (valeurs mesurées au tout début de l'étude), tout en n'ayant aucun effet sur les concentrations d'adiponectine (149).

Une relation en forme de U semble clairement présente entre la consommation de **breuvages alcoolisés** (notamment le vin ou la bière) et l'inflammation chronique de faible intensité (3). Les effets les plus protecteurs correspondraient à la prise d'une à deux consommations par jour. Cependant, on ne sait pas à l'heure actuelle si ces effets

protecteurs sont principalement attribuables au contenu en alcool ou bien, dans le cas particulier du vin rouge, à certaines composantes telles que les composés phénoliques (3).

Alimentation globale

Les **profils alimentaires** (traduction de « *dietary patterns* », parfois aussi appelés « patrons alimentaires ») sont des facteurs nutritionnels qui intègrent la consommation de plusieurs aliments ou groupes d'aliments et qui reflètent donc l'alimentation dans sa globalité (32, 150). Globalement, deux types d'approches permettent l'identification de profils alimentaires en épidémiologie nutritionnelle : 1) une approche *a priori*, caractérisée par le calcul de scores ou d'indices basés sur des caractéristiques nutritionnelles prédéterminées comme les recommandations alimentaires (ex : « *Healthy Eating Index* ») et 2) une approche *a posteriori*, ou exploratoire, dans laquelle les profils sont identifiés au moyen de procédures statistiques à partir de données sur la consommation alimentaire recueillies dans une population donnée (« *data-driven approach* ») (151, 152). Une récente revue systématique de la littérature a regroupé les résultats de 46 études ayant identifié des profils alimentaires par l'une ou l'autre des approches décrites ci-haut (153). Cette revue systématique supporte les conclusions de précédentes revues (non-systématiques) de la littérature (3, 32) comme quoi les **profils alimentaires de type « Prudent »**, caractérisés principalement par la consommation de fruits et légumes, de produits céréaliers à grains entiers et de poisson, sont associés à un profil inflammatoire favorable (concentrations plus faibles de biomarqueurs inflammatoires comme la hs-CRP et l'IL-6 et concentrations plus élevées de biomarqueurs anti-inflammatoires comme l'adiponectine). À l'inverse, les **profils alimentaires de type « Western »**, caractérisés entre autres par la consommation de viande rouge, de viande transformée, de produits céréaliers raffinés, de boissons alcoolisées, de sucreries et de boissons gazeuses, sont associés à un profil inflammatoire défavorable (153).

Dans le même ordre d'idées, les individus adoptant une **alimentation de type végétarienne** auraient de plus faibles concentrations de biomarqueurs pro-inflammatoires que les individus adoptant une alimentation de type non-végétarienne (154, 155). Toutefois, il est important de reconnaître que le végétarisme est un terme relativement large pouvant inclure plusieurs sous-catégories de pratiques alimentaires (156) et que certaines habitudes

de vie comme la pratique d'activité physique peuvent différer entre les végétariens et les omnivores (3). Ces éléments ont potentiellement une influence sur les résultats observés.

En résumé, sur la base de la littérature actuelle (3, 32, 157, 158), on constate que les **facteurs nutritionnels globalement considérés comme bénéfiques pour la santé** (ex : fruits et légumes) semblent associés à un profil inflammatoire favorable. À l'inverse, les **facteurs nutritionnels globalement considérés comme néfastes pour la santé** (ex : boissons sucrées) semblent associés à une détérioration du profil inflammatoire. La littérature actuelle démontre également que plusieurs questions demeurent non résolues quant aux effets de l'alimentation sur l'inflammation. Comme le soulignent Calder *et al.* (3) dans leur vaste revue de la littérature publiée en 2011, la plupart des associations décrites ci-haut devront être confirmées dans le futur avec des études utilisant une méthodologie adéquate. On constate par ailleurs que d'autres facteurs nutritionnels non mentionnés dans la présente section ont été sous-explorés en lien avec l'inflammation et/ou font l'objet d'une controverse. Je pense ici aux produits laitiers, dont j'aurai l'occasion de discuter plus tard.

CHAPITRE 2 : ALIMENTATION ET INFLAMMATION – PROBLÉMATIQUE GÉNÉRALE

2.1 Objectif général et hypothèse

Sur la base des informations présentées au chapitre précédent, on peut dire que l'étude des liens entre divers facteurs nutritionnels et l'inflammation est loin d'être un sujet clos. L'**objectif général** établi dans le cadre de mon projet de doctorat était donc le suivant :

Étudier l'impact de l'alimentation sur l'inflammation et ses mécanismes sous-jacents.

L'**hypothèse générale** émise en lien avec cet objectif était :

Les facteurs nutritionnels globalement considérés comme bénéfiques pour la santé sont associés à des effets anti-inflammatoires, alors que les facteurs nutritionnels globalement considérés comme néfastes pour la santé sont associés à des effets pro-inflammatoires.

2.2 Cadre méthodologique du projet

Tel qu'illustré à la **Figure 2.1**, il est important de noter que les facteurs nutritionnels auxquels je fais référence dans l'hypothèse générale, qu'ils soient considérés ou non comme bénéfiques pour la santé, comprennent autant de simples **nutriments** que des **aliments** ou encore des **profils alimentaires** reflétant l'alimentation dans sa globalité. La figure 2.1 vise aussi à illustrer que les nutriments qui ont été précisément ciblés dans le cadre de mon projet de doctorat sont les acides gras alimentaires, avec un accent un peu plus particulier sur les AGPI oméga-3 d'origine marine. Dans la catégorie des aliments, mes travaux ont spécifiquement porté sur les produits laitiers. En ce qui concerne les profils alimentaires, prenez note qu'ils n'étaient pas prédéterminés, car ils ont été identifiés en cours d'analyse par une approche guidée par les données (« *data-driven approach* »). Je ne décrirai donc pas ces profils alimentaires pour le moment.

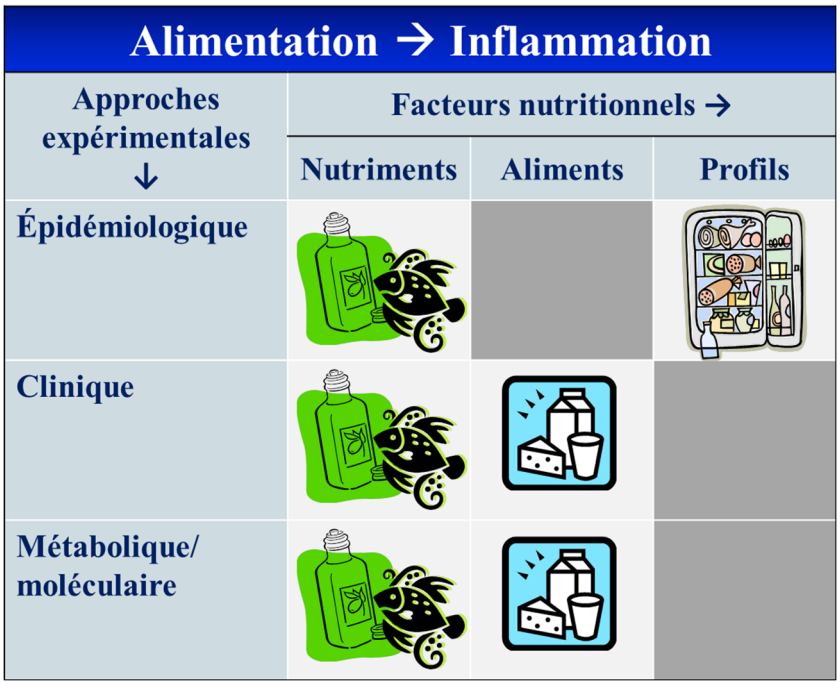

Figure 2.1 : Illustration résumant l'ensemble des travaux réalisés dans le cadre du présent projet de doctorat portant sur l'impact de l'alimentation sur l'inflammation et ses mécanismes sous-jacents. Le terme « alimentation » sous-entend l'étude de différentes catégories de facteurs nutritionnels incluant des nutriments (acides gras, dont les oméga-3), des aliments (produits laitiers) et des profils alimentaires. Ces divers facteurs nutritionnels sont étudiés en lien avec l'inflammation en utilisant différentes approches expérimentales, soit une approche épidémiologique, une approche clinique et une approche métabolique.

Un autre point fort important à noter, comme le dévoile le titre de ma thèse, est que trois approches expérimentales différentes ont été retenues pour étudier la relation entre divers facteurs nutritionnels et l'inflammation : 1) une **approche épidémiologique**, 2) une **approche clinique** et 3) une **approche métabolique** (synonyme de moléculaire). La figure 2.1 illustre d'ailleurs précisément quels facteurs nutritionnels ont été étudiés selon quelles approches. Alors que l'utilisation d'une approche épidémiologique permet seulement de dresser un portrait des associations entre l'alimentation et l'inflammation au sein d'une ou de plusieurs populations, les approches « clinique » et « métabolique » permettent respectivement, au sein d'une étude d'intervention nutritionnelle, de mieux comprendre les relations causales entre l'alimentation et le profil inflammatoire ainsi que les mécanismes à

l'origine de la réponse physiologique engendrée par l'intervention. C'est donc en suivant cette ligne de pensée allant du général (études populationnelles) au spécifique (études mécanistiques) que je vous présente les **trois volets** de mon projet de doctorat. Ces volets sont répartis comme suit :

D'abord, chacun des volets comprend **quatre chapitres** : le premier chapitre présente la problématique propre au volet, le deuxième et le troisième chapitre présentent les travaux (articles scientifiques) réalisés dans le cadre de ce volet et le quatrième chapitre constitue une conclusion spécifique au volet.

Ainsi, les chapitres 3 à 6 (volet 1) portent sur l'étude des **associations, dans un contexte épidémiologique, entre certains facteurs nutritionnels** (d'une part les acides gras oméga-3, d'autre part des profils alimentaires) **et l'inflammation chez deux nations autochtones de la province de Québec**.

Les chapitres 7 à 10 (volet 2) portent sur l'étude de l'**impact de la consommation de produits laitiers sur l'inflammation** dans un contexte clinique ainsi que métabolique.

Les chapitres 11 à 14 (volet 3) portent sur l'étude de l'**impact de la consommation de divers acides gras alimentaires sur l'inflammation** également dans un contexte clinique ainsi que métabolique.

Enfin, je vous rappelle que le chapitre 15 constitue la conclusion générale regroupant les trois volets de mon projet de doctorat.

CHAPITRE 3 : ÉTUDE DES ASSOCIATIONS ENTRE L'ALIMENTATION ET L'INFLAMMATION CHEZ LES NATIONS AUTOCHTONES DU NORD-DU-QUÉBEC – PROBLÉMATIQUE

Pour débuter le présent chapitre, voici un bref portrait des différentes nations autochtones constituant le Québec, incluant l'introduction des deux nations à l'étude. Les raisons de l'intérêt de notre équipe de recherche envers les autochtones seront ensuite exposées.

3.1 Portrait des nations autochtones du Québec

La province de Québec comprend 11 nations autochtones incluant à la fois les Amérindiens (10 nations) et les Inuits (1 nation) (159). Les Amérindiens, aussi appelés *Premières Nations*, se subdivisent en deux familles linguistiques et culturelles : 1) la famille algonquienne, qui inclut les Abénaquis, les Algonquins, les Attikameks, les Cris, les Innus, les Malécites, les Micmacs et les Naskapis et 2) la famille iroquoïenne, qui inclut les Hurons-Wendats et les Mohawks (160). Les Inuits forment un groupe ethnique distinct des Amérindiens puisqu'ils sont issus d'une vague de peuplement plus tardive (160). Sur le plan démographique, les 11 nations autochtones représentent près de 92 000 individus, ce qui équivaut à environ 1% de la population québécoise (160).

Les Cris de la Baie-James et les Inuits du Nunavik sont les deux nations autochtones qui ont été spécifiquement ciblées dans le cadre du présent projet de doctorat. Ces deux nations habitent la région administrative du Nord-du-Québec. Tel qu'illustré à l'**annexe A**, les Cris sont répartis dans 9 communautés situées sur la côte est de la Baie-James ou de la Baie d'Hudson ou encore à l'intérieur des terres, près de la ville de Chibougamau (160). Cette nation autochtone initialement semi-nomade dont le nom signifie « le peuple de chasseurs » est originaire des plaines de l'Ouest canadien (160). La population crie s'élève actuellement à près de 17 000 individus (160). Les Inuits, pour leur part, habitent le Nunavik, qui se définit comme le territoire québécois situé au nord du 55^{e} parallèle (160). Cette nation autochtone initialement nomade dont le nom signifie « les êtres humains » est actuellement constituée de quelque 11 000 individus (160). La population inuite est répartie dans 14

villages côtiers situés sur les rives de la Baie d'Hudson, du Détroit d'Hudson et de la Baie d'Ungava (annexe A) (160).

3.2 Enquêtes de santé chez les nations autochtones du Nord-du-Québec

Il est important de souligner que les Cris de la Baie-James et les Inuits du Nunavik ont fait l'objet de grandes enquêtes de santé au cours de la dernière décennie. D'une part, l'enquête *« Nituuchischaayihtitaau Aschii Multi-Community Environment-and-Health Study in Eeyou Istchee »* a été réalisée entre 2005 et 2009 chez les Cris de la Baie-James. Cette enquête avait pour but de recueillir de l'information sur l'état de santé des Cris, leur mode de vie, leurs conditions sociales ainsi que les conditions environnementales auxquelles ils sont exposés (contaminants), puis d'évaluer les associations entre ces différents facteurs (161). D'autre part, l'*« Enquête de santé auprès des Inuits du Nunavik 2004 – Qanuippitaa? Comment allons-nous? »* avait comme objectif général de mettre à jour les informations concernant l'état de santé et de bien-être de la population inuite du Nord-du-Québec (162). L'accès à un large éventail de données recueillies dans le cadre des enquêtes chez les Cris et les Inuits explique en partie pourquoi le volet épidémiologique de mon projet de doctorat a précisément porté sur ces deux nations autochtones. Cependant, les raisons réelles de l'intérêt de notre équipe de recherche envers ces nations autochtones dans le contexte de l'alimentation et de l'inflammation sont beaucoup plus profondes. En voici les détails.

3.3 Transition nutritionnelle et désordres métaboliques multifactoriels

Au cours des 50 à 60 dernières années, les nations autochtones du Nord-du-Québec, tout comme celles de l'ensemble de l'Amérique du Nord, ont fait face à d'énormes changements socioculturels et économiques incluant leur établissement dans des communautés permanentes (sédentarisation), le passage à une économie basée sur les salaires et l'accès aux technologies et moyens de transport facilitant les communications et la commercialisation avec les grands centres urbains (160-164). Ces changements sociaux ont donné lieu à une importante transition nutritionnelle, un concept désignant le passage très rapide d'une alimentation traditionnelle, caractérisée par la consommation d'aliments locaux obtenus par des activités de chasse, de pêche et de cueillette, vers une alimentation de type « américanisée », caractérisée par la consommation d'aliments du commerce

importés des régions urbaines (163, 165-168). En termes de nutriments, la transition nutritionnelle se définit comme un déclin dans la consommation d'aliments à haute densité nutritive, notamment riches en AGPI oméga-3 à longue chaîne, protéines, vitamines et minéraux (vitamine A, vitamine D, fer), en faveur de la consommation d'aliments à haute densité énergétique, notamment riches en sucres simples et en AGS (166, 167, 169, 170). Il importe aussi de souligner que le concept de transition nutritionnelle, tel que défini par son « créateur », le Dr. Barry Popkin, englobe non seulement des changements rapides et profonds dans l'alimentation d'une population, mais aussi des changements dans les niveaux d'activité physique (171). Cependant, l'aspect de l'activité physique ne fait pas l'objet de la présente thèse.

Les Cris, les Inuits ainsi que les autres nations autochtones à travers l'Amérique du Nord ont été passablement épargnés des désordres métaboliques multifactoriels comme le diabète de type 2 avant la survenue du phénomène de transition nutritionnelle (172-175). Toutefois, parallèlement à ce phénomène, on note depuis plusieurs décennies une forte augmentation de la prévalence de l'obésité (176-180), de l'hypertension (178), du diabète de type 2 (179, 181-183) et des MCV (178, 184) dans ces populations. Par exemple, Dannenbaum *et al.* (181) ont indiqué que la prévalence du diabète de type 2 chez les Cris de la Baie-James âgés de 20 ans et plus avait augmenté de 330% sur une période de 16 ans, passant de 5,2% en 1989 à 17,3% en 2005. En comparaison, la prévalence du diabète de type 2 chez l'ensemble des Québécois (excluant les Autochtones) a été estimée à 3,7% en 2005 (185).

3.4 Prévalence de l'inflammation chez les nations autochtones du Nord-du-Québec

Les désordres métaboliques multifactoriels dont la prévalence ne cesse d'augmenter dans les nations autochtones depuis les dernières décennies sont tous reliés à l'inflammation systémique et chronique de faible intensité, tel que décrit dans l'introduction générale et à la section 1.4 du chapitre 1. Jusqu'à récemment, on ne connaissait pas spécifiquement la prévalence de l'inflammation chronique de faible intensité chez les nations autochtones du Nord-du-Québec. Une partie de mes travaux de doctorat a permis de déterminer cette prévalence à partir des données recueillies dans les enquêtes de santé *« Nituuchischaayihtitaau Aschii »* et *« Qanuippitaa? »*. Dans le cas des Cris, ces travaux se

rapportent au premier des deux objectifs de l'étude présentée au chapitre 4. Dans le cas des Inuits, ces travaux constituent l'objectif principal de l'étude présentée à l'annexe B.

En bref, bien que la prévalence des désordres métaboliques multifactoriels augmente grandement dans *l'ensemble* des nations autochtones, mes travaux ont permis de constater que cette augmentation est associée à une prévalence de l'inflammation chronique de faible intensité (concentrations de la hs-CRP entre 3,0 et 10,0 mg/L) très élevée chez les Cris (47%), mais au moins 2 fois plus faible chez les Inuits (21% ; **Figure 3.1**). La prévalence de l'inflammation chronique de faible intensité chez les Inuits est d'ailleurs semblable à celle de la population canadienne caucasienne d'après des données d'Anand *et al.* (84) publiées en 2004 (25%). Sur la base de ces résultats plutôt surprenants, on peut se questionner à savoir quels sont les principaux déterminants du statut inflammatoire dans chacune de ces deux populations autochtones.

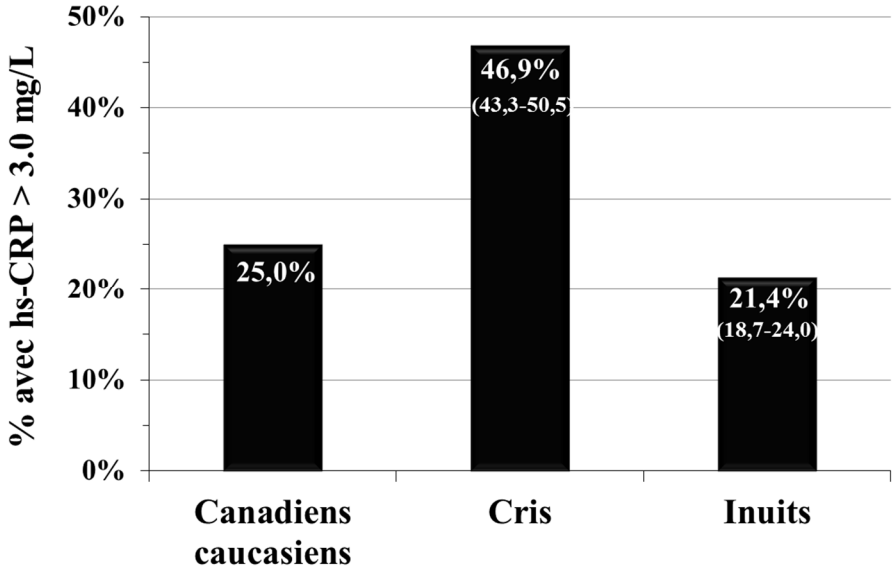

Figure 3.1 : Prévalence de concentrations élevées de la hs-CRP (> 3,0 mg/L) chez les Canadiens caucasiens, les Cris de la Baie-James et les Inuits du Nunavik. Les données entre parenthèses sont les intervalles de confiance à 95% (si disponibles). La valeur correspondant aux Canadiens caucasiens provient d'un article publié par Anand *et al.* (84). Les valeurs chez les Cris et les Inuits proviennent de deux articles publiés par Labonté *et al.* (186, 187), présentés respectivement au chapitre 4 et à l'annexe B de la présente thèse. Abréviations : hs-CRP, protéine C-réactive mesurée par dosage ultra-sensible.

3.5 Alimentation et inflammation chez les nations autochtones du Nord-du-Québec

Considérant le caractère unique et l'ampleur de la transition nutritionnelle vécue par les populations autochtones du Nord-du-Québec ainsi que le rôle de plus en plus reconnu de l'alimentation dans la modulation du profil inflammatoire, il y a fort à parier que certains facteurs nutritionnels représentent d'importants déterminants du profil inflammatoire chez les Cris et les Inuits. Toutefois, les facteurs nutritionnels exerçant une influence sur le profil inflammatoire de ces populations n'ont jamais été documentés à ce jour. L'accès aux données recueillies dans les enquêtes de santé *« Nituuchischaayihtitaau Aschii »* et *« Qanuippitaa? »* représentait alors une opportunité unique pour évaluer les associations entre l'alimentation et l'inflammation chez les nations autochtones du Nord-du-Québec. J'ajouterais de surcroît que très peu d'études ont évalué les associations entre l'alimentation et l'inflammation chez les nations autochtones de l'ensemble de l'Amérique du Nord. À ma connaissance, les études publiées jusqu'à maintenant sur le sujet ont été réalisées seulement chez les populations autochtones de l'Alaska, aux États-Unis (188-190). Notez qu'il sera brièvement question des résultats de ces études au cours des prochains chapitres.

3.6 Objectif et hypothèses des travaux réalisés

Sur la base des informations présentées précédemment, le **premier objectif spécifique** du présent projet de doctorat était :

Évaluer, dans un contexte épidémiologique, les associations entre l'alimentation des populations autochtones du Nord-du-Québec et les concentrations sanguines des biomarqueurs de l'inflammation.

Il importe de souligner que les associations entre l'alimentation et les biomarqueurs de l'inflammation ont été évaluées en tenant compte de facteurs nutritionnels différents dans chacune des deux nations autochtones à l'étude (**Figure 3.2**). Des hypothèses spécifiques à chacune de ces deux populations ont donc été émises en lien avec l'objectif ci-haut. Les justifications entourant le choix des facteurs nutritionnels retenus, et par le fait même des hypothèses, sont fournies plus bas.

Hypothèse chez les **Cris de la Baie-James :**

1. Les concentrations érythrocytaires d'acides gras oméga-3 à longue chaîne sont associées à un profil inflammatoire favorable.

Hypothèses chez les **Inuits du Nunavik :**

1. Les profils alimentaires caractérisés par la consommation d'aliments traditionnels et d'aliments « sains » sont associés à un profil inflammatoire favorable.

2. Les profils alimentaires caractérisés par la consommation d'aliments transformés et/ou à haute densité énergétique sont associés à un profil inflammatoire détérioré.

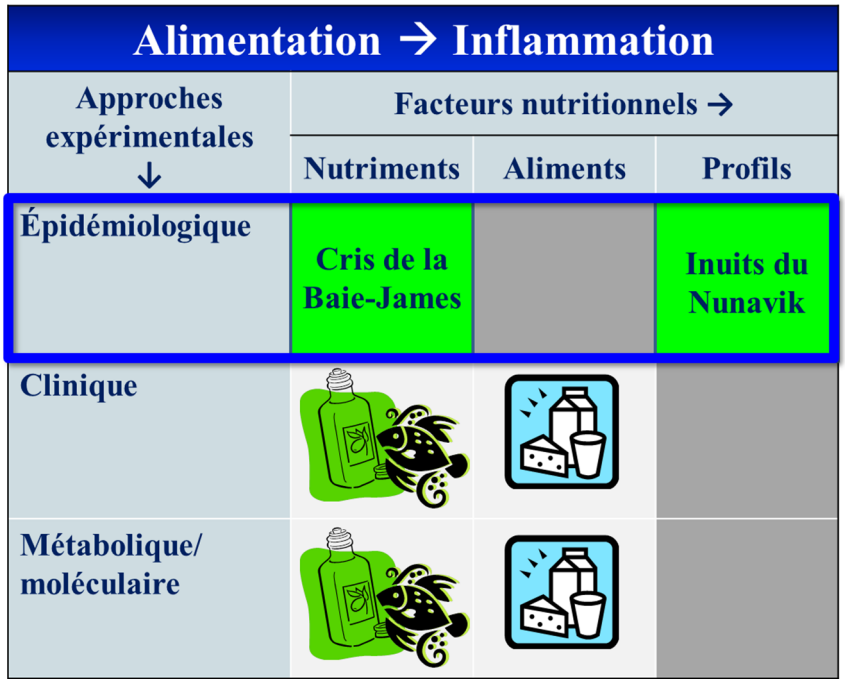

Figure 3.2 : Illustration mettant en lumière les travaux réalisés dans le cadre du **premier volet** du présent projet de doctorat, dont le but était d'évaluer, dans un contexte épidémiologique, les associations entre certains facteurs nutritionnels et l'inflammation chez deux nations autochtones de la province de Québec.

Mes travaux en lien avec les Cris, publiés dans la revue *European Journal of Clinical Nutrition* (Labonté *et al.* 2014;68:1042-7), sont présentés au chapitre 4. Mes travaux en lien

avec les Inuits, publiés dans la revue *Journal of the Academy of Nutrition and Dietetics* (Labonté *et al.* 2014;114:1208-15.e3), sont présentés au chapitre 5.

Bien que l'étude de facteurs nutritionnels similaires chez les Cris de la Baie-James et chez les Inuits du Nunavik aurait permis de mieux comparer les résultats obtenus dans ces deux populations, la réalité en a décidé autrement. Autant chez les Cris que chez les Inuits, l'idée première était d'identifier des profils alimentaires à partir des données alimentaires auto-rapportées recueillies dans le cadre des enquêtes de santé *« Nituuchischaayihtitaau Aschii »* et *« Qanuippitaa? »*, puis d'associer les profils alimentaires au profil inflammatoire. Puisque les profils alimentaires offrent une vision globale de l'alimentation, leur identification peut s'avérer fort utile dans le contexte d'études observationnelles où les effets d'un seul nutriment, aliment ou groupe d'aliments peuvent difficilement être isolés (150).

Toutefois, l'information recueillie sur l'alimentation de la population crie était plus ou moins détaillée, particulièrement en ce qui concerne les aliments du commerce, restreignant ainsi la possibilité d'obtenir des profils alimentaires complets. Pour cette raison, chez les Cris, nous avons plutôt décidé de nous attarder à des biomarqueurs valides et objectifs des apports en produits marins, soit les concentrations érythrocytaires d'acides gras oméga-3 à longue chaîne (incluant l'acide eicosapentaénoïque [EPA ; « *eicosapentaenoic acid* » ; C20:5 n-3] et l'acide docosahexaénoïque [DHA ; « *docosahexaenoic acid* » ; C22:6 n-3]) (191-193). La mesure de biomarqueurs nutritionnels permet ainsi d'éliminer les biais et erreurs de mesures attribuables à la collecte de données alimentaires « subjectives » (194). Par ailleurs, tel que décrit dans l'introduction du prochain chapitre, nous avons cru intéressant d'évaluer la manière dont les acides gras oméga-3 sont reliés à l'inflammation dans une population avec un statut modérément élevé en ces acides gras (4% des acides gras totaux dans les phospholipides plasmatiques), soit se situant à mi-chemin entre les concentrations sanguines très faibles des Québécois caucasiens (2%) et celles très élevées des Inuits et autres populations circumpolaires (8%) (195). Pour émettre notre hypothèse, nous nous sommes basés sur les associations inverses entre les acides gras oméga-3 et le profil inflammatoire observées dans diverses populations dans un bon nombre d'études

observationnelles précédentes. Davantage de détails à ce sujet sont encore une fois fournis dans l'introduction du prochain chapitre.

Chez les Inuits, l'éventail plus large de données alimentaires recueillies nous a permis de poursuivre notre première idée et d'identifier des profils alimentaires afin de les associer au profil inflammatoire. Afin d'émettre nos hypothèses, nous nous sommes basés sur le fait que les populations inuites ont été passablement épargnées des désordres métaboliques associés à l'inflammation tant qu'elles ont adopté une alimentation traditionnelle (voir section 3.3 ci-haut) (172-174). Nous nous sommes aussi basés sur les études réalisées dans diverses populations ayant démontré que l'adhésion à des profils alimentaires qualifiés de « Prudents » sont inversement associés aux biomarqueurs pro-inflammatoires, alors que les profils alimentaires qualifiés de « Western » sont positivement associés aux biomarqueurs pro-inflammatoires (voir section 1.5.2 du chapitre 1) (153).

Enfin, en ce qui concerne le chapitre 5 (étude chez les Inuits), il est important de noter que des facteurs du risque cardiovasculaire autres que les biomarqueurs inflammatoires ont également été évalués (ex : lipides et lipoprotéines plasmatiques). Cela explique pourquoi le titre de ce chapitre/publication scientifique n'est pas directement relié au sujet de l'inflammation.

CHAPITRE 4 :

ASSOCIATIONS ENTRE LES CONCENTRATIONS ÉRYTHROCYTAIRES D'ACIDES GRAS OMÉGA-3 ET LES CONCENTRATIONS PLASMATIQUES DE BIOMARQUEURS INFLAMMATOIRES CHEZ LA POPULATION CRIE DU QUÉBEC

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RÉSUMÉ

Cette étude transversale avait pour but d'évaluer la prévalence de concentrations élevées de la hs-CRP ainsi que les associations entre les concentrations érythrocytaires d'acides gras oméga-3 et les concentrations plasmatiques de biomarqueurs inflammatoires chez les Cris de la Baie-James (province de Québec). Un échantillon de 744 Cris (18-91 ans) provenant de 7 communautés de l'est de la Baie-James a été inclus dans les analyses. Les associations entre les concentrations érythrocytaires d'acides gras oméga-3 et les concentrations plasmatiques de la hs-CRP, de l'IL-6 et de TNF-α ont été évaluées à l'aide de modèles linéaires généralisés (GLM) tenant compte du sexe, de l'âge et du tour de taille. Un score inflammatoire arbitraire a été créé sur la base de la somme des quartiles des concentrations de la hs-CRP, de l'IL-6 et de TNF-α (écart = 3 – 12). La prévalence de concentrations élevées de la hs-CRP (> 3 mg/L) était de 46,9% (IC 95% 43,3-50,5). Les concentrations érythrocytaires d'acide docosapentaénoïque (DPAn-3) étaient inversement associées à la hs-CRP, au TNF-α et au score inflammatoire (*P* pour la tendance < 0,02), alors que les concentrations érythrocytaires d'EPA et de DHA n'étaient pas associées à l'inflammation (*P* pour la tendance > 0,18). Chez les participants avec des concentrations érythrocytaires de DPAn-3 au-dessus de la médiane de la population, les probabilités d'avoir un score inflammatoire élevé (≥ 9) étaient de 0,67 (IC 95% 0,48-0,93) comparativement aux participants en-dessous de la médiane de la population. Ces résultats indiquent que l'inflammation systémique de faible intensité est hautement prévalente chez les Cris de la Baie-James et que des concentrations élevées de DPAn-3 sont associées à un plus faible risque d'inflammation systémique dans cette population.

TITLE PAGE

TITLE

Association of red blood cell n-3 polyunsaturated fatty acids with plasma inflammatory biomarkers among the Quebec Cree population

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RUNNING TITLE: n-3 fatty acids and inflammation among Cree

ABSTRACT

Background/Objectives: We examined the prevalence of elevated plasma high-sensitivity C-reactive protein (hs-CRP) concentrations and associations with red blood cell (RBC) long-chain n-3 polyunsaturated fatty acids (LCn-3PUFA) in the James Bay Cree population from the province of Quebec (Canada). **Subjects/Methods:** A total of 744 Cree adults (18-91 years) from seven communities of Eastern James Bay were included in these cross-sectional analyses. Associations between RBC LCn-3PUFA and pro-inflammatory markers (hs-CRP, interleukin-6 [IL-6] and tumor necrosis factor-alpha [TNF-α]) were assessed by using multivariate general linear models with adjustment for sex, age, and waist circumference. An arbitrary inflammation score was defined based on the sum of the quartiles of hs-CRP, IL-6 and TNF-α concentrations (range=3-12). **Results:** Elevated hs-CRP concentrations (> 3 mg/L) were present in 46.9% (95% confidence interval [CI], 43.3-50.5) of the James Bay Cree population. RBC docosapentaenoic acid (DPAn-3; C22:5n-3) was inversely associated with hs-CRP, TNF-α and the inflammation score (all *P* trend < 0.02), whereas eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) in RBC were not associated with inflammation (all *P* trend > 0.18). Among participants with RBC DPAn-3 levels above the median of the population, odds ratio of having an elevated inflammation score (≥ 9) was 0.67 (95% CI, 0.48-0.93) compared with participants below the median. **Conclusions:** Results indicate that low-grade systemic inflammation is highly prevalent and that higher RBC DPAn-3 levels are associated with a lower risk of systemic inflammation in the James Bay Cree population. **Keywords:** C-reactive protein, Cree, inflammation, n-3 polyunsaturated fatty acids, docosapentaenoic acid

INTRODUCTION

Detrimental impact of westernization of Canadian First Nations' diet and lifestyle on metabolic disorders such as obesity, type 2 diabetes, and cardiovascular diseases (CVD) is well known.[1-5] Cardiometabolic disorders are associated with a chronic low-grade inflammatory state,[6] often reflected by increased concentrations of pro-inflammatory mediators such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Previous studies have shown that elevated CRP concentrations (> 3.0 mg/L) are present in more than 50% of First Nations adult communities from Ontario, Canada, with even higher values noted among women.[7,8] Pro-inflammatory biomarker concentrations have also been positively associated with age,[7] plasma levels of persistent organic pollutants (POPs)[9] and several features of the metabolic syndrome (MetS) including adiposity indices, fasting glucose and insulin[7, 10] in Oji-Cree communities from northwestern Ontario. However, the prevalence and correlates of elevated pro-inflammatory biomarkers concentrations among the Cree communities of Eastern James Bay (Quebec, Canada) have not been described yet.

Cross-sectional studies conducted primarily in Caucasian populations have shown a consistent inverse association between fish consumption or the intake of long-chain n-3 polyunsaturated fatty acids (LCn-3PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), estimated from dietary questionnaires or blood levels, and inflammatory biomarker concentrations.[11-19] To the best of our knowledge, studies having investigated these associations among Aboriginals so far have been limited to Inuit populations.[20, 21] Although blood levels of LCn-3PUFA have previously been associated with traditional risk factors for CVD among First Nations communities such as the Cree population,[22] the extent to which LCn-3PUFA are associated with systemic inflammation in these communities remains unknown. Plasma phospholipid levels of EPA+DHA among the James Bay Cree population (3.9%) fall between levels seen among Aboriginal circumpolar populations such as Nunavik Inuit (northern Quebec; 8.0%) and levels observed in non-Aboriginal Quebecers (1.8%).[23] Investigating associations between blood levels of LCn-3PUFA and inflammation in the James Bay Cree population therefore provides information

on how LCn-3PUFA relate to inflammation in a population with a moderately elevated LCn-3PUFA status.

The first objective of the present study was to determine the prevalence of elevated high-sensitivity (hs)-CRP concentrations among the James Bay Cree population from the province of Quebec, Canada. The second objective was to assess the association between red blood cell (RBC) LCn-3PUFA, used as a biological marker of LCn-3PUFA dietary intake,[24-26] and inflammatory biomarkers concentrations.

SUBJECTS AND METHODS

Population and study design

Data collection for the present cross-sectional study was carried out between summer 2005 and summer 2009 as part of the "Nituuchischaayihtitaau Aschii Multi-Community Environment and Health Study in Iiyiyiu Aschii", a health survey conducted among 7 coastal and inland Cree communities from Eastern James Bay (Quebec, Canada). The method used for selecting participants has been described previously.[27] As shown in **Figure 1**, the final study sample included 744 subjects for the analyses of hs-CRP and 743 subjects for the analyses of IL-6 and TNF-α.

The study protocol was approved by the Research Ethics Committee of the Centre Hospitalier de l'Université Laval, and accepted by the Committees of McGill and McMaster Universities, as well as by the Research Committee of the Cree Board of Health and Social Services of James Bay. All participants provided written informed consent.

Clinical data

Participants' medical files were reviewed to determine the prevalence of type 2 diabetes and the use of anti-inflammatory, antihypertensive, antidiabetic and lipid lowering medications over the 12-month period that preceded the beginning of the study.

Blood samples as well as anthropometric and physiological measurements were obtained during a 2.5-h clinical session conducted by a research nurse. Participants were asked to fast for at least 8 h prior to blood sample collection. Collected blood samples were

temporarily frozen at either -20°C or -80°C. Aliqots of samples were also sent to the Centre Hospitalier Universitaire de Québec, in Québec City, where they were stored at ≤ -80°C. Body weight, waist circumference, body mass index, body fat percentage and blood pressure measurements were described previously.[27, 29] Elevated waist circumference was defined as ≥ 90 cm in men and ≥ 80 cm in women according to the International Diabetes Federation (IDF) classification of central obesity for First Nations.[30] The presence of the MetS was determined using the IDF classification in First Nations.[30]

Red blood cell fatty acids determination

Individual LCn-3PUFA were quantified in RBC membranes phospholipids by gas-liquid chromatography and were expressed as percent of total fatty acids. Fatty acids determination has been previously described elsewhere.[27]

Measurement of hs-CRP, IL-6, and TNF-α

Inflammatory biomarkers were analyzed in batches on samples stored at -80ºC. Plasma hs-CRP concentrations were measured by nephelometry as previously described.[31] TNF-α and IL-6 were measured in EDTA-plasma using a human TNF-α and a human IL-6 ELISA kit (Quantikine HS, R&D System, Minneapolis, MN), respectively. An arbitrary inflammation score was created to reflect an overall inflammatory profile. The score ranges from 3 to 12 points and corresponds to the sum of the individual quartiles of hs-CRP, IL-6 and TNF-α concentrations (Q1 = 1 point; Q2 = 2 points; Q3 = 3 points; Q4 = 4 points). Thus, the inflammation score is positively associated with low-grade systemic inflammation.

Other biochemical measurements

Analyses of total cholesterol (total-C), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerol (TG), fasting insulin, fasting glucose, toxic metals (i.e. lead, mercury, cadmium), POPs and seroprevalence of zoonoses have been described previously.[27, 29, 32] Seroprevalence of zoonoses (positive or negative) was considered positive when an individual tested positive for at least one of the pathogens tested. Equivocal values were classified in the negative category.

Lifestyle and socioeconomic data

An individual questionnaire administered by a local interviewer either in *Iiyiyiuyimuwin* or in English was used to collect information on gender, age, and smoking habits. Physical activity was assessed using the short version of the International Physical Activity Questionnaire (IPAQ).[33]

Dietary intake

An 81-item interviewer-administered semi-quantitative food frequency questionnaire (FFQ) inquiring on the consumption of traditional and commercial food items was used to estimate the daily frequency of consumption of fish and alcoholic beverages. Details on the FFQ are provided in **Supplementary File 1**.

Statistical analyses

Elevated plasma hs-CRP concentrations were defined as > 3.0 mg/L, as suggested by the American Heart Association.[34] An elevated inflammation score was defined as ≥ 9 points, which is the equivalent of having a value above the median of the population for each of the three pro-inflammatory markers analyzed (hs-CRP, IL-6 and TNF-α).

Chi-square tests were used to compare the prevalence of elevated plasma hs-CRP concentrations between subgroups of individuals stratified on the basis of sex (men/women), age (18-39 versus ≥ 40 years), waist circumference (low/high), and MetS (yes/no).

Multivariate general linear models (SAS PROC GLM) were used to assess the association between quartiles of RBC EPA, DPAn-3 and DHA levels and inflammation. Covariates considered in the models were sex (male/female), age (continuous), and waist circumference (continuous). Adjusted means (95% confidence intervals [CI]) were computed by quartiles of RBC LCn-3PUFA for each inflammatory marker. Adjusting for body weight, body mass index (BMI) or body fat instead of waist circumference led to similar results (data not shown). Further adjustments for RBC total n-6 and *trans* fatty acids, for environmental contaminants (POPs, including polychlorinated biphenyls and

organochlorine pesticides), for toxic metals (cadmium, mercury, lead), for the presence of type 2 diabetes or for the use of anti-inflammatory, anti-hypertensive, antidiabetic or lipid-lowering medication also had no influence on the observed results (data not shown). These variables were therefore not retained in final analyses of the data. Tests for trend across quartiles of RBC LCn-3PUFA were included in all models using the CONTRAST option "linear". Median scores in each quartile category were used for the trend analysis.

Associations between RBC levels of individual LCn-3PUFA and the consumption of fish were assessed using Spearman correlation coefficients.

Multivariate logistic regression analysis was used to characterize the odds of having an elevated inflammation score according to demographic, anthropometric, biochemical, lifestyle and nutritional (i.e. RBC LCn-3PUFA) variables. Odds ratio (OR) and 95% CI were calculated for each variable individually, using a multivariate model that included sex, age (continuous), and waist circumference (low/high) as covariates. The number of subjects in each model varied depending on the outcome of interest but was always greater than or equal to 719 participants after exclusion of missing covariates. Details on the number and percent of missing data for each covariable are provided in **Supplementary File 2**. A single multivariate logistic model was used to determine which risk factors overall remained significant and independent predictors of a high inflammation score.

Continuous data are presented as means (95% CI) unless stated otherwise. Non-normally distributed variables were natural log-transformed prior to analysis. Thus, geometric means (95% CI) are shown for these variables. Statistical analyses were performed using the SAS software (version 9.2; SAS Institute Inc., Cary, NC). Statistical significance was set at a *P* value < 0.05.

RESULTS

Table 1 shows characteristics of the study participants by sex. Participants (55% women) had a mean age of 39.6 years (95% CI 38.5-40.8), a mean BMI of 33.2 kg/m^2 (95% CI 32.7-33.7), and half were occasional or daily smokers (52%; data not shown). Prevalence of utilisation of lipid-lowering, antidiabetic, anti-inflammatory or anti-hypertensive

medication ranged from 11% to 25%. Type 2 diabetes prevalence in men and women combined was 23%.

The prevalence of hs-CRP concentrations > 3.0 mg/L was 46.9% (95% CI 43.3-50.5) and was higher among women (56.5%, 95% CI 51.6-61.3) than among men (35.1%, 95% CI 30.0-40.3, $P < 0.0001$). The prevalence of hs-CRP concentrations > 3.0 mg/l was also higher among individuals with a high vs normal waist circumference (48.3% vs 18.0%, $P = 0.0002$) and among individuals with MetS vs no MetS (51.4% vs 41.3%, $P = 0.006$). The proportion of individuals with hs-CRP concentrations > 3.0 mg/L did not differ according to age (18-39 years, 47.3% vs. 40 years and older, 46.4%, $P = 0.79$).

Table 2 shows the age, sex and waist circumference-adjusted inflammatory biomarker concentrations across quartiles of RBC EPA, DPAn-3 and DHA. Hs-CRP, TNF-α and the inflammation score were lower across increasing quartiles of RBC DPAn-3 (all P trend < 0.02), while IL-6 showed no statistical association with RBC DPAn-3 (P trend = 0.77). RBC EPA and DHA levels were not associated with any inflammatory biomarker (all P trend > 0.18). The correlation between fish consumption estimated by the FFQ and RBC DPAn-3 was lower overall ($r = 0.19$, $P < 0.0001$) than for EPA or DHA ($r = 0.42$ and $r = 0.43$, respectively, both $P < 0.0001$).

Risk factors for an elevated inflammation score in the James Bay Cree population were identified using a multivariate logistic model. Female sex (vs. male, OR = 2.27, 95% CI 1.64-3.15) and high waist circumference (vs. normal waist, OR = 3.16, 95% CI 1.20-8.31) increased the odds of having an elevated inflammation score in the James Bay Cree population. Insulin concentrations (log) (OR = 1.76, 95% CI 1.33-2.35) were also positively associated, while community of residence (inland vs. coastal, OR = 0.43, 95% CI 0.30-0.62), HDL-C concentrations (OR = 0.39, 95% CI 0.22-0.68), and RBC DPAn-3 levels above the median of the population (vs. levels below the median, OR = 0.67, 95% CI 0.48-0.92) were inversely associated with the odds of having an inflammation score ≥ 9 (all $P < 0.02$). Each of these variables was considered individually, after adjustment for age, sex, and waist circumference. The following variables showed no association with the inflammation score: LDL-C, TG, fasting glucose, systolic blood pressure, diastolic blood pressure, physical activity, smoking status, drinking status, type 2 diabetes, environmental

contaminants, toxic metals, the use of anti-inflammatory, antihypertensive, antidiabetic or lipid-lowering medication and RBC EPA and DHA. As shown in **Table 3**, when considering all significant risk factors simultaneously in a single multivariate model, only sex, community of residence, HDL-C, insulin (log) and RBC DPAn-3 remained independently associated with the odds of having an elevated inflammation score (all $P < 0.03$). The direction of the associations remained the same as described above.

DISCUSSION

To the best of our knowledge, this study is the first to determine the prevalence of elevated plasma hs-CRP concentrations in the James Bay Cree population from northern Quebec and to assess the associations between RBC LCn-3PUFA and inflammation in a Canadian First Nations community.

Our data suggest that almost half (≈ 47%) of the James Bay Cree population has elevated hs-CRP concentrations. This prevalence is almost twice that of Canadians of European origin (25.0%)[8] and more than twice that of the Inuit population from northern Quebec (21.4%).[35] On the other hand, this prevalence is comparable to the prevalence observed in First Nations communities from Ontario, Canada (54.8% in Anand et al.[8] or 51% for women and 32% for men, based on a cut-off of 3.8 mg/L, in Connelly et al.[7]). Consistent with previous studies in First Nations[7, 8] and other Aboriginal communities such as the Inuit,[35] we showed that the prevalence of elevated hs-CRP concentrations is considerably higher among women than among men. Interestingly, in contrast with studies that support a positive association between age and low-grade systemic inflammation,[7, 36] we showed that the prevalence of elevated hs-CRP concentrations did not differ according to age in the James Bay Cree population. Other risk factors appear to outweigh the impact of age on inflammation in this population. Hs-CRP concentrations over 3 mg/L have been associated with an increased risk of coronary heart disease (CHD) in the general population,[34] but further investigations are warranted to determine how elevated hs-CRP concentrations relate to future CHD events in First Nations communities. Nevertheless, observations from our study and other studies in First Nations communities highlight that systemic inflammation is a serious health concern that needs to be addressed in the context of the high prevalence of CHD in these populations.

So far, no study has documented the association between blood levels of LCn-3PUFA and inflammatory biomarkers in Canadian First Nations populations. Few studies conducted in other populations,[14-18, 20] but not all,[19, 37] have shown inverse associations between RBC, plasma or serum EPA and/or DHA and blood hs-CRP concentrations. Similar to our results, three out of five studies showed no association between IL-6 concentrations and plasma or RBC EPA and DHA,[17, 20, 37] while the other two studies showed inverse associations.[15, 19] Only two studies assessed TNF-α in addition to hs-CRP and IL-6 and showed no association with EPA and DHA measured in plasma.[17, 19] Makhoul et al.[20] have shown in a cohort of Yup'ik Eskimos, who consume very large amounts of EPA and DHA, that the inverse association between EPA and hs-CRP was stronger at values > 3% of total fatty acids in erythrocytes. For DHA, a strong inverse association with hs-CRP was observed at DHA values over 7% of total fatty acids, while the association appeared to be non-existing below a value of 7%. In the present study, none of the participants had RBC EPA > 3% of total fatty acids and only one participant had RBC DHA > 7% (not shown). This may explain the lack of association between RBC EPA and DHA and inflammatory biomarkers in the present study. Moreover, residual but very strong confounding effects of obesity may have masked associations between RBC EPA and DHA and low-grade systemic inflammation. Obesity indices including waist circumference and BMI were indeed higher across increasing quartiles of RBC EPA and DHA in the present study (all P trend $\leq$ 0.0001, data not shown), while RBC DPAn-3 showed no such association with obesity indices.

Multivariate logistic regression analysis has shown that the association between RBC DPAn-3 and inflammation in the James Bay Cree population was independent of the community of residence (coastal vs. inland) as well as of well-known correlates of inflammation among Aboriginal populations such as female sex, HDL-C (inverse), and insulin.[8, 10, 38] The inverse association between RBC DPAn-3 and inflammation seen in the James Bay Cree population is consistent with results from studies in other populations.[14, 16-18, 37] The absence of association between RBC DPAn-3 and IL-6 concentrations in the present study is also consistent with data from Sun et al.[37] In agreement with observations in Finnish and US populations,[16, 18, 37] blood DPAn-3 in the present study was less strongly correlated with fish intake than EPA or DHA. This suggests that endogenous chain

elongation from EPA may have a greater impact on blood DPAn-3 levels than direct dietary consumption.[39, 40] Although blood DPAn-3 appears as a poor marker for fish intake,[16] it is more strongly correlated with inflammation than EPA or DHA.[16, 17, 37] Little is known thus far on the mechanisms by which DPAn-3 may exert anti-inflammatory effects. DPAn-3 may reduce the expression of inflammatory genes or potentiate the production of DPA-related D-series resolvins and neuroprotectins,[40] which in turn may inhibit the production of inflammatory mediators as is the case with DHA-related pro-resolving molecules.[41] Nonetheless, intervention and experimental studies are warranted to confirm the anti-inflammatory effects of DPAn-3 and to elucidate the underlying mechanisms for these effects.

The present study has a number of strengths and limitations that need to be pointed out. The use of validated objective biomarkers of dietary LCn-3PUFA intake[24-26] in a large number of subjects is a strength. Indeed, LCn-3PUFA estimates derived from dietary questionnaires are more prone to subjective measurement errors and bias.[42] However, due to the cross-sectional design of the study, no causal relationship can be established. Although several factors known to influence pro-inflammatory processes such as environmental contaminants and medication use were taken into account in the analyses, we cannot exclude the possibility of residual confounding by unconsidered or unmeasured factors. The relatively low participation rate in the study (42%) may limit the representativeness of the sample to the whole James Bay Cree population. Nevertheless, our results regarding the prevalence of elevated hs-CRP concentrations among this population corroborate those observed among other Canadian First Nations.

In conclusion, elevated hs-CRP concentrations are highly prevalent among the James Bay Cree population, particularly among women and abdominally obese subjects. RBC DPAn-3, but not EPA or DHA, is inversely associated with inflammatory biomarkers in Cree adults even after adjustment for important covariates. Further longitudinal and interventional investigations are required to confirm this apparent anti-inflammatory effect of DPAn-3 seen in the James Bay Cree population.

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CONFLICT OF INTEREST STATEMENT

Marie-Eve Labonté has received funding from the CBHSSJB through Niskamoon Corporation for the present work. The other authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

Éric Dewailly designed the research and collected data; Marie-Ève Labonté performed statistical analyses, interpreted the data, and wrote the manuscript; Benoît Lamarche, Michel Lucas and Patrick Couture participated to analysis and interpretation of data; Benoît

Lamarche has had full access to the data in the study and has primary responsibility for the final content of the paper. All authors critically reviewed the manuscript and approved its final version.

REFERENCES

1. Haman F, Fontaine-Bisson B, Batal M, Imbeault P, Blais JM, Robidoux MA. Obesity and type 2 diabetes in Northern Canada's remote First Nations communities: the dietary dilemma. *Int J Obes (Lond)* 2010; **34 Suppl 2:** S24-31.

2. Garriguet D. Obesity and the eating habits of the Aboriginal population. *Health Rep* 2008; **19**(1)**:** 21-35.

3. Dannenbaum D, Kuzmina E, Lejeune P, Torrie J, Gangbe M. Prevalence of diabetes and diabetes-related complications in First Nations communities in northern Quebec (Eeyou Istchee), Canada. *Can J Diabetes* 2008; **32**(1)**:** 46-52.

4. Shah BR, Hux JE, Zinman B. Increasing rates of ischemic heart disease in the native population of Ontario, Canada. *Arch Intern Med* 2000; **160**(12)**:** 1862-1866.

5. Young TK, Reading J, Elias B, O'Neil JD. Type 2 diabetes mellitus in Canada's first nations: status of an epidemic in progress. *CMAJ* 2000; **163**(5)**:** 561-566.

6. Scrivo R, Vasile M, Bartosiewicz I, Valesini G. Inflammation as "common soil" of the multifactorial diseases. *Autoimmun Rev* 2011; **10**(7)**:** 369-374.

7. Connelly PW, Hanley AJ, Harris SB, Hegele RA, Zinman B. Relation of waist circumference and glycemic status to C-reactive protein in the Sandy Lake Oji-Cree. *Int J Obes (Lond)* 2003; **27**(3)**:** 347-354.

8. Anand SS, Razak F, Yi QL, Davis B, Jacobs R, Vuksan V *et al.* C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. *Arterioscler Thromb Vasc Biol* 2004; **24**(8)**:** 1509-1515.

9. Imbeault P, Findlay CS, Robidoux MA, Haman F, Blais JM, Tremblay A *et al.* Dysregulation of cytokine response in Canadian First Nations communities: is there an association with persistent organic pollutant levels? *PLoS One* 2012; **7**(7)**:** e39931.

10. Liu J, Young TK, Zinman B, Harris SB, Connelly PW, Hanley AJ. Lifestyle variables, non-traditional cardiovascular risk factors, and the metabolic syndrome in an Aboriginal Canadian population. *Obesity (Silver Spring)* 2006; **14**(3)**:** 500-508.

11. Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N *et al.* Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr* 2004; **134**(7)**:** 1806-1811.

12. Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysohoou C, Skoumas Y *et al.* Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. *J Am Coll Cardiol* 2005; **46**(1)**:** 120-124.

13. He K, Liu K, Daviglus ML, Jenny NS, Mayer-Davis E, Jiang R *et al.* Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). *Am J Cardiol* 2009; **103**(9)**:** 1238-1243.

14. Micallef MA, Munro IA, Garg ML. An inverse relationship between plasma n-3 fatty acids and C-reactive protein in healthy individuals. *Eur J Clin Nutr* 2009; **63**(9)**:** 1154-1156.

15. Farzaneh-Far R, Harris WS, Garg S, Na B, Whooley MA. Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study. *Atherosclerosis* 2009; **205**(2)**:** 538-543.

16. Reinders I, Virtanen JK, Brouwer IA, Tuomainen TP. Association of serum n-3 polyunsaturated fatty acids with C-reactive protein in men. *Eur J Clin Nutr* 2012; **66**(6)**:** 736-741.

17. Kalogeropoulos N, Panagiotakos DB, Pitsavos C, Chrysohoou C, Rousinou G, Toutouza M *et al.* Unsaturated fatty acids are inversely associated and n-6/n-3 ratios

are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. *Clin Chim Acta* 2010; **411**(7-8)**:** 584-591.

18. Mozaffarian D, Lemaitre RN, King IB, Song X, Spiegelman D, Sacks FM *et al.* Circulating long-chain omega-3 fatty acids and incidence of congestive heart failure in older adults: the cardiovascular health study: a cohort study. *Ann Intern Med* 2011; **155**(3)**:** 160-170.

19. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F *et al.* Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab* 2006; **91**(2)**:** 439-446.

20. Makhoul Z, Kristal AR, Gulati R, Luick B, Bersamin A, Boyer B *et al.* Associations of very high intakes of eicosapentaenoic and docosahexaenoic acids with biomarkers of chronic disease risk among Yup'ik Eskimos. *Am J Clin Nutr* 2010; **91**(3)**:** 777-785.

21. Makhoul Z, Kristal AR, Gulati R, Luick B, Bersamin A, O'Brien D *et al.* Associations of obesity with triglycerides and C-reactive protein are attenuated in adults with high red blood cell eicosapentaenoic and docosahexaenoic acids. *Eur J Clin Nutr* 2011; **65**(7)**:** 808-817.

22. Dewailly E, Blanchet C, Gingras S, Lemieux S, Holub BJ. Cardiovascular disease risk factors and n-3 fatty acid status in the adult population of James Bay Cree. *Am J Clin Nutr* 2002; **76**(1)**:** 85-92.

23. Dewailly E, Blanchet C, Gingras S, Lemieux S, Holub BJ. Fish consumption and blood lipids in three ethnic groups of Quebec (Canada). *Lipids* 2003; **38**(4)**:** 359-365.

24. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *Am J Clin Nutr* 2007; **86**(1)**:** 74-81.

25. Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. *Lipids* 1997; **32**(7)**:** 697-705.

26. 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**(10)**:** 2012-2022.

27. Valera B, Dewailly E, Poirier P. Impact of mercury exposure on blood pressure and cardiac autonomic activity among Cree adults (James Bay, Quebec, Canada). *Environ Res* 2011; **111**(8)**:** 1265-1270.

28. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 2003; **107**(3)**:** 363-369.

29. Nieboer E, Dewailly E, Egeland GM, Château-Degat M-L, Bonnier-Viger Y. *Nituuchischaayihtitaau Aschii. Multi-Community Environment-and-Health Longitudinal Study in Eeyou Istchee: Eastmain and Wemindji. Technical report: summary of 2007 activities, results and recommendations.* In: Nieboer E, Robinson E, Petrov K (eds). Public Health Report Series 4 on the Health of the Population. Cree Board of Health and Social Services of James Bay: Chisasibi, QC, 2011.

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

31. 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**(20)**:** 2474-2480.

32. Sampasa-Kanyinga H, Levesque B, Anassour-Laouan-Sidi E, Cote S, Serhir B, Ward BJ *et al.* Zoonotic infections in native communities of James Bay, Canada. *Vector Borne Zoonotic Dis* 2012; **12**(6)**:** 473-481.

33. International Physical Activity Questionnaire (IPAQ). *Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) – Short and Long Forms November 2005*. Available from http://www.ipaq.ki.se/scoring.pdf (date accessed July 23, 2013).

34. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M *et al.* Markers of inflammation and cardiovascular disease application to clinical and public health practice - A statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. *Circulation* 2003; **107**(3)**:** 499-511.

35. Labonte ME, Dewailly E, Chateau-Degat ML, Couture P, Lamarche B. Population-based study of high plasma C-reactive protein concentrations among the Inuit of Nunavik. *Int J Circumpolar Health* 2012; **71**: 19066. doi: 10.3402/ijch.v71i0.19066.

36. Bruunsgaard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokines. *Curr Opin Hematol* 2001; **8**(3)**:** 131-136.

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

38. Rowley K, Walker KZ, Cohen J, Jenkins AJ, O'Neal D, Su Q *et al.* Inflammation and vascular endothelial activation in an Aboriginal population: relationships to coronary disease risk factors and nutritional markers. *Med J Aust* 2003; **178**(10)**:** 495-500.

39. Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J Nutr* 2012; **142**(3)**:** 614S-625S.

40. Kaur G, Cameron-Smith D, Garg M, Sinclair AJ. Docosapentaenoic acid (22:5n-3): a review of its biological effects. *Prog Lipid Res* 2011; **50**(1)**:** 28-34.

41. Borgeson E, Godson C. Resolution of inflammation: therapeutic potential of pro-resolving lipids in type 2 diabetes mellitus and associated renal complications. *Front Immunol* 2012; **3:** 318.

42. Potischman N. Biologic and methodologic issues for nutritional biomarkers. *J Nutr* 2003; **133 Suppl 3:** 875S-880S.

TABLES

Table 1. Characteristics of a random sample of the James Bay Cree population

Characteristics	Men[1]		Women[1]	
Age (years)	40.3	(38.5-42.0)	39.1	(37.6-40.6)
Weight (kg)	97.0	(94.9-99.1)	90.1	(88.2-92.0)
BMI (kg/m^2)	31.8	(31.1-32.4)	34.3	(33.7-35.0)
Body fat (%)	32.9	(31.9-33.9)	44.6	(43.9-45.2)
Waist circumference (cm)	109.9	(108.2-111.5)	110.5	(109.0-112.0)
Cholesterol (mmol/L)				
Total-C	4.8	(4.7-4.9)	4.5	(4.4-4.6)
LDL-C	2.8	(2.8-2.9)	2.5	(2.5-2.6)
HDL-C	1.19	(1.16-1.22)	1.29	(1.25-1.32)
Total-C/HDL-C ratio	4.1	(4.0-4.2)	3.6	(3.5-3.6)
Triacylglycerol (mmol/L)	1.5	(1.4-1.6)	1.4	(1.3-1.4)
Blood pressure (mmHg)				
Systolic	124.5	(122.9-126.0)	119.4	(117.9-120.9)
Diastolic	76.6	(75.5-77.7)	71.9	(70.8-72.9)
Inflammatory markers				
hs-CRP (mg/L)	2.0	(1.8-2.2)	2.9	(2.7-3.2)
IL-6 (pg/mL)	2.1	(2.0-2.3)	2.7	(2.5-2.8)
TNF-α (pg/mL)	2.3	(2.1-2.5)	2.6	(2.4-2.8)
Inflammation score[2]	6.9	(6.7-7.2)	8.0	(7.8-8.2)
Insulin (pmol/L)	122.0	(114.0-130.5)	148.7	(141.1-156.8)
Fasting glucose (mmol/L)[3]	5.6	(1.2)	5.5	(1.0)
Physical activity (MET)[3,4]	4380	(9473)	4230	(6225)
Smoking (%)				
Never	9.7		9.4	
Ex	40.8		36.5	
Occasionally	11.2		13.1	
Daily	38.3		41.0	
Drinking (%)				

Never	51.7	60.3
< once per week	18.7	19.0
≥ once per week to < each two days	16.5	12.8
≥ each two days	7.8	4.7
Daily	5.3	3.2
Medication (yes, %)		
Antiinflammatory	20.4	27.3
Antihypertensive	24.0	25.6
Antidiabetic	12.6	17.8
Lipid-lowering	11.7	11.2
Diabetes (yes, %)	21.3	23.8

Abbreviations: BMI, body mass index; C, cholesterol; HDL, high density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LDL, low-density lipoprotein; MET, metabolic equivalent of task; TNF-α, tumor necrosis factor-alpha.

[1]n=316-333 for men and n=387-411 for women, depending on the variable. Values are arithmetic means (95% confidence intervals) for normally distributed variables and geometric means (95% confidence intervals) for non-normally distributed variables, unless stated otherwise.

[2]Score ranging from 3 to 12 points, corresponding to the sum of the quartiles in which individuals are present for hs-CRP, IL-6 and TNF-α concentrations (Q1 = 1 point; Q2 = 2 points; Q3 = 3 points; Q4 = 4 points).

[3]The variable was not normally distributed even after log transformation. Median values (interquartile range) are thus shown.

[4]MET represents MET-minutes per week.

Table 2. Inflammatory biomarkers concentrations across quartiles of RBC n-3 fatty acid levels among the James Bay Cree population[1]

	Quartiles of RBC LCn-3PUFA								
	Q1		Q2		Q3		Q4		*P* trend
EPA (%)[2]	0.32		0.41		0.52		0.78		
hs-CRP (mg/L)	2.25	(1.97-2.56)	2.53	(2.24-2.86)	2.54	(2.25-2.87)	2.30	(2.00-2.65)	0.82
IL-6 (pg/mL)	2.51	(2.31-2.73)	2.42	(2.23-2.61)	2.22	(2.05-2.40)	2.35	(2.14-2.57)	0.18
TNF-α (pg/mL)	2.46	(2.18-2.79)	2.33	(2.07-2.61)	2.55	(2.27-2.86)	2.37	(2.07-2.71)	0.92
Inflammation score[3]	7.46	(7.13-7.79)	7.51	(7.20-7.81)	7.47	(7.17-7.78)	7.30	(6.95-7.66)	0.55
DPAn-3 (%)[2]	1.78		1.97		2.15		2.41		
hs-CRP (mg/L)	2.71	(2.39-3.06)	2.45	(2.18-2.76)	2.35	(2.08-2.65)	2.14	(1.89-2.42)	0.009
IL-6 (pg/mL)	2.43	(2.24-2.63)	2.35	(2.18-2.54)	2.31	(2.13-2.49)	2.40	(2.22-2.60)	0.77
TNF-α (pg/mL)	2.85	(2.54-3.20)	2.44	(2.18-2.73)	2.03	(1.81-2.27)[4]	2.46	(2.19-2.76)	0.02
Inflammation score[3]	7.78	(7.47-8.09)	7.48	(7.18-7.78)	7.17	(6.87-7.48)[4]	7.32	(7.01-7.63)	0.02
DHA (%)[2]	2.54		3.07		3.63		4.58		
hs-CRP (mg/L)	2.17	(1.91-2.48)	2.41	(2.13-2.73)	2.63	(2.32-2.96)	2.42	(2.09-2.80)	0.26
IL-6 (pg/mL)	2.36	(2.17-2.57)	2.32	(2.14-2.51)	2.38	(2.20-2.57)	2.44	(2.22-2.68)	0.61
TNF-α (pg/mL)	2.53	(2.23-2.87)	2.22	(1.97-2.50)	2.67	(2.39-3.00)	2.29	(1.99-2.63)	0.73
Inflammation score[3]	7.52	(7.19-7.85)	7.31	(7.00-7.62)	7.63	(7.33-7.93)	7.28	(6.92-7.65)	0.67

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha.

[1]n=729 for hs-CRP and n=728 for IL-6, TNF-α and the inflammation score. Associations were assessed using the general linear model (GLM) procedure. Geometric means (95% confidence intervals) are shown for hs-CRP, IL-6, and TNF-α. Arithmetic means (95% confidence intervals) are shown for the inflammation score. Means are adjusted for age, sex, and waist circumference.

[2]Values are expressed as percent of total fatty acids in erythrocyte membrane phospholipids and represent the median percentage in each quartile.

[3]Score ranging from 3 to 12 points, corresponding to the sum of the quartiles in which individuals are present for hs-CRP, IL-6 and TNF-α concentrations (Q1 = 1 point; Q2 = 2 points; Q3 = 3 points; Q4 = 4 points).

[4]Significantly lower than Q1 ($P < 0.05$), as determined by the Tukey adjustment within the GLM procedure.

Table 3. Adjusted odds ratio for an elevated inflammation score in the James Bay Cree population according to demographic, anthropometric, biochemical, lifestyle and nutritional risk factors[1]

Variables	Multivariate OR inflammation score ≥ 9[2]	95% CI	*P*
Sex			
Men	1.00	(ref)	
Women	2.32	(1.64-3.28)	< 0.0001
Waist circumference (cm)[3]			
Low	1.00	(ref)	
High	1.74	(0.63-4.80)	0.29
Community			
Coastal	1.00	(ref)	
Inland	0.38	(0.26-0.56)	< 0.0001
HDL-C (mmol/L)	0.52	(0.29-0.93)	0.03
Log insulin (pmol/L)	1.73	(1.26-2.37)	0.0007
RBC DPAn-3 levels (%)			
< 50th percentile	1.00	(ref)	
≥ 50th percentile	0.67	(0.48-0.93)	0.02

Abbreviations: CI, confidence interval; DPA, docosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; OR, odds ratio; RBC, red blood cell; Ref, reference group.

[1]OR and 95% CI were determined using a multivariate logistic regression model in SAS simultaneously taking into account all shown variables. OR for each variable are thus adjusted for all other variables in the table.

[2]The inflammation score is a score ranging from 3 to 12 points, corresponding to the sum of the quartiles in which individuals are present for hs-CRP, IL-6 and TNF-α concentrations (Q1 = 1 point; Q2 = 2 points; Q3 = 3 points; Q4 = 4 points). An elevated inflammation score (≥ 9) is the equivalent of having a value above the median of the population for each of the pro-inflammatory markers analyzed, i.e. hs-CRP, IL-6 and TNF-α.

[3]High waist circumference cut-offs were ≥ 90 cm in men and ≥ 80 cm in women.

FIGURES

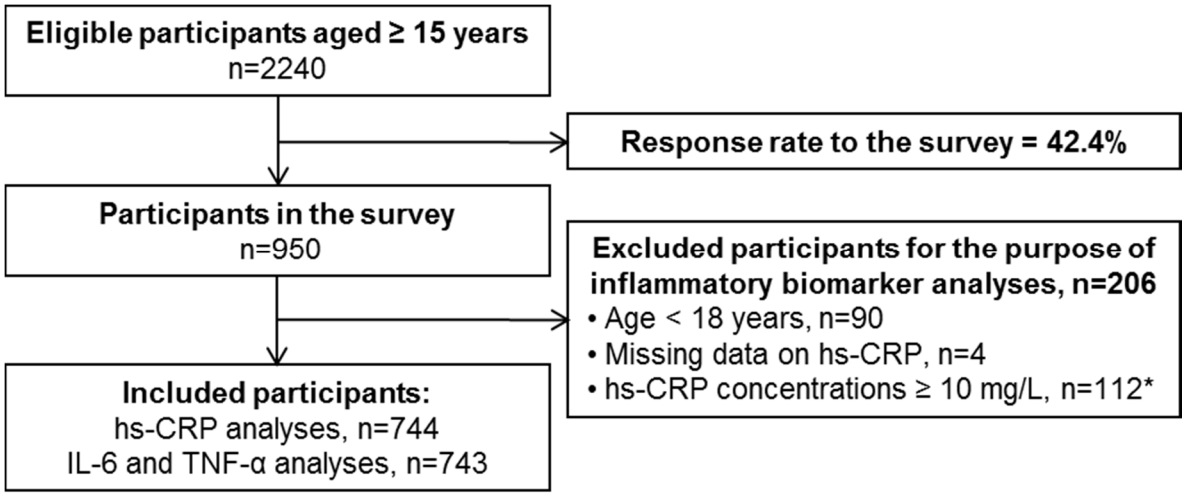

Figure 1. Flow of participants through the Nituuchischaayihtitaau Aschii Multi-Community Environment and Health Study in Iiyiyiu Aschii. Abbreviations: hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha. *112 participants with hs-CRP concentrations ≥ 10 mg/L were excluded based on a clinical criteria indicative of the presence of an active acute infection.[28]

SUPPLEMENTARY FILES

Supplementary File 1. Details on the interviewer-administered semi-quantitative food frequency questionnaire

Participants reported their usual food intake on an 81-item interviewer-administered semi-quantitative food frequency questionnaire (FFQ). The FFQ was divided into two sections: 53 items on traditional foods consumed during the past year (i.e. consumption of game animals, fish, birds and berries; taking into account seasonal variation) and 28 items on commercial foods eaten during the last 30 days. Interviewers and translators were selected from local communities and received appropriate training in dietary assessment techniques. Research team members also reviewed the FFQ to ensure that all questions were answered adequately and appropriately. Food intakes derived from the FFQ are expressed in terms of daily frequency of consumption. An example of the FFQ can be viewed in another report.[1]

[1]Nieboer E, Dewailly E, Egeland GM, Château-Degat M-L, Bonnier-Viger Y. *Nituuchischaayihtitaau Aschii. Multi-Community Environment-and-Health Longitudinal Study in Eeyou Istchee: Eastmain and Wemindji. Technical report: summary of 2007 activities, results and recommendations.* In: Nieboer E, Robinson E, Petrov K (eds). Public Health Report Series 4 on the Health of the Population. Chisasibi, QC: Cree Board of Health and Social Services of James Bay, 2011, pp. 280-289

Supplementary File 2. Details on the number and percent of missing data for each covariable considered in the multivariate logistic regression analysis

- n=0 (no missing data) for sex, age, community, glucose concentrations, insulin concentrations, presence of type 2 diabetes and use of anti-inflammatory, anti-hypertensive, antidiabetic or lipid-lowering medication;
- n=1 (0.1%) for high-density lipoprotein cholesterol (HDL-C), triglycerides, red blood cell (RBC) eicosapentaenoic acid (EPA), RBC docosapentaenoic acid (DPAn-3), RBC docosahexaenoic acid (DHA), and environmental contaminants (persistent organic pollutants, including polychlorinated biphenyls and organochlorine pesticides);
- n=3 (0.4%) for toxic metals (cadmium, mercury, lead);
- n=7 (0.9%) for low-density lipoprotein cholesterol (LDL-C);
- n=14 (1.9%) for systolic and diastolic blood pressure and waist circumference;
- n=17 (2.3%) for drinking habits;
- n=18 (2.4%) for smoking;
- n=24 (3.2%) for physical activity

CHAPITRE 5 :

UN PROFIL ALIMENTAIRE TRADITIONNEL EST ASSOCIÉ À DES CONCENTRATIONS PLASMATIQUES DE CHOLESTÉROL ÉLEVÉES CHEZ LES INUITS DU NUNAVIK

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Traditional dietary pattern is associated with elevated cholesterol among the Inuit of Nunavik

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RÉSUMÉ

Cette étude transversale avait pour but d'évaluer les associations entre des profils alimentaires et les facteurs du risque cardiovasculaire (incluant les biomarqueurs inflammatoires) chez les Inuits du Nunavik (province de Québec). La collecte des mesures cliniques, des échantillons sanguins et des données nutritionnelles à l'aide d'un questionnaire de fréquence alimentaire (FFQ) a été effectuée dans le cadre du *2004 Nunavik Inuit Health Survey*. Un échantillon de 666 Inuits âgés de 18 ans ou plus a été inclus dans les analyses. Les profils alimentaires ont été identifiés par une analyse en composantes principales. Les associations entre les profils alimentaires et les facteurs du risque cardiovasculaire ont été évaluées à l'aide de modèles linéaires généralisés (GLM) tenant compte du sexe, de l'âge, du tour de taille et d'autres variables potentiellement confondantes. Quatre profils alimentaires ont été identifiés, soit les profils « Traditionnel », « Western », « À faible valeur nutritive » et « Prudent ». Le profil « Traditionnel » était positivement associé aux concentrations de cholestérol (C)-total, de LDL-C, d'apolipoprotéine B100, de LDL oxydées ainsi qu'au diamètre majeur des LDL (*P* pour la tendance ≤ 0,04), mais ne montrait aucune association avec le ratio C-total : HDL-C ni avec les biomarqueurs inflammatoires (*P* pour la tendance ≥ 0,19). Le profil « À faible valeur nutritive » était positivement associé aux LDL oxydées (*P* = 0,04), mais inversement associé à la hs-CRP (*P* < 0,0001). Les profils « Western » et « Prudent » ne montraient aucune association avec les facteurs du risque cardiovasculaire. Ces résultats démontrent qu'une forte adhésion à un profil alimentaire traditionnel chez les Inuits du Nunavik n'est pas associée à des changements importants dans les facteurs du risque cardiovasculaire, sauf pour une légère augmentation des concentrations de cholestérol, fort possiblement attribuable à une plus grande consommation d'acides gras oméga-3 d'origine marine. Les profils alimentaires reflétant l'introduction récente des aliments du commerce dans l'alimentation des Inuits du Nunavik semblent exercer une influence non significative sur les facteurs du risque cardiovasculaire.

TITLE PAGE

TITLE

Traditional dietary pattern is associated with elevated cholesterol among the Inuit of Nunavik

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KEYWORDS: Dietary patterns; cardiovascular disease risk factors; Inuit; principal component analysis; traditional diet

ABSTRACT

The present cross-sectional study assessed the associations between dietary patterns and cardiovascular disease (CVD) risk factors among Nunavik Inuit. This study was conducted as part of the 2004 Nunavik Inuit Health Survey, which included the collection of clinical measurements, plasma samples and diet information from a food frequency questionnaire. A sample of 666 Inuit aged 18 years and over was included in analyses. Dietary patterns were generated by principal component analysis. Multivariate general linear models adjusting for sex, age, waist circumference and other potential confounders were used to examine associations between dietary patterns and CVD risk factors. Four distinct patterns were identified, namely the traditional, Western, nutrient-poor food and healthy patterns. The traditional pattern showed positive associations with plasma total-cholesterol (C), low-density lipoprotein (LDL)-C, apolipoprotein B100, LDL peak particle diameter and oxidized-LDL (all P trend ≤ 0.04), but showed no association with the total-C/high-density lipoprotein-C ratio or with inflammatory biomarkers (all P trend ≥ 0.19). The nutrient-poor food pattern was positively associated with oxidized-LDL (P=0.04), but inversely associated with high-sensitivity C-reactive protein (P<0.0001). The Western and healthy patterns showed no association with any CVD risk factor. Our data show that high adherence to a traditional pattern among Nunavik Inuit is not associated with important changes in CVD risk factors, with the exception of a slight elevation in cholesterol concentrations, most likely attributable to increased omega-3 fatty acid intake. Dietary patterns reflecting the recent introduction of market foods in the Inuit diet appear to exert a trivial impact on CVD risk factors.

INTRODUCTION

The nutrition transition concept describes the rapid dietary and physical activity changes in transitional countries.[1] Among Canadian Inuit populations, the nutrition transition took place over the last 50 years.[2,3] This transition is characterized by a decline in the consumption of nutrient-dense traditional foods originally gathered by Inuit from their local environment concurrent with an increase in the consumption of non-nutrient-dense/energy-dense market foods.[2-4] This shift towards westernized diets high in total fat, refined carbohydrates and sugar[2,5] from market foods parallels an increase in the prevalence of contemporary metabolic disorders including obesity,[6,7] cardiovascular diseases (CVD),[8] and diabetes[9] in the Inuit population.

The identification of overall dietary patterns, which takes into consideration possible interactions between nutrients or food items,[10] reflects the complexity of the diet and may provide insightful information on its relationship with CVD risk factors. Only a few studies have examined the association between dietary patterns and CVD risk factors among aboriginal communities including Alaska Natives,[11,12] Ojibwa-Cree from northwestern Ontario, Canada,[13] and Greenlandic Inuit.[14] Among Inuit from Nunavik (Northern Quebec, Canada), studies on CVD risk factors have focused mainly on specific nutrients such as n-3 fatty acids and *trans* fatty acids,[15,16] or specific food groups, such as dairy products and fish.[17,18] The relationship of dietary patterns, rather than specific nutrients or food items, with CVD risk factors has never been determined in the Nunavik Inuit population.

Thus, the purpose of the present study was to derive dietary patterns from principal component analysis (PCA) and to evaluate their association with key CVD risk factors, including plasma lipids-lipoproteins and inflammatory biomarkers, among Nunavik Inuit. We hypothesized that westernized dietary patterns among Inuit from Nunavik are associated with a deteriorated CVD risk factor profile.

METHODS

Population and study design

Data collection for the present cross-sectional study was carried out aboard the Canadian Coast Guard ship (CCGS) Amundsen from August 27th to October 1st 2004, as part of the Nunavik Inuit Health Survey entitled "Qanuippitaa? – How are we?". The ship visited the 14 Inuit communities of Nunavik. The target population was permanent residents of Nunavik aged 18 years and over. Residents of collective dwellings (i.e. hotels, hospitals, jails) were excluded as well as households in which no Inuit was aged 18 years and over. The survey used a complex two-stage stratified random sampling of private Inuit households across the 14 communities of Nunavik. Further details on the sampling process are reported elsewhere.[19] The present study sample included 666 Inuit adults. Flow of participants and reasons for exclusion are provided in **Supplementary File 1**. The survey was approved by ethics committees of Laval University and *Institut national de santé publique du Québec*. Participants provided written informed consent after watching a video describing the study.

Clinical and biological measurements

Clinical and biological measurements were collected during a 3-h clinical session aboard the CCGS Amundsen, as previously described.[6,7] Diagnosis of type 2 diabetes status was based on self-report.

Participants were instructed to fast for at least 8 h prior to venipuncture. After centrifugation and storage in -80°C freezers onboard the CCGS Amundsen, collected blood samples were sent at the Centre Hospitalier de l'Université Laval for analyses of the lipid-lipoprotein and inflammatory profiles. Methods used for the analysis of total-cholesterol (C), low-density lipoprotein (LDL)-C, high-density lipoprotein (HDL)-C, triglycerides (TG), apolipoprotein (apo)-AI, apoB100, LDL peak particle diameter (LDL-PPD) and plasma high-sensitivity C-reactive protein (hs-CRP) concentrations have been reported previously.[7,16,20] Plasma oxidized-LDL concentrations were measured by enzyme-linked immunosorbent assay by use of monoclonal antibody mAb-4E6 (Mercodia AB, Uppsala,

Sweden). Plasma concentrations of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were measured using commercial enzyme-linked immunosorbent assay kits (R&D Systems Inc., Minneapolis, MN, USA). Coefficients of variation were 7.8% for IL-6 and 8.4% for TNF-α.

Lifestyle and socioeconomic information

Participants completed interviewer- and self-administered questionnaires in order to document their lifestyle and socioeconomic characteristics (e.g. smoking habits, education level).[19] Classification of participants according to smoking status, drinking habits, physical activity levels and education level has been described previously by our group.[21]

Dietary assessment

Diet information was collected during a face-to-face interview with a food frequency questionnaire (FFQ) adapted for use in the Inuit population, as previously described.[19] The FFQ was available in Inuktitut, English and French. It inquired on the consumption of 69 food items and beverages divided into two major categories: traditional Inuit foods (25 items) and market foods (44 items). Detailed information on the FFQ, including computerization of the collected data, is provided elsewhere.[22]

Dietary patterns

Food items from the FFQ were aggregated into 27 predefined food groups based on food groupings used in previous studies that identified dietary patterns among circumpolar populations.[11,23] Food items included in each food group can be viewed in **Supplementary File 2**. Daily intakes (g/day) for each food group were logarithmically transformed (log[x+1]) prior to PCA in order to achieve normality. Dietary patterns were generated by PCA on the basis of the 27 food groups by means of the varimax orthogonal procedure.[24] The number of factors to retain was determined using Eigenvalues ≥ 1, the Scree plot, and interpretability of the factors. Seven factors had Eigenvalues ≥ 1. Based on the Scree plot and the interpretability of the factors, four factors were retained for subsequent analysis of the association between dietary patterns and CVD risk factors. Dietary patterns were labeled according to food groups with high loading on each factor. Factor loadings with

absolute values ≥ 0.30 were considered as significantly contributing to a given dietary pattern. For each participant, a factor score for each pattern was calculated by using SAS SCORE procedure. Scores were calculated by summing the observed intakes of food groups weighted by their respective factor loadings. Dietary pattern scores were categorized into quartiles.

Statistical analyses

Associations between quartiles of dietary patterns and characteristics of participants were assessed using general linear models (SAS PROC GLM) for continuous variables or the Cochran-Armitage trend test for categorical variables. Multivariate general linear models (SAS PROC GLM) were used to assess the association between quartiles of dietary patterns and CVD risk factors. Potential confounders considered in the models were sex (male/female), age (continuous), waist circumference (continuous), physical activity (< or ≥ 3.5 h/week for at least one season in the previous year), smoking status (non, ex-, or current smokers), drinking habits (never, light: < 1 drink/d, moderate: 1-2 drinks/d, or heavy drinkers: > 2 drinks/d), education level (less, equal to, or more than completed high school), diabetes (yes/no), and use of lipid-lowering medication (yes/no). Clinical relevance guided the choice of covariates. Only covariates that showed an association with a given CVD risk factor at $P \leq 0.10$ were kept in the final model. Adjusted means ± SEM were computed by quartiles of dietary patterns for each CVD risk factor. Adjustment for body weight, body weight and height, body mass index or body fat instead of waist circumference had no impact on the results, unless stated otherwise. Tests for trend across quartiles of adherence to each dietary pattern were performed within the multivariate models using the CONTRAST option "linear". Median scores in each quartile category were used for the trend analysis. Assumptions for use of general linear models were verified for each of the CVD risk factors and variables were natural log-transformed prior to analysis when required. Statistical analyses were performed using the Statistical Analysis Software (version 9.2, 2008, SAS Institute Inc, Cary, NC). Statistical significance was set at a P value of < 0.05.

RESULTS AND DISCUSSION

Table 1 shows characteristics of the study participants. Participants were mainly women (55%), young (36 ± 14 years), overweight (BMI: 27.5 ± 5.7 kg/m^2), and current smokers (75%). Mean plasma lipid and blood pressure profiles were within normal ranges. Self-reported prevalence of diabetes was 5.3%.

Four dietary patterns were identified with PCA (**Table 2**). These four patterns explained 38.5% of the variance in food intake. The variation accounted for each factor was 17% for traditional, 10.2% for Western, 6.4% for nutrient-poor food, and 4.9% for healthy patterns. Consistent with observations from previous studies,[11,12,23] the traditional dietary pattern reflects the consumption of local foods obtained from hunting, fishing and gathering activities (**Table 2**) and is associated with older age among the Nunavik Inuit population (*P* trend = 0.0003; **Supplementary File 3**). On the other hand, the Western, nutrient-poor food and healthy patterns reflect the important contribution of market foods in the actual Inuit diet. The Western pattern loaded high on products typically consumed in Western diets such as potatoes, store-bought meat and alternatives (red meat, processed and fatty meat, poultry, and eggs), refined grain products, and fried food. The nutrient-poor food pattern was characterized by high loadings for energy-dense, nutrient-poor foods including potato chips, soft drinks, fried food and sweets and desserts. The healthy pattern was characterized by higher intakes of store-bought foods recognized as "healthy food choices" including 100% fruit juice, fruits, vegetables, dairy products, whole grain products, as well as legumes and nuts. The nutrient-poor food and healthy patterns were inversely associated with age (both $P \leq 0.04$; Supplementary File 3), reflecting a decline in the consumption of traditional foods in favour of the consumption of market foods among younger adults in the Nunavik Inuit population.

As shown in **Table 3**, plasma total-C, LDL-C, apoB100, and oxidized-LDL concentrations as well as LDL-PPD increased across quartiles of traditional pattern, independently of potential confounders (all *P* trend ≤ 0.04). Consistent with our results, Eilat-Adar et al.[11] observed that adherence to a traditional dietary pattern tended to be associated with higher LDL-C concentrations in the Inupiat of Alaska after adjustment for confounders similar to the ones we have considered in the present study. On the other hand, a study conducted in

Alaskan Yup'ik showed no difference in LDL-C concentrations between the lowest (< 10% of energy) and highest (> 31% of energy) quintiles of traditional food intake after adjustment for age, sex and BMI.[12] ApoB-100, LDL-PPD and oxidized-LDL were not assessed in these two previous studies.[11,12]

The association noted between the traditional dietary pattern and LDL concentrations may intuitively appear as undesirable in the context of CVD prevention. However, although total-C and LDL-C concentrations increased across quartiles of traditional pattern, no difference was found between quartiles in the total-C/HDL-C ratio, a stronger risk factor for ischemic heart disease risk and mortality than total-C alone,[25,26] due to a concomitant trend towards an increase in HDL-C concentrations (P trend = 0.11 and P trend = 0.04 when adjusted for body weight instead of waist circumference). This observation is consistent with observations from previous studies in Alaska Natives[11,12] and suggests the presence of a potentially higher capacity for reverse cholesterol transport. We also stress that the Inuit population from Nunavik has high HDL-C concentrations, which makes them less prone to changes attributable to dietary variation. Furthermore, the increase in LDL-PPD across quartiles of traditional pattern also suggests a lower risk of CVD.[27] The positive association observed between traditional pattern and oxidized-LDL was expected considering that traditional foods represent a source of polychlorinated biphenyls which, together with LDL-C concentrations, have previously been shown as positive predictors of the variance in oxidized-LDL in the Inuit from the village of Salluit, Nunavik.[28] However, it is stressed that oxidized-LDL concentrations remain very low in Inuit from Nunavik.[28] Plasma LDL-C concentrations have been shown to increase with the consumption of high doses of long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA).[29,30] Relative concentrations of n-3 LC-PUFA from marine sources in erythrocyte membranes are valid biomarkers of n-3 LC-PUFA intake.[31-33] Accordingly, n-3 LC-PUFA in erythrocytes increased across quartiles of traditional pattern in the present study (data not shown). Based on this and on a previous report from our group,[15] we hypothesize that the positive association noted between traditional dietary pattern and LDL-C and apoB100 even after adjustment for confounders such as age may be partly attributable to a higher consumption of n-3 LC-PUFA among Inuit showing a greater degree of adherence to the traditional pattern. In sum, the traditional pattern is associated with increased plasma cholesterol

concentrations but also with larger LDL and shows no association with the total-C/HDL-C ratio and biomarkers of inflammation. These observations suggest overall that adherence to a traditional dietary pattern among Inuit from Nunavik is not associated with an increased risk of CVD, especially if we consider that concentrations of cholesterol and other CVD risk factors in the Inuit population may not have similar CVD risk implications than in other populations. Nevertheless, these associations need to be further investigated in longitudinal studies.

The nutrient-poor food pattern in the present study of Inuit from Nunavik was positively associated with oxidized-LDL concentrations (*P* trend = 0.04, Table 3), but, unexpectedly, showed an inverse association with hs-CRP concentrations even after adjustment for abdominal obesity (*P* trend < 0.0001). However, this association was no longer significant after adjustment for body weight instead of waist circumference (*P* trend = 0.09). Food groups loading high on the nutrient-poor food pattern comprise food items that were important sources of industrially-produced *trans* fatty acids (TFA) when the survey was conducted. Consumption of these food items has previously been positively correlated with blood TFA in the Inuit population from Nunavik[16] and there is evidence that TFA consumption promotes low-grade systemic inflammation.[34] Recent data from our group indicated that age and waist circumference are among the most significant and independent correlates of elevated hs-CRP concentrations in the Inuit population from Nunavik.[21] Residual confounding effects of age and waist circumference/obesity may therefore account, at least partly, for this inverse association between the nutrient-poor food pattern and low-grade systemic inflammation. Indeed, hs-CRP concentrations may be lower in Inuit with a high degree of adherence to the nutrient-poor food pattern only because they are considerably younger and have a significantly lower mean waist circumference than Inuit with a low degree of adherence to this pattern (Supplementary File 3). This, however, warrants further investigation in the future.

Adherence to the Western and healthy patterns was not associated with any CVD risk factor (all *P* trend ≥ 0.08, Table 3). The absence of association between the Western, the healthy and even the nutrient-poor food dietary patterns and the vast majority of CVD risk factors investigated in this study suggests that these "contemporary" dietary patterns have

limited impact on CVD risk factors in Nunavik Inuit once other socio-demographic, lifestyle or anthropometric characteristics have been considered. A similar inference has been suggested by Eilat-Adar et al.[11] after having observed no clinically meaningful association between various dietary patterns and inflammatory biomarkers among Inupiat of Alaska. We cannot exclude the possibility that the lack of association between the Western, nutrient-poor food and healthy dietary patterns and CVD risk factors is attributable to limitations inherent to the cross-sectional design and dietary assessment in this study. Indeed, dietary assessment tools such as FFQ are subjected to substantial measurement errors and biases.[35] It is also stressed that the cultural context of Nunavik made it difficult to collect complete dietary information, as paying attention to details regarding the food being consumed (e.g. food brands) is not a priority for the Inuit people of Nunavik.[36] Dietary pattern analysis (PCA) is also complicated in interpretation and translation to the individual level. This may contribute to some of the lack of findings with CVD risk factors.

Strengths and limitations inherent to this study need to be pointed out. First, causality cannot be inferred from the observed associations due to the cross-sectional design of the study. Second, as in all observational studies, residual confounding by unconsidered or unmeasured factors may have occurred although adjustment for several covariates was taken into account in the analyses. Third, PCA involves a substantial part of subjectivity.[37] However, primary dietary data obtained from the FFQ were collapsed into food groups based on previous studies that have identified dietary patterns among circumpolar populations.[11,23] Eigenvalues and the Scree plot were used as criteria to determine the final number of patterns to retain. A specific cut-off value was used to determine food groups that significantly contributed to each pattern and the labeling of the patterns was based on food groups loading high on each pattern as well as labels previously used in the literature. These steps limit subjectivity and also allow data replication in future studies.

CONCLUSIONS

A high adherence to a traditional dietary pattern among Nunavik Inuit is not associated with important changes in CVD risk factors, with the exception of a slight elevation in cholesterol concentrations, most likely attributable to increased omega-3 fatty acid intake.

Dietary patterns reflecting the recent introduction of market foods in the diet of the Inuit population appear to exert only a trivial impact on CVD risk factors. Further longitudinal, prospective investigations are needed to confirm the present findings in the Inuit population from Nunavik and other circumpolar populations.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

REFERENCES

1. Popkin BM. Nutritional patterns and transitions. *Popul Dev Rev.* 1993;19(1):138-157.

2. Kuhnlein HV, Receveur O, Soueida R, Egeland GM. Arctic Indigenous Peoples experience the nutrition transition with changing dietary patterns and obesity. *J Nutr.* 2004;134(6):1447-1453.

3. Sheikh N, Egeland GM, Johnson-Down L, Kuhnlein HV. Changing dietary patterns and body mass index over time in Canadian Inuit communities. *Int J Circumpolar Health.* 2011;70(5):511-519.

4. Blanchet C, Dewailly E, Ayotte P, Bruneau S, Receveur O, Holub BJ. Contribution of selected traditional and market foods to the diet of Nunavik Inuit women. *Can J Diet Prac Res.* 2000;61(2):50-59.

5. Hopping BN, Mead E, Erber E, Sheehy C, Roache C, Sharma S. Dietary adequacy of Inuit in the Canadian Arctic. *J Hum Nutr Diet.* 2010;23(suppl 1):27-34.

6. Chateau-Degat ML, Dewailly E, Louchini R, et al. Cardiovascular burden and related risk factors among Nunavik (Quebec) Inuit: insights from baseline findings in the circumpolar Inuit health in transition cohort study. *Can J Cardiol.* 2010;26(6):190-196.

7. Chateau-Degat ML, Dewailly E, Charbonneau G, et al. Obesity risks: towards an emerging Inuit pattern. *Int J Circumpolar Health.* 2011;70(2):166-177.

8. Dewailly E, Chateau-Degat ML, Ekoé JM, Ladouceur R, Rochette L. *Status of Cardiovascular Disease and Diabetes in Nunavik. Nunavik Inuit Health Survey 2004, Qanuippitaa? How are we?* Québec, Institut national de santé publique du Québec (INSPQ) & Nunavik Regional Board of Health and Social Services (NRBHSS); 2007.

9. Ayach BB, Korda H. Commentary: Type 2 Diabetes Epidemic in First Nations People of Canada. *Ethn Dis.* 2010;20(3):300-303.

10. Kant AK. Dietary patterns and health outcomes. *J Am Diet Assoc.* 2004;104(4):615-635.

11. Eilat-Adar S, Mete M, Nobmann ED, et al. Dietary Patterns are Linked to Cardiovascular Risk Factors but Not to Inflammatory Markers in Alaska Eskimos. *J Nutr.* 2009;139(12):2322-2328.

12. Bersamin A, Luick BR, King IB, Stern JS, Zidenberg-Cherr S. Westernizing diets influence fat intake, red blood cell fatty acid composition, and health in remote Alaskan Native communities in the center for Alaska Native health study. *J Am Diet Assoc.* 2008;108(2):266-273.

13. Gittelsohn J, Wolever TMS, Harris SB, Harris-Giraldo R, Hanley AJG, Zinman B. Specific patterns of food consumption and preparation are associated with diabetes and obesity in a native Canadian community. *J Nutr.* 1998;128(3):541-547.

14. Munch-Andersen T, Olsen DB, Søndergaard H, et al. Metabolic profile in two physically active Inuit groups consuming either a western or a traditional Inuit diet. *Int J Circumpolar Health.* 2012;71:17342. doi: 10.13402/ijch.v7li0.17342.

15. Dewailly E, Blanchet C, Lemieux S, et al. n-3 Fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik. *Am J Clin Nutr.* 2001;74(4):464-473.

16. Counil E, Julien P, Lamarche B, Chateau-Degat ML, Ferland A, Dewailly E. Association between trans-fatty acids in erythrocytes and pro-atherogenic lipid profiles among Canadian Inuit of Nunavik: possible influences of sex and age. *Br J Nutr.* 2009;102(5):766-776.

17. Ferland A, Lamarche B, Chateau-Degat ML, et al. Dairy product intake and its association with body weight and cardiovascular disease risk factors in a population in dietary transition. *J Am Coll Nutr.* 2011;30(2):92-99.

18. Dewailly E, Blanchet C, Gingras S, Lemieux S, Holub BJ. Fish consumption and blood lipids in three ethnic groups of Quebec (Canada). *Lipids.* 2003;38(4):359-365.

19. Rochette L, Blanchet C. *Methodological Report. Nunavik Inuit Health Survey 2004, Qanuippitaa? How are we?* Québec, Institut national de santé publique du Québec (INSPQ) & Nunavik Regional Board of Health and Social Services (NRBHSS); 2007.

20. Pirro M, Bergeron J, Dagenais GR, 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(20):2474-2480.

21. Labonte ME, Dewailly E, Chateau-Degat ML, Couture P, Lamarche B. Population-based study of high plasma C-reactive protein concentrations among the Inuit of Nunavik. *Int J Circumpolar Health.* 2012;71:19066. doi: 10.3402/ijch.v71i0.19066.

22. Lucas M, Proust F, Blanchet C, et al. Is marine mammal fat or fish intake most strongly associated with omega-3 blood levels among the Nunavik Inuit? *Prostaglandins Leukot Essent Fatty Acids.* 2010;83(3):143-150.

23. Bjerregaard P, Jeppesen C. Inuit dietary patterns in modern Greenland. *Int J Circumpolar Health.* 2010;69(1):13-24.

24. Jolliffe IT. *Principal Component Analysis, 2nd ed.* New York (NY): Springer-Verlag; 2002.

25. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A Prospective-Study of Cholesterol, Apolipoproteins, and the Risk of Myocardial-Infarction. *N Engl J Med.* 1991;325(6):373-381.

26. Lewington S, Whitlock G, Clarke R, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet.* 2007;370(9602):1829-1839.

27. Lamarche B, Tchernof A, Mauriege P, et al. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *JAMA.*1998;279(24):1955-1961.

28. Belanger MC, Dewailly E, Berthiaume L, et al. Dietary contaminants and oxidative stress in Inuit of Nunavik. *Metabolism.* 2006;55(8):989-995.

29. 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.

30. Jacobson TA, Glickstein SB, Rowe JD, Soni PN. Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review. *J Clin Lipidol.* 2012;6(1):5-18.

31. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *Am J Clin Nutr.* 2007;86(1):74-81.

32. Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. *Lipids.* 1997;32(7):697-705.

33. 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(10):2012-2022.

34. Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr.* 2004;79(6):969-973.

35. Willet WC. *Nutritional Epidemiology. 2nd ed.* New York: Oxford University Press; 1998.

36. Blanchet C, Rochette L. *Nutrition and Food Consumption among the Inuit of Nunavik. Nunavik Inuit Health Survey 2004, Qanuippitaa? How are we?* Québec, Institut national de santé publique du Québec (INSPQ) & Nunavik Regional Board of Health and Social Services (NRBHSS); 2008.

37. Newby PK, Tucker KL. Empirically derived eating patterns using factor or cluster analysis: a review. *Nutr Rev.* 2004;62(5):177-203.

TABLES

Table 1. Characteristics of study participants from the 2004 Nunavik Inuit Health Survey[a,b]

Characteristics	Total n[c]	
Males (%)	666	44.6
Age (years)	666	36.4 ± 13.6
Weight (kg)	648	70.0 ± 15.9
BMI (kg/m^2)	648	27.5 ± 5.7
% Body fat	647	27.3 ± 10.4
Waist circumference (cm)	657	91.6 ± 13.6
Cholesterol (mg/dL)[d]		
Total-C	657	193.0 ± 38.9
LDL-C	657	108.2 ± 34.0
HDL-C	657	63.6 ± 17.9
Total-C/HDL-C ratio	657	3.1 (3.0-3.2)[e]
Triglycerides (mg/dL)[d]	657	93.4 (89.9-97.0)[e]
Apolipoproteins		
ApoB100 (mg/dL)[d]	659	95.0 ± 24.4
ApoAI (mg/dL)[d]	659	169.9 ± 29.8
LDL peak particle diameter (Å)	659	255.8 ± 2.7
Oxidized-LDL (U/L)	642	51.6 ± 15.4
Blood pressure (mmHg)		
Systolic	661	117.7 ± 14.3
Diastolic	661	73.8 ± 9.5
Inflammatory markers		
Hs-CRP (mg/L)	658	1.2 (1.1-1.3)[e]
IL-6 (pg/mL)	642	1.8 (1.7-1.9)[e]
TNF-α (pg/mL)	642	1.9 (1.8-2.0)[e]
Fasting glucose (mg/dL)[d]	657	80.6 (79.5-81.7)[e]
Fasting insulin (μIU/mL)[d]	655	7.4 (7.1-7.8)[e]
Physical activity (≥ 3.5 h/wk, %)[f]	636	45.4
Smoking (current, %)	666	74.5
Drinking (≥ 1 drink/day, %)	535	26
Education level (≥ high school, %)	666	27.2
Diabetes (yes, %)	658	5.3

[a] Apo = apolipoprotein; BMI = body mass index; C = cholesterol; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; IL-6 = interleukin 6; LDL = low density lipoprotein; TNF-α = tumor necrosis factor-alpha.

[b] Values are means ± SD unless stated otherwise.

[c] Total number of participants in the study sample for whom data was available.

[d] To convert cholesterol to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113; to convert apolipoproteins to g/L, multiply by 0.01; to convert fasting glucose to mmol/L, multiply by 0.0555 and to convert fasting insulin to pmol/L, multiply by 6.945.

[e] Values are geometric means (95% confidence intervals).

[f] Physical activity ≥ 3.5 h/week for at least one season in the previous year.

Table 2. Factor-loading matrix for four dietary patterns derived from principal component analysis among Inuit of the 2004 Nunavik Health Survey[a]

	Dietary patterns			
Food groups	Traditional (factor 1)	Western (factor 2)	Nutrient-poor food (factor 3)	Healthy (factor 4)
Fish and seafood	**0.69**	—	—	0.16
Land animals meat and organ	**0.68**	—	0.15	—
Game birds meat	**0.68**	—	—	—
Marine mammal meat	**0.67**	—	—	—
Marine mammal fat	**0.58**	—	-0.17	—
Wild berries	**0.55**	—	—	—
Eggs from game birds	**0.55**	—	—	—
Potatoes	—	**0.70**	—	0.22
Store-bought red meat	—	**0.58**	**0.31**	0.24
Vegetables	—	**0.55**	—	**0.50**
Processed meat	—	**0.54**	0.22	—
Poultry	—	**0.52**	—	—
Chicken eggs	—	**0.51**	—	-0.16
Refined grain products	—	**0.37**	**0.34**	0.24
Potato chips	—	—	**0.70**	—
Carbonated beverages	—	—	**0.70**	—
Fried food	—	**0.41**	**0.51**	0.22
Fruit juice	—	—	0.24	**0.63**
Fruits	0.15	**0.31**	—	**0.56**
Dairy products	—	—	—	**0.56**
Whole grain products	—	—	**-0.42**	**0.43**
Hot beverages	—	—	—	—
Sweets and desserts	—	—	**0.35**	0.27
Bannock	0.24	—	**-0.33**	—
Flavored drinks	—	—	0.29	—
Legumes and nuts	0.15	0.15	-0.16	**0.37**
Spread fat	—	0.18	—	0.16

[a] Absolute values < 0.15 were omitted from the table for simplicity. Food groups with absolute factor loadings ≥ 0.30 (in bold) were considered as significantly contributing to the pattern.

Table 3. Dietary patterns associations with cardiovascular disease risk factors among Inuit of the 2004 Nunavik Health Survey (n = 666)[a,b]

	Traditional			Western			Nutrient-poor food			Healthy		
Biomarkers	**Q1**	**Q4**	***P* trend**	**Q1**	**Q4**	***P* trend**	**Q1**	**Q4**	***P* trend**	**Q1**	**Q4**	***P* trend**
Total-C (mg/dL)[c,d,e]	158.6±1.0	168.4±1.0	0.003	166.6±1.0	168.4±1.0	0.65	166.9±1.0	162.5±1.0	0.58	163.9±1.0	163.7±1.0	0.67
LDL-C (mg/dL)[c,d,f]	78.4±1.0	86.8±1.0	0.001	85.2±1.0	85.0±1.0	0.87	83.1±1.0	83.1±1.0	0.60	82.5±1.0	81.9±1.0	0.74
HDL-C (mg/dL)[c]	62.0±2.3	65.2±2.3	0.11	63.7±2.3	64.1±2.4	0.82	63.6±2.3	62.4±2.3	0.38	63.9±2.3	61.6±2.3	0.07
Total-C/HDL-C ratio[d]	2.96±1.03	2.98±1.03	0.57	3.05±1.04	3.04±1.03	0.78	2.99±1.03	3.07±1.04	0.18	2.96±1.04	3.03±1.03	0.42
Triglycerides (mg/dL)[c,d,g,h]	88.1±1.0	82.2±1.0	0.58	85.4±1.0	90.3±1.0	0.52	88.3±1.0	86.6±1.0	0.97	89.5±1.1	94.0±1.1	0.50
ApoB100 (mg/dL)[c,d]	80.1±1.0	85.3±1.0	0.02	85.3±1.0	85.7±1.0	0.99	84.4±1.0	83.5±1.0	0.86	83.2±1.0	84.4±1.0	0.88
ApoAI (mg/dL)[c,d]	168.7±1.0	173.2±1.0	0.14	169.0±1.0	171.3±1.0	0.49	172.3±1.0	167.3±1.0	0.14	169.6±1.0	170.3±1.0	0.56
LDL peak particle diameter (Å)	255.1±0.3	255.6±0.3	0.04	254.8±0.3	255.4±0.3	0.08	255.2±0.3	255.0±0.3	0.39	255.3±0.3	255.0±0.3	0.19
Oxidized-LDL (U/L)[d]	44.2±1.0	48.0±1.0	0.02	46.8±1.0	48.2±1.0	0.57	45.3±1.0	48.5±1.0	0.04	45.8±1.0	46.0±1.0	0.96
Hs-CRP (mg/L)[d,i]	1.38±1.10	1.18±1.10	0.19	1.12±1.10	1.15±1.10	0.60	1.58±1.10	0.94±1.10	<0.0001	1.25±1.10	1.22±1.10	0.91
IL-6 (pg/mL)[d]	1.84±1.06	1.78±1.06	0.74	1.83±1.06	1.73±1.06	0.26	1.81±1.07	1.81±1.06	0.93	1.78±1.06	1.85±1.06	0.55
TNF-α (pg/mL)[d]	1.76±1.04	1.74±1.05	0.86	1.98±1.05	1.92±1.05	0.28	1.84±1.05	1.89±1.05	0.89	1.93±1.04	1.94±1.05	0.76

[a] A dietary pattern score was computed for each participant for each of the four dietary patterns derived from principal component analysis. Within each dietary pattern, participants were classified into quartiles based on their individual score. Associations were assessed using the general linear model (GLM) procedure. Means ± SEM are each adjusted for one or more of the following potential confounders: sex (male/female), age (continuous), waist circumference (continuous), physical activity (< or ≥ 3.5 h/week for at least one season in the previous year), smoking status (non, ex-, or current smokers), drinking habits (never, light, moderate, or heavy drinkers), education level (less, equal to, or more than completed high school), diabetes (yes/no), and use of lipid-lowering medication

(yes/no). Covariates differed between biomarkers since they were kept in the final model when they were associated at $P \leq 0.10$ with the dependent variable. Thus, total-C and LDL-C are adjusted for age, waist circumference, diabetes, education level and lipid-lowering medication in all four dietary patterns. Total-C is also further adjusted for sex and drinking habits (except for the healthy pattern, in which total-C is not further adjusted for sex). HDL-C and total-C/HDL-C ratio are adjusted for sex and waist circumference in all four dietary patterns. HDL-C is also adjusted for age, smoking status, drinking habits and lipid-lowering medication, while total-C/HDL-C ratio is further adjusted for diabetes and education level. TG are adjusted for waist circumference and smoking status in all four dietary patterns and are further adjusted for lipid-lowering medication in the healthy pattern. ApoB100 is adjusted for age, waist circumference, drinking habits, diabetes and education level (except for the nutrient-poor food and healthy patterns, in which apoB100 is not adjusted for drinking habits). ApoAI is adjusted for sex, age, waist circumference and smoking status in all four dietary patterns. LDL-peak particle diameter is adjusted for sex and lipid-lowering medication in all four patterns and is further adjusted for waist circumference in the nutrient-poor food pattern. Oxidized-LDL are adjusted for age, waist circumference, drinking habits, diabetes and education level (except for the nutrient-poor food pattern, in which oxidized-LDL are not adjusted for drinking habits). Hs-CRP is adjusted for age and waist circumference (except for the nutrient-poor food pattern, in which hs-CRP is not adjusted for age). IL-6 is adjusted for age, sex and waist circumference in all four patterns. TNF-α is adjusted for age only in the traditional and nutrient-poor food patterns and for age and sex in the Western and healthy patterns.

[b] Apo = apolipoprotein; C = cholesterol; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; IL-6 = interleukin 6; LDL = low density lipoprotein; TNF-α = tumor necrosis factor-alpha.

[c] To convert cholesterol to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113 and to convert apolipoproteins to g/L, multiply by 0.01.

[d] Variables were log-transformed prior to analysis. Geometric means ± SEM are shown for these variables.

[e] In the traditional pattern, total-C concentrations differed between quartiles ($P = 0.007$). More specifically, total-C concentrations were significantly lower in Q1 compared with Q3 (169.6 ± 1.0) and Q4 (shown above), as determined by the Tukey adjustment within the GLM procedure ($P < 0.05$).

[f] In the traditional pattern, LDL-C concentrations differed between quartiles ($P = 0.009$). More specifically, LDL-C concentrations were significantly lower in Q1 compared with Q3 (85.5 ± 1.0) and Q4 (shown above), as determined by the Tukey adjustment within the GLM procedure ($P < 0.05$).

[g] Inuit who had not been fasting for at least 8 h prior to blood sample collection ($n = 89$) were excluded from analyses for triglycerides.

[h] In the traditional pattern, TG concentrations differed between quartiles ($P = 0.01$). More specifically, TG concentrations were significantly higher in Q3 (97.4 ± 1.0) compared with Q2 (86.9 ± 1.0) and Q4 (shown above), as determined by the Tukey adjustment within the GLM procedure ($P < 0.05$).

[i] In the nutrient-poor food pattern, hs-CRP concentrations differed between quartiles ($P = 0.0009$). More specifically, hs-CRP concentrations were significantly higher in Q1 (shown above) compared with Q2 (1.34 ± 1.10), Q3 (1.11 ± 1.10) and Q4 (shown above), as determined by the Tukey adjustment within the GLM procedure ($P < 0.05$). Hs-CRP concentrations were also significantly higher in Q2 compared with Q4 ($P < 0.05$).

SUPPLEMENTARY FILES

Supplementary File 1. Reasons for non-response to the FFQ in the Nunavik Inuit Health Survey 2004 among the 891 individuals who signed the consent form[a]

Food Frequency Questionnaire	
Reason for non-response	**No. of subjects**
No time, too late[b]	30
Inadequate interview[c]	19
Refusal[d]	15
Did not show up[e]	15
Tired[f]	11
Day 2 run-in period[g]	11
Handicapped[h]	7
Unspecified[i]	6
Home visit[j]	5
Confused[k]	1
Erroneous age[l]	1
Was forgotten[m]	1
Total	122

[a] Among 677 households visited by the research team (number decided prior to the survey), 521 households agreed to participate, giving a response rate of 77.8%. The 521 households represented 1294 eligible individuals (men and non-pregnant women aged between 18 and 74 years), among whom 891 individuals signed the consent form and 769 of them also completed a food frequency questionnaire (FFQ). Reasons for non-response to the FFQ are provided in the Table and are adapted from pages 193-194, 196 of reference 19. We further excluded non-Inuit individuals (n = 17), and individuals who had missing data for at least one of the 27 food groups created for the identification of dietary patterns, as factor scores could not be determined for those participants (n = 86). The final study sample for the present work thus included 666 Inuit adults.

[b] It was too late and the ship had to leave for another village. The staff did not have time to complete the test or the questionnaire.

[c] The dietary questionnaires of some participants were rejected due to a lack of information. Some participants struggled to recall or figure out their food consumption with exactness.

[d] The participant consented to some tests or questionnaires but refused a specific survey instrument.

[e] The participant had signed a consent form at home but did not go to the ship for data collection.

[f] The participant was too tired to continue.

[g] At the end of the first day of data collection (August 31st, 2004), it was decided to temporarily alleviate the workload of nurses and interviewers to allow better integration of all activities. Hence, on the second day, the food frequency test was only given in the afternoon.

[h] The participant was not surveyed because of a physical or a mental handicap.

[i] Reason was not recorded by the nurse or the interviewer.

[j] The participant could not come aboard the ship and had to be seen at home. Most of the time this situation concerned elderly people and participants with physical restrictions. Hence, a maximum of testing and questionnaire completion was conducted, but it was sometimes impossible to complete each survey instrument due to a lack of time and the logistics.

[k] The participant's answers were confusing and not considered valid.

[l] One participant was considered to be 17 years old whereas the date of birth showed that the participant was actually 18. None of the clinical tests or nutrition questionnaires was administered.

[m] The participant had to go through a large number of tests and questionnaires in a short period of time. On a few occasions, some questionnaires or tests were forgotten and not administered.

Supplementary File 2. Food groupings used in the dietary pattern analyses among Inuit participants in the 2004 Nunavik Health Survey

Food groups	Food Items
Marine mammal meat and organ	Beluga meat (fresh, cooked or frozen), dried beluga, beluga muktuk, seal meat (fresh, cooked or frozen, without blubber), walrus meat, parts of beluga, seal or walrus (liver, kidney, etc.), igunak (seal)
Marine mammal fat	Beluga or seal misirak/blubber
Fish and seafood	Arctic char, cod, white fish, trout, salmon, other fish (pike, cisco, walleye, etc.), dried fish, clams, molluscs, mussels, oysters, scallops, urchin (all fresh, frozen or cooked), canned fish (salmon, tuna)
Land animals meat and organ	Caribou meat (fresh, cooked or frozen), dried caribou meat, caribou liver, kidney and other parts, other game animals (bear, fox, hare; fresh, cooked or frozen)
Game birds meat	Ptarmigan, partridge, goose, other birds (pintail, scoter, merganser)
Eggs from game birds	Game birds eggs
Wild berries	Wild berries
Store-bought red meat	Beef (steak, ground, canned, stewed or corned), pork, other meat (lamb, veal, etc.)
Processed meat	Luncheon or sliced meats (ham, salami, bologna, etc.), bacon, sausages, wieners
Poultry	Chicken/turkey (breast, legs)
Chicken eggs	Chicken eggs
Fruits (excluding juice)	Apples, pears, bananas, oranges, grapefruit, other fresh fruits, canned fruit
Fruit juice	100% fruit juice (canned or frozen)
Flavored drinks	Tang[a], fruit punch, Kool-Aid[a], Sunny Delight[b], Gatorade[c]
Vegetables	Carrots, turnips, broccoli, cauliflower, cabbage, tomatoes (fresh or canned), mixed vegetables (fresh, canned or frozen), other vegetables (peas, corn, etc.)
Potatoes	Potatoes (fresh)
Dairy products	Milk (whole, 3.25, 2% or skim including evaporated milk) as drink, in cereals, in coffee or in tea, yogurt, cheese (slice, cheddar, mozzarella, processed cheese products)
Refined grain products	White bread, cold cereals (cornflakes, etc.), rice, macaroni, spaghetti, macaroni and cheese dinner
Whole grain products	Whole wheat bread, hot cereals (oatmeal, etc.)
Bannock	Bannock
Legumes and nuts	Beans, peas, chickpeas, peanut butter, nuts, seeds
Sweets and desserts	Ice cream, cakes, doughnuts, pies, cookies, syrup, jam, honey, marmalade, candy bars, candies, white sugar in tea or coffee
Carbonated beverages	Regular or diet soda beverages

Potato chips	Potato chips
Fried food	Fried chicken or nuggets, French fries
Spread fat	Butter or margarine on bread
Hot beverages	Tea, herbal tea, coffee

[a] Kraft Foods Group.

[b] Sunny Delight Beverages Co.

[c] PepsiCo.

Supplementary File 3. Dietary patterns associations with socio-demographic, lifestyle, and anthropometric characteristics among Inuit of the 2004 Nunavik Health Survey (n = 666)[a]

	Traditional			Western			Nutrient-poor food			Healthy		
Characteristics	**Q1**	**Q4**	***P* trend[b]**	**Q1**	**Q4**	***P* trend[b]**	**Q1**	**Q4**	***P* trend[b]**	**Q1**	**Q4**	***P* trend[b]**
Age (years)[c]	34.4 ± 1.0	39.6 ± 1.0	0.0003	36.4 ± 1.1	34.6 ± 1.1	0.27	49.5 ± 0.8	27.0 ± 0.8	<0.0001	37.9 ± 1.1	35.1 ± 1.1	0.04
Waist circumference (cm)[d]	91.4 ± 1.1	92.4 ± 1.1	0.23	91.4 ± 1.1	91.6 ± 1.1	0.84	96.3 ± 1.1	88.5 ± 1.0	<0.0001	91.6 ± 1.1	93.2 ± 1.1	0.12
Sex (men)	67 (40.1)	86 (51.8)	0.02	74 (44.3)	88 (53)	0.37	74 (44.6)	75 (44.9)	0.55	85 (50.9)	69 (41.6)	0.08
Physical activity (≥ 3.5 h/wk)[e]	66 (41.3)	80 (50.3)	0.14	65 (40.9)	85 (53.5)	0.08	71 (44.9)	82 (50.9)	0.35	57 (36.3)	93 (57.4)	<0.0001
Current smoking	136(81.4)	118 (71.1)	0.04	129 (77.3)	126 (75.9)	0.99	91 (54.8)	145 (86.8)	<0.0001	131 (78.4)	120 (72.3)	0.06
Drinking status (≥ 1 drink/d)	44 (31)	28 (21.5)	0.22	26 (19.7)	37 (27.8)	0.03	18 (15.9)	47 (31.5)	0.01	33 (25.6)	36 (26.3)	0.99
Education level (≥ high school)	33 (19.8)	48 (28.9)	0.08	42 (25.2)	54 (32.5)	0.24	40 (24.1)	44 (26.4)	0.96	33 (19.8)	46 (27.7)	0.05
Diabetes (yes)	10 (6.1)	11 (6.6)	0.53	8 (4.9)	6 (3.7)	0.92	27 (16.4)	0 (0)	<0.0001	6 (3.6)	12 (7.4)	0.22

[a] A dietary pattern score was computed for each participant for each of the four dietary patterns derived from principal component analysis. Within each dietary pattern, participants were classified into quartiles based on their individual score. Associations were assessed using the general linear model (GLM) procedure for continuous variables and the Cochran-Armitage trend test for categorical variables. Unadjusted means ± SEM are presented for age and waist circumference. Number of individuals and percent values (in parentheses) are shown for categorical variables. Percentages are unadjusted for other variables.

[b] *P* values for categorical variables were obtained using the Cochran-Armitage trend test.

[c] In the traditional and nutrient-poor food patterns, age differed between quartiles ($P = 0.003$ and < 0.0001, respectively). In the traditional pattern, age was significantly higher in Q4 (shown above) compared with Q1 (shown above) and Q2 (35.1 ± 1.0), as

determined by the Tukey adjustment within the GLM procedure ($P < 0.05$). In the nutrient-poor food pattern, age was significantly higher in Q1 (shown above) compared with Q2 (36.5 ± 0.8), Q3 (32.7 ± 0.8) and Q4 (shown above) ($P < 0.05$). Age was also significantly higher in Q2 compared with Q3 and Q4 and in Q3 compared with Q4 ($P < 0.05$).

[d] In the nutrient-poor food pattern, waist circumference differed between quartiles ($P < 0.0001$). More specifically, waist circumference was significantly higher in Q1 (shown above) compared with Q2 (91.2 ± 1.0), Q3 (90.6 ± 1.0) and Q4 (shown above), as determined by the Tukey adjustment within the GLM procedure ($P < 0.05$).

[e] Physical activity ≥ 3.5 h/week for at least one season in the previous year.

CHAPITRE 6 : ÉTUDE DES ASSOCIATIONS ENTRE L'ALIMENTATION ET L'INFLAMMATION CHEZ LES NATIONS AUTOCHTONES DU NORD-DU-QUÉBEC – CONCLUSION

Ce premier volet de mon projet de doctorat visait à évaluer, pour la toute première fois, les associations entre l'alimentation et les concentrations sanguines des biomarqueurs inflammatoires chez deux nations autochtones du Nord-du-Québec. Le contexte de l'importante transition nutritionnelle vécue récemment par ces populations m'amenait à croire de prime abord que la qualité de leur alimentation puisse être grandement et significativement associée à leur profil inflammatoire. L'étude chez les Cris de la Baie-James a permis d'observer, dans une population dont le statut en oméga-3 est modérément élevé, que les concentrations érythrocytaires d'acide docosapentaénoïque (DPAn-3 ; « *docosapentaenoic acid* » ; C22:5 n-3) sont associées de façon favorable au profil inflammatoire. D'un point de vue nutritionnel, il faut toutefois retenir que le DPAn-3 corrèle moins bien avec les apports alimentaires en produits marins que l'EPA ou le DHA. Chez les Inuits du Nunavik, contre toute attente, un profil alimentaire « À faible valeur nutritive » caractérisé principalement par la consommation d'aliments camelote était inversement associé aux concentrations de la hs-CRP. Mis à part ces résultats très spécifiques observés dans chacune des deux nations autochtones à l'étude, force a été de constater, de façon globale, qu'il y a absence d'associations convaincantes entre différents facteurs nutritionnels et le profil inflammatoire des Cris de la Baie-James et des Inuits du Nunavik. Nos hypothèses ne peuvent donc pas être confirmées.

Un point sur lequel je désire mettre l'accent suite à la réalisation de ces travaux dans un cadre épidémiologique est que certaines caractéristiques des participants (ex : âge, tour de taille) peuvent représenter d'importants facteurs confondants dans l'étude des associations entre l'alimentation et le profil inflammatoire d'une population. Mes travaux ont en effet permis de constater que de tels facteurs confondants peuvent possiblement masquer et même dénaturer les associations attendues entre l'alimentation et l'inflammation. De plus, il est relativement évident que certains de ces facteurs confondants sont en fait d'importants déterminants du profil inflammatoire des populations étudiées. Il apparaît donc important

de prendre en considération ces facteurs confondants/déterminants dans les études futures. Cette prise en considération ne signifie pas seulement d'ajuster les analyses pour ces facteurs confondants/déterminants. Je crois plutôt qu'il faudrait prévoir *a priori* la réalisation d'analyses en fonction de groupes déterminés sur la base des facteurs confondants/déterminants du profil inflammatoire identifiés dans les présents travaux. Un exemple plutôt simple pourrait être, chez les Inuits, d'identifier des profils alimentaires séparément chez les sujets jeunes et chez les sujets plus âgés, puis d'évaluer les associations entre les profils identifiés et l'inflammation. Cela pourrait permettre de vérifier, dans chacun des groupes d'âges, s'il y a bel et bien présence ou non d'une association inverse entre l'adhésion à un profil alimentaire de type « À faible valeur nutritive » (advenant le cas où un tel profil est identifié) et la hs-CRP. La planification d'analyses en fonction de différents groupes de sujets permettrait également de vérifier comment l'alimentation est associée à l'inflammation en mettant l'accent sur les sous-groupes les plus à risque d'inflammation chronique de faible intensité, c'est-à-dire sur les sujets qui pourraient le plus bénéficier de modifications alimentaires dans le but d'améliorer leur statut inflammatoire. Dans cet ordre d'idées, Makhoul *et al.* (190) ont obtenu des résultats fort intéressants dans une étude chez les Esquimaux Yup'ik de l'Alaska. Ils ont démontré que la forte association positive entre l'obésité et les concentrations de la hs-CRP observée chez l'ensemble des Yup'ik était substantiellement atténuée, voire presque nulle, chez les individus avec un IMC au-dessus de 28 kg/m^2 présentant des concentrations érythrocytaires élevées d'EPA et de DHA (90^e percentile de la population). Makhoul *et al.* (190) suggèrent donc que des apports élevés en EPA et DHA pourraient en quelque sorte « protéger » les individus souffrant d'embonpoint ou d'obésité contre l'inflammation systémique de faible intensité.

Par ailleurs, je crois que le développement et l'utilisation d'outils d'évaluation alimentaire complets et valides représente un aspect important à considérer dans les futures études chez les nations autochtones. Bien que je reconnaisse qu'apparemment tout ait été fait en sorte que les outils d'évaluation alimentaire (questionnaires de fréquence) utilisés chez les Cris de la Baie-James et les Inuits du Nunavik soient adaptés autant que possible aux populations étudiées, une validation formelle de ces outils n'a pas été réalisée. En tant que personne qui n'a jamais mis les pieds en terrain autochtone, je ne peux pas vraiment me

prononcer sur la faisabilité d'une telle validation chez ces nations. Toutefois, l'utilisation d'outils validés de façon formelle pourrait permettre de recueillir des données alimentaires plus complètes (je pense ici aux aliments du commerce chez les Cris de la Baie-James, dont les données limitées ont fait en sorte que nous n'avons pas procédé à l'analyse en composantes principales) et/ou de réduire les erreurs de mesure et biais attribuables à l'utilisation de données alimentaires auto-rapportées. De plus, considérant que ces populations semblent peu enclines à porter attention aux détails des aliments qu'elles consomment (voir la discussion du chapitre 5), le fait de mesurer certains biomarqueurs nutritionnels comme nous l'avons fait chez les Cris pourrait s'avérer une avenue particulièrement intéressante pour complémenter la cueillette de données alimentaires auto-rapportées. Considérer à la fois des données nutritionnelles subjectives (questionnaire alimentaire) et objectives (biomarqueurs) dans une même étude pourrait permettre de vérifier si un même facteur nutritionnel est associé de façon similaire au profil inflammatoire dépendamment de la manière dont il est mesuré.

Ensuite, il faut souligner que les biomarqueurs inflammatoires qui ont été évalués autant chez les Cris que chez les Inuits étaient spécifiquement des biomarqueurs pro-inflammatoires (hs-CRP, IL-6, TNF-α). Considérant que le profil inflammatoire est en réalité une balance entre des biomarqueurs pro- et anti-inflammatoires, les futures études portant sur le sujet de l'alimentation et de l'inflammation dans les populations autochtones du Nord-du-Québec auront sans doute avantage à inclure l'évaluation de biomarqueurs anti-inflammatoires, dont l'adiponectine.

Au risque de me répéter, il est important de se rappeler que les deux études présentées aux chapitres précédents sont de nature transversale et représentent le tout premier portrait, à un moment donné, des associations entre l'alimentation et le profil inflammatoire des nations autochtones du Nord-du-Québec. Notre équipe de recherche était également la première, à notre connaissance, à avoir évalué la prévalence de l'inflammation chronique de faible intensité chez ces nations autochtones. De plus amples investigations, notamment de nature prospective longitudinale, sont requises afin de suivre l'évolution du statut inflammatoire de ces populations et d'évaluer à quel point leur alimentation dans le présent peut influencer leur risque futur d'inflammation chronique de faible intensité. Le défi des études

futures sera donc d'arriver à déterminer si le rôle de l'alimentation sur le profil inflammatoire des nations autochtones du Nord-du-Québec est bel et bien mineur ou bien si ce rôle est simplement masqué par certains facteurs confondants ou certaines limites méthodologiques. Ces investigations seront nécessaires avant qu'on puisse, éventuellement, arriver à établir des recommandations alimentaires visant la réduction de l'inflammation chronique de faible intensité chez les populations autochtones du Nord-du-Québec, notamment chez les Cris chez qui la prévalence d'un phénotype pro-inflammatoire est très élevée.

CHAPITRE 7 : ÉTUDE DE L'IMPACT DES PRODUITS LAITIERS SUR L'INFLAMMATION – PROBLÉMATIQUE

Plusieurs lignes directrices officielles en matière de nutrition (« Bien manger avec le Guide alimentaire canadien », « *MyPlate* » aux États-Unis, « La santé vient en mangeant – Le guide alimentaire pour tous » en France, etc.) considèrent que les produits laitiers font partie intégrante d'une alimentation saine. À l'inverse, une recherche bien simple sur le Web en utilisant un moteur de recherche « grand public » nous permet rapidement de constater que les régimes anti-inflammatoires populaires préconisent de limiter, voire d'éliminer la consommation de produits laitiers. Mythe ou réalité? C'est particulièrement dans le but de clarifier ce sujet controversé qu'une partie de mon projet de doctorat a porté sur l'étude des liens entre la consommation de produits laitiers et l'inflammation.

Avant d'aller plus loin, je tiens à préciser que le terme « produits laitiers » désigne ici le lait, le yogourt et le fromage de source bovine. Je n'aborderai donc pas les effets sur l'inflammation d'autres produits laitiers animaux (ex : lait de chèvre) ou bien de substituts végétaux (ex : boissons de soya, boissons d'amandes).

7.1 Produits laitiers et inflammation : état des connaissances

7.1.1 Études de cultures cellulaires avec des bactéries lactiques

Des études de cultures cellulaires réalisées dans les années 1990 ont montré une augmentation de la production de diverses cytokines pro-inflammatoires par des cellules mononucléaires du sang périphérique (PBMC, « *peripheral blood mononuclear cells* ») suite à leur incubation avec des bactéries lactiques contenues entre autres dans le yogourt (196-198). Par exemple, Solis-Pereyra *et al.* (196) ont observé que l'incubation de PBMC pendant 48 heures avec le *Lactobacillus bulgaricus*, le *Streptococcus thermophilus* ou une combinaison des deux souches bactériennes avait stimulé la production de l'interféron-gamma (IFN-γ), de TNF-α et de l'IL-1β de l'ordre de 5 à 30 fois comparativement à des PBMC cultivés en l'absence de bactéries lactiques ($P < 0{,}001$). Ces données sont peut-être, du moins en partie, à l'origine de la croyance populaire comme quoi les produits laitiers seraient des aliments pro-inflammatoires. Ces observations ne sont tout de même pas

totalement surprenantes considérant que les bactéries, de manière générale, constituent un élément déclencheur du processus inflammatoire par les cellules immunitaires. Il faut aussi retenir que ces études *ex vivo* ne reflètent pas nécessairement ce qui survient réellement *in vivo* lorsque les produits laitiers sont consommés en tant qu'aliments entiers. Alors, jetons un coup d'œil aux études chez l'humain.

7.1.2 Études observationnelles

À ma connaissance, seulement six études observationnelles ont à ce jour évalué les associations entre la consommation de produits laitiers et le profil inflammatoire chez les adultes (**Tableau 7.1**) (199-204). Ces six études sont toutes de nature transversale et elles sont relativement récentes, ayant été publiées au cours des sept dernières années.

Une seule de ces six études s'est intéressée uniquement à la consommation totale de produits laitiers sans donner de spécifications sur le(s) type(s) et le contenu en matières grasses des produits laitiers consommés par 772 hommes et femmes à haut risque cardiovasculaire (199). Après ajustement pour diverses variables potentiellement confondantes (âge, genre, IMC, diabète, tabagisme et utilisation de statines, d'anti-inflammatoires non stéroïdiens et d'aspirine), les concentrations de la hs-CRP étaient plus faibles à travers les tertiles de consommation de produits laitiers, allant de moins de 282 g/jour dans le tertile 1 jusqu'à plus de 527 g/jour dans le tertile 3 (P pour la tendance = 0,005). Aucune association n'a été observée dans le cas de l'IL-6.

Deux des six études se sont intéressées à la fois à la consommation totale de produits laitiers, à la consommation de produits laitiers à faible teneur en matières grasses ainsi qu'à la consommation de produits laitiers plus riches en matières grasses (200, 201). Panagiotakos *et al.* (200) ont démontré, chez 3042 hommes et femmes de la région d'Attica, en Grèce, que les consommateurs de 11 à 14 portions de produits laitiers par semaine avaient des concentrations de la hs-CRP, de l'IL-6 et de TNF-α respectivement 16%, 5% et 12% plus faibles que les consommateurs de moins de 8 portions par semaine ($P < 0,05$). Chez les consommateurs de plus de 14 portions de produits laitiers par semaine, ces différences étaient respectivement de 29%, 9% et 20% ($P < 0,05$). Des analyses de régression linéaire multiple ont par ailleurs révélé que les associations inverses entre la

consommation de produits laitiers et les concentrations des trois biomarqueurs pro-inflammatoires demeuraient présentes en évaluant séparément les produits laitiers faibles en matières grasses (2% de M.G. ou moins ou bien décrits comme faibles en M.G. par le producteur ; $P \leq 0,03$ pour les trois biomarqueurs) ainsi que riches en matières grasses (plus de 2% de M.G. ; $P = 0,06$ pour la hs-CRP et $P \leq 0,05$ pour l'IL-6 et le TNF-α). Ces associations inverses étaient observées indépendamment de nombreuses variables potentiellement confondantes incluant, entre autres, l'âge, le sexe, le tabagisme, le niveau d'activité physique et l'IMC.

L'étude d'Esmaillzadeh et Azadbakht (201) chez 486 femmes d'âge mûr n'a quant à elle démontré aucune association entre la consommation totale de produits laitiers et les concentrations de biomarqueurs pro-inflammatoires incluant la hs-CRP, l'IL-6, le TNF-α et la SAA. Toutefois, en accord avec les résultats de Panagiotakos *et al.* (200), les concentrations de la hs-CRP, de l'IL-6 et de TNF-α étaient plus faibles à travers les quintiles de consommation de lait et/ou de yogourt contenant moins de 2% de matières grasses (33 g/jour, en moyenne, dans le quintile 1 jusqu'à 152 g/jour dans le quintile 5) après ajustement pour l'âge, l'IMC, le tour de taille et d'autres variables potentiellement confondantes (201). Il n'y avait aucune association entre la consommation de produits laitiers faibles en matières grasses et la SAA. Dans le cas des produits laitiers contenant au moins 2% de matières grasses (incluant la crème, le fromage à la crème et la crème glacée), aucune association n'a été observée avec les concentrations de la hs-CRP et de l'IL-6, alors qu'une association positive a été observée avec les concentrations de TNF-α et de la SAA.

Deux études se sont plutôt intéressées aux associations entre les produits laitiers faibles ou riches en matières grasses et les concentrations d'adiponectine (202, 203). Dans une cohorte de 220 femmes méditerranéennes apparemment en santé et âgées en moyenne de 48 ans, Yannakoulia *et al.* (202) ont démontré que la consommation de produits laitiers à faible teneur en matières grasses ne corrélait pas avec les concentrations d'adiponectine de haut poids moléculaire ($r = 0,07$; $P = 0,30$) ou bien d'adiponectine totale ($r = 0,11$; $P = 0,13$) après ajustement pour l'âge, le pourcentage de masse adipeuse, le tour de taille, le tabagisme, l'activité physique, le statut ménopausal et le fait d'avoir déclaré un faible apport énergétique. Toutefois, une seconde étude de Yannakoulia *et al.* (203) réalisée dans

cette même cohorte a démontré que l'adhésion à un profil alimentaire caractérisé par des apports élevés en produits laitiers faibles en matières grasses et en produits céréaliers à grains entiers était positivement associée aux concentrations plasmatiques d'adiponectine (β normalisé = 0,18 ; P = 0,03) indépendamment de l'âge, du tour de taille et du tabagisme. En ce qui concerne les produits laitiers riches en matières grasses, les deux études de Yannakoulia *et al.* (202, 203) ont démontré que la consommation de ce type de produits laitiers à proprement parler ou encore que l'adhésion à un profil alimentaire caractérisé par des apports élevés en ces produits laitiers n'étaient pas associées aux concentrations d'adiponectine.

Enfin, la plus récente des six études s'est uniquement intéressée à la consommation de fromage, considéré comme un produit laitier riche en matières grasses, chez 1752 hommes et femmes iraniens (204). Il a été observé que les individus consommant du fromage 7 fois ou plus par semaine avaient des concentrations de la hs-CRP environ 9% plus élevées que les individus consommant du fromage moins de 7 fois par semaine. Toutefois, l'analyse réalisée n'était pas ajustée pour diverses variables potentiellement confondantes et la hs-CRP était le seul biomarqueur inflammatoire évalué, ce qui limite la portée des résultats.

En somme, les quelques études transversales réalisées jusqu'à maintenant nous indiquent que la consommation totale de produits laitiers ainsi que la consommation de produits laitiers à faible teneur en matières grasses ne semblent pas associées à une détérioration du profil inflammatoire chez les adultes. La consommation de produits laitiers à faible teneur en matières grasses semble même inversement associée à l'inflammation. Dans le cas des produits laitiers riches en matières grasses, les résultats sont plutôt flous, car des associations allant dans toutes les directions ont été observées à travers cinq études ayant spécifiquement abordé la question (200-204).

Tableau 7.1 : Études transversales ayant évalué les associations entre la consommation de produits laitiers et l'inflammation chez les adultes [a]

Premier auteur, année	Sexe	Âge (années)	Produits laitiers évalués	Association entre les produits laitiers et l'inflammation
Salas-Salvado, 2008	F / M	55-80	Consommation totale (type de produit / contenu en M.G. non spécifiés)	↓ hs-CRP ≠ IL-6
Panagiotakos, 2010	F / M	18-89	**i**) Consommation totale	↓ hs-CRP, IL-6, TNF-α
			ii) À faible teneur en M.G. (≤ 2% ou décrits comme faibles en M.G. par le producteur)	↓ hs-CRP, IL-6, TNF-α
			iii) Riches en M.G. (> 2%)	↓ hs-CRP (tendance), IL-6, TNF-α
Esmaillzadeh, 2010	F	40-60	**i**) Consommation totale	≠ hs-CRP, IL-6, TNF-α, SAA
			ii) À faible teneur en M.G. (lait et/ou yogourt < 2% M.G.)	↓ hs-CRP, IL-6, TNF-α ≠ SAA
			iii) Riches en M.G. (≥ 2%, incluant crème, fromage à la crème et crème glacée)	≠ hs-CRP, IL-6 ↑ TNF-α, SAA
Yannakoulia, 2008 (*Eur J Endocrinol*)	F	18-84	**i**) À faible teneur en M.G.	≠ adiponectine
			ii) Riches en M.G.	≠ adiponectine
Yannakoulia, 2008 (*Metabolism*)	F	18-84	**i**) Profil alimentaire caractérisé par des apports élevés en produits laitiers à faible teneur en M.G.	↑ adiponectine
			ii) Profil alimentaire caractérisé par des apports élevés en produits laitiers riches en M.G.	≠ adiponectine
Sadeghi, 2014	F / M	≈ 38	Fromage	↑ hs-CRP

[a] Abréviations : F, féminin ; hs-CRP, protéine C-réactive mesurée par dosage ultra-sensible ; IL-6, interleukine-6 ; M, masculin ; M.G., matières grasses ; SAA, protéine amyloïde A sérique ; TNF-α, facteur de nécrose tumorale alpha ; ↓, association inverse significative ; ≠, aucune association ; ↑, association positive significative.

7.1.3 Études d'intervention

La nature transversale des études décrites précédemment ne permet pas d'établir de relation de cause à effet entre la consommation de produits laitiers et l'inflammation. Afin d'obtenir des réponses concernant la véritable influence que les produits laitiers exercent sur le profil inflammatoire, il s'avère fort pertinent de se tourner vers les études d'intervention nutritionnelle randomisées et contrôlées. Ces études sont en effet considérées comme celles ayant le cadre le plus rigoureux dans le domaine de la recherche en nutrition afin d'établir des rapports de causalité entre l'alimentation et la santé.

Normalement, la présente sous-section servirait à décrire les différentes études d'intervention nutritionnelle randomisées et contrôlées publiées jusqu'à maintenant sur le sujet des produits laitiers et de l'inflammation. Toutefois, puisque la mise en commun de ces études représentait un des objectifs poursuivis dans le cadre de mon doctorat, je n'en discuterai pas pour le moment. Je vous amène sans plus tarder à l'aperçu des travaux réalisés.

7.2. Avant-goût des travaux réalisés et énoncé des objectifs et hypothèses

7.2.1 Revue systématique des études d'intervention sur les produits laitiers et l'inflammation

Considérant la controverse médiatique entourant les produits laitiers et l'inflammation et notre désir de se forger l'idée la plus réaliste possible sur le sujet, notre équipe de recherche a entrepris la réalisation d'une revue systématique de la littérature dont l'**objectif** précis était le suivant :

Synthétiser les résultats des études d'intervention nutritionnelle randomisées et contrôlées qui avaient pour objectif d'évaluer l'impact de la consommation de produits laitiers de source bovine (lait, yogourt, fromage) sur les concentrations sanguines de biomarqueurs inflammatoires chez les adultes, et ce, comparativement à une diète faible en produits laitiers (intervention témoin).

Cet objectif est entièrement relié au **« contexte clinique »** du second volet de mon projet de doctorat (**Figure 7.1**). Il est à noter que nous n'avions **pas émis d'hypothèse** en lien avec cet objectif. Le prochain chapitre présente les détails de la revue systématique, publiée dans la revue scientifique *American Journal of Clinical Nutrition* (Labonté *et al.* 2013;97:706-17).

Il est important de souligner que cette revue de la littérature inclut les études d'intervention nutritionnelle randomisées contrôlées qui ont été publiées en ligne avant le 29 juin 2012. D'autres études pertinentes ont nécessairement été publiées depuis. Afin de respecter un ordre logique de présentation, je rattacherai les résultats de ces nouvelles études à ceux issus de la revue systématique en conclusion du présent volet, au chapitre 10.

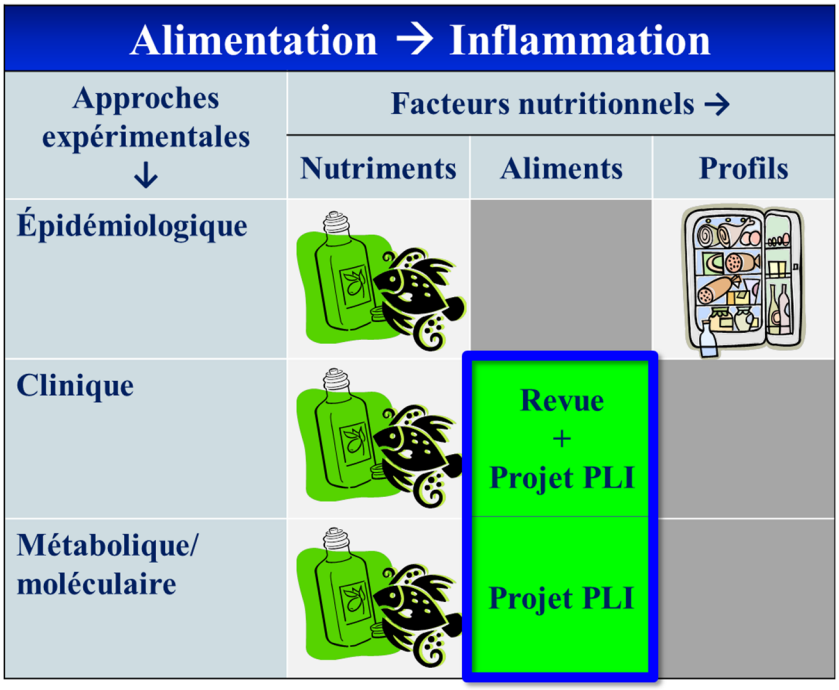

Figure 7.1 : Illustration mettant en lumière les travaux réalisés dans le cadre du **second volet** du présent projet de doctorat, dont le but était d'évaluer l'impact de la consommation de produits laitiers sur l'inflammation dans un contexte clinique ainsi que métabolique (expression de gènes inflammatoires). Abréviations : PLI, projet de recherche intitulé « Produits laitiers et inflammation » ; Revue, revue systématique des études d'intervention nutritionnelle randomisées et contrôlées sur les produits laitiers et l'inflammation.

En complément de la revue systématique, notre équipe de recherche a réalisé l'une des rares études d'intervention nutritionnelle portant sur l'impact de la consommation de produits laitiers sur l'inflammation qui, d'une part, a considéré l'inflammation en tant que mesure principale (« *primary outcome* ») et, d'autre part, a évalué les mécanismes sous-jacents à la réponse inflammatoire (mesure de l'expression de gènes inflammatoires en plus des biomarqueurs inflammatoires circulants). Il s'agit précisément de l'étude PLI, que voici.

7.2.2 Projet PLI : Produits Laitiers et Inflammation

Mon projet de doctorat m'aura permis de contribuer à la toute première étude d'intervention nutritionnelle multicentrique, et par le fait même la plus grande étude conçue selon un devis en chassé-croisé, à avoir évalué l'impact de la consommation de produits laitiers sur l'inflammation en tant que **mesure principale (« *primary outcome* »)** chez des adultes présentant une inflammation systémique de faible intensité.

L'**objectif** établi en lien avec mon projet de doctorat était :

Évaluer l'impact de la consommation de produits laitiers sur les concentrations sanguines de biomarqueurs inflammatoires ainsi que sur l'expression de gènes inflammatoires dans les cellules sanguines complètes chez des hommes et des femmes en santé, mais présentant une inflammation systémique de faible intensité.

L'**hypothèse** émise en lien avec cet objectif était :

La consommation de produits laitiers influence de façon favorable le profil inflammatoire ainsi que l'expression de gènes inflammatoires dans les cellules sanguines complètes.

Le chapitre 9 présente la méthodologie et les résultats du projet PLI, qui ont été publiés dans la revue *Journal of Nutrition* (Labonté *et al.* 2014;144:1760-7). L'objectif ci-haut est à la fois relié au **« contexte clinique »** et au **« contexte métabolique »** du second volet de mon projet de doctorat (Figure 7.1). Il s'agit de la toute première étude, à notre connaissance, à avoir évalué l'expression de gènes inflammatoires dans le sang en réponse à la consommation de produits laitiers. Contrairement à d'autres tissus, les cellules

sanguines sont facilement disponibles, ce qui les rend très pratiques pour étudier l'expression de gènes (205, 206).

CHAPITRE 8 :

IMPACT DES PRODUITS LAITIERS SUR LES BIOMARQUEURS INFLAMMATOIRES : UNE REVUE SYSTÉMATIQUE DES ÉTUDES D'INTERVENTION NUTRITIONNELLE RANDOMISÉES CONTRÔLÉES CHEZ LES ADULTES EN SURPOIDS OU OBÈSES

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Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults

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http://ajcn.nutrition.org/content/97/4/706.long

RÉSUMÉ

Nous avons réalisé une revue systématique des études d'intervention nutritionnelle randomisées contrôlées qui avaient pour objectif d'évaluer l'impact de la consommation de produits laitiers (lait, yogourt, fromage) sur les concentrations sanguines de biomarqueurs inflammatoires chez les adultes. La recherche systématique de la littérature a été effectuée sur *PubMed* en avril 2012. Cette recherche était limitée aux études réalisées chez l'humain et publiées en anglais. Les études incluant des femmes enceintes ou allaitantes ainsi que les études ne comprenant pas d'intervention « témoin » faible en produits laitiers ont été exclues. Huit études réalisées chez des individus en surpoids ou obèses ont été retenues. La seule étude qui avait identifié le changement dans le profil inflammatoire comme sa mesure principale a démontré que la consommation de produits laitiers améliorait les concentrations de biomarqueurs pro- et anti-inflammatoires comparativement à une diète témoin faible en produits laitiers. Trois des sept études dans lesquelles l'inflammation était une mesure secondaire ou indéterminée ont montré des améliorations de certains biomarqueurs inflammatoires (hs-CRP, IL-6 ou TNF-α) suite à la consommation de produits laitiers, alors que les quatre autres études n'ont montré aucun effet. Cette revue systématique suggère que la consommation de produits laitiers n'exerce aucun effet néfaste sur les biomarqueurs inflammatoires chez les individus en surpoids ou obèses. Plusieurs limites méthodologiques nous empêchent de déterminer si les produits laitiers exercent un impact bénéfique ou tout simplement neutre sur l'inflammation. Des études additionnelles spécifiquement conçues pour évaluer les effets des produits laitiers sur l'inflammation et ses mécanismes sous-jacents sont sans aucun doute requises.

TITLE PAGE

TITLE

Impact of dairy products on biomarkers of inflammation: A systematic review of randomized controlled nutritional intervention studies in overweight and obese adults

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RUNNING HEAD: Impact of dairy products on inflammation

ABBREVIATIONS: CHO, carbohydrate; CRP, C-reactive protein; CVD, cardiovascular disease; hs-CRP, high-sensitivity CRP; MCP-1, monocyte chemoattractant protein-1; MetS, metabolic syndrome; s-TNFR, soluble TNF-α receptor

ABSTRACT

Background: Recent data from cross-sectional studies suggest that consumption of dairy products is inversely associated with low-grade systemic inflammation, but a cause-and-effect relationship can only be confirmed with results from randomized controlled trials. **Objective:** We reviewed the results of randomized controlled nutritional intervention studies that have assessed the impact of dairy products consumption (i.e. milk, yogurt, and/or cheese) on biomarkers of inflammation in adults (≥ 18 y). **Design:** We performed a systematic literature search in PubMed in April 2012, which was limited to randomized controlled trials in humans and published in English. Studies that included pregnant or lactating women or that did not include a low-dairy control intervention were excluded. **Results:** Eight trials all conducted in overweight or obese adults were included in the review. The only study that had identified change in the inflammatory profile as its primary outcome measure showed that dairy foods consumption improved pro- and anti-inflammatory biomarkers concentrations compared with the low-dairy control diet. Three out of the seven studies in which inflammation was a secondary or undefined outcome showed improvement in key inflammatory biomarkers, i.e. C-reactive protein, interleukin-6, or tumor necrosis factor-α after dairy products consumption, while the other four studies showed no effect. **Conclusions:** Dairy products consumption does not exert adverse effects on biomarkers of inflammation in overweight or obese adults. Several methodological factors and limitations among existing studies do not allow differentiating between a beneficial or neutral impact of dairy products on inflammation. Further studies specifically designed to assess inflammation-related outcomes are warranted. **Keywords:** C-reactive protein, dairy, inflammatory markers, milk, randomized controlled trials, review

INTRODUCTION

Low-grade systemic inflammation is now considered a key etiological factor in the development and progression of several multifactorial disorders including atherosclerosis (1), metabolic syndrome (MetS) (2, 3), type 2 diabetes (4-6), and cardiovascular diseases (CVD) (7). Elevated plasma concentrations of C-reactive protein (CRP) and of the pro-inflammatory cytokines TNF-α and IL-6 have been associated with an increased risk of CVD (8-13). From a mechanistic perspective, chemokines such as monocyte chemoattractant protein-1 (MCP-1) have been shown to mediate recruitment of monocytes at sites of vascular inflammation, thereby promoting atherosclerosis (14). On the other hand, proteins such as adiponectin have anti-inflammatory and anti-atherogenic properties (15, 16).

In addition to age (17), sex (18), obesity (19), smoking habits (20), alcohol consumption (21) and physical activity (22), increasing evidence suggests that diet plays a major role in the modulation of the inflammatory profile (23). The extensive and detailed review from Calder *et al.* (23) has shown that food groups or nutrients that are part of a healthy diet, including fruits and vegetables, whole grains, fish, fiber, omega-3 fatty acids, vitamin C, vitamin E and carotenoids may protect against low-grade systemic inflammation. In contrast, saturated fatty acids, *trans* fatty acids, as well as dietary patterns characterized by high intakes of red and processed meats, sweets, soft drinks, fried snacks or refined grains have been shown to promote a pro-inflammatory state (23). While diet composition has been shown to modify pro- and anti-inflammatory processes through many mechanisms in different cell types including adipocytes (24) and peripheral blood mononuclear cells (25), more studies on this topic are clearly warranted (26).

Recent cross-sectional studies suggest that the consumption of dairy products is inversely associated with low-grade systemic inflammation (27-29). Indeed, Panagiotakos *et al.* (28) have shown in the ATTICA study that CRP, IL-6 and TNF-α concentrations of individuals consuming more than 14 servings of dairy products per wk (i.e. > 2 servings/d) were respectively 29%, 9%, and 20% lower than those of individuals consuming less than 8 servings per wk (≤ 1 serving/d). This inverse association was independent of potential

confounders such as age, gender, smoking, physical activity, BMI, and other dietary factors.

The cross-sectional nature of these studies precludes definite conclusions on the cause-and-effect relationship between dairy foods consumption and inflammatory outcomes, which can only be investigated through rigorously controlled randomized clinical trials. The purpose of the present systematic review of the literature was to summarize results of randomized controlled nutritional intervention studies in adults that assessed the impact of dairy products consumption on biomarkers of inflammation.

METHODS OF THE REVIEW

The present review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (30).

Study eligibility criteria

The review includes randomized controlled trials published in English that assessed the impact of bovine dairy products consumption (i.e. milk, cheese, and yogurt) on serum or plasma inflammatory biomarkers concentrations of adult men and women compared with low-dairy (control) interventions. No restriction was imposed on publication date or publication status for inclusion in the review.

It was *a priori* determined that studies with the following characteristics would be excluded from the review: studies that included pregnant or lactating women, studies that included patients suffering from severe inflammation-related disorders (e.g. cancer, Crohn's disease, arthritis), studies that did not include a low-dairy control intervention, studies in which test interventions consisted of donkey milk, goat milk or soy beverages only, and studies in which test interventions consisted of high-fat or high-sugar dairy products only (i.e. butter, cream, ice cream), as these products are generally not consumed on the basis of their nutritional value.

Search strategy

A systematic literature search was conducted in PubMed using the following terms: (dairy OR milk OR cheese OR yogurt) AND (food OR product OR intake OR consumption) AND (inflammation OR inflammatory markers OR C-reactive protein OR cytokines) NOT (pregnant OR pregnancy OR lactating OR "breast milk"). The following limits were activated during the search: Humans, All Adult: 19+ years, Randomized Controlled Trial, and English.

The database search was performed on April 2nd, 2012 by one review author (M-EL). The title and abstract of retrieved articles were screened in order to assess the eligibility of studies. The reference list of each article selected through the electronic search was scanned for the identification of other possibly relevant articles. The entire search and articles' screening process was re-run on June 29th, 2012 independently by a second reviewer (CR) to ensure that all relevant articles had been retrieved and to look for newly published articles. The search was also conducted in Embase (www.embase.com) and the Cochrane Library (www.thecochranelibrary.com) at that time to look for possibly relevant articles that may not have been recorded in PubMed. Results from the different searches were then compared and discordances were resolved by consensus.

Data extraction

For each study included in the review, one review author (M-EL) extracted data on study design, context of the interventions (duration, type of dairy products tested, control intervention characteristics), primary outcome (i.e. change in inflammatory profile as the primary outcome or not), population characteristics (gender, age, weight status, baseline CRP concentrations), and results on inflammatory biomarkers concentrations.

Assessment of the risk of bias within studies

Based on the Cochrane Collaboration's tool for assessing the risk of biased results within studies (31), the following items were evaluated for each study: randomized sequence generation, allocation concealment, blinding of participants, personnel, and outcome assessment, incomplete outcome data, selective reporting, and other possible bias (i.e.

carry-over effect in crossover studies and baseline imbalance in factors strongly related to outcome measures). The risk of bias within studies was independently assessed by two review authors (M-EL, CR). Authors thereafter compared their results and discordances were resolved by consensus.

RESULTS

Study selection

As shown in **Figure 1**, the database search first retrieved 91 research articles. The title and abstract of each article was screened and 82 articles were discarded because they did not meet the eligibility criteria. The full text of nine articles was retrieved for detailed evaluation. Five studies met the inclusion criteria and were included in the review. Two additional research articles that met eligibility criteria were identified by checking the reference list of the five selected articles. Thus, a total of seven research articles reporting the results of eight nutritional intervention trials were identified for inclusion in the review (32-38). The re-run of the search in PubMed as well as in other databases generated the same list of eligible papers and no new study was identified.

Study characteristics

A summary of the eight nutritional intervention trials included in the present review is provided in **Table 1**. Briefly, all eight trials were randomized controlled trials published in English between August 2005 and October 2011, of which two had a crossover design (37, 38) and six had a parallel design (32-36). All trials included overweight or obese adults aged 18 y and older. Seven out of the eight trials included both men and women (32-35, 37, 38), whereas one study included women only (36). The duration of the interventions ranged from 4 wk (1 mo) in Zemel *et al.* (37) to 48 wk (≈ 1 y) in Thompson *et al.* (33). Because the interventions differed markedly between studies (duration, type of dairy products tested, characteristics of the control intervention), no meta-analysis was performed. The following paragraphs provide a narrative description of each study. The description is categorized according to whether or not inflammation was the primary outcome of the study. This is based on the argument that sample size calculations for a primary outcome other than

inflammation may not have been adequate for the analysis of inflammation as secondary outcome.

Studies with change in inflammatory profile as primary outcome

Using a parallel-group design, Stancliffe *et al.* (32) randomly assigned 40 overweight and obese adults with MetS to one of two isoenergetic weight-maintenance diets that lasted 12 wk each: an adequate-dairy diet (> 3.5 servings/d of dairy, of which 3 were provided to participants by the research team and 2 were milk and/or yogurt) was compared to a low-dairy diet (< 0.5 serving/d) during which participants were provided with 3 daily servings of pre-packaged non-dairy foods. These pre-packaged non-dairy foods were selected by subjects from a list of low-sodium varieties or soy-based substitutes of luncheon meats, packaged fruit cups, granola bars and peanut butter crackers, as previously described (32). Both diets were constructed and matched to achieve macronutrient and fiber intakes that were comparable to the estimated intake in the United States (values reported by authors ≈ 35% energy from fat, ≈ 49% energy from carbohydrate (CHO), ≈ 16% energy from protein and 8-12 g/d of fiber). However, by design, the two diets differed in terms of calcium intake (difference of approximately 600 mg) and dairy-derived proteins (difference of 28-35 g). By wk 12, the adequate-dairy diet significantly reduced TNF-α (-35%, $P < 0.01$), IL-6 (-21%, $P < 0.02$), MCP-1 (-24%, $P < 0.02$), and CRP concentrations (-47%, $P < 0.02$), while it concurrently increased adiponectin concentrations (+53%, $P < 0.005$). For the majority of the inflammatory biomarkers, effects were present after only one wk of treatment and progressed over time. In general, these effects were also more pronounced in obese subjects than in the overweight subgroup. Although there was no change in body weight, the adequate-dairy diet led to reductions in adiposity indices, i.e. fat mass, trunk fat, as well as waist circumference (all $P < 0.05$). The low-dairy diet had no significant impact on inflammatory biomarkers, body weight or adiposity indices.

Studies with change in inflammatory profile as secondary or undefined outcome

Thompson *et al.* (33), using a parallel design, allocated 90 obese men and women (BMI between 30-40 kg/m^2) to one of three diets: 1) a high-dairy diet (4 daily servings with at least 2 as fluid milk; percent fat unspecified), 2) a high-dairy diet similar to the first, except

for a higher fiber content and a lower glycemic index, and 3) a control diet (2 daily servings of dairy). All diets were designed to provide a 500 kcal/d deficit and lasted 48 wk. Subjects bought and prepared their own food according to a meal plan. The change in body weight was the primary outcome of the study and the change in high sensitivity (hs)-CRP concentrations was a pre-specified secondary outcome. Analyses of compliance showed that on average, 3.13, 3.12, and 1.38 daily servings of dairy products were consumed by the subjects in the high-dairy, high-dairy/high-fiber and control groups, respectively. All three groups consumed equal amounts of energy ($P = 0.81$). The proportion of energy from fat and CHO was similar in the control and high-dairy diets, but percent energy from fat was lower and percent energy from CHO was higher in the high-dairy/high-fiber diet compared with the other two groups ($P < 0.05$). Percent energy from protein was lower in the control group compared with the two high-dairy groups ($P < 0.05$). Results showed that all three diets led to significant reductions from baseline in hs-CRP concentrations (-17.0%, -28.6%, and -22.2% in the high-dairy, high-dairy/high-fiber and control groups, respectively, all $P < 0.0001$), but no between-group difference was observed ($P = 0.66$). The magnitude of weight loss was similar in all three groups ($P = 0.45$).

Zemel and Sun (34), using archived samples from two randomized controlled parallel trials (39, 40), retrospectively assessed the impact of high-dairy diets on plasma CRP and adiponectin concentrations in healthy obese subjects (BMI between 30-40 kg/m^2). The primary outcome of the first study (39) consisted of changes in body fat. Briefly, 39 African-American men and women were randomized to consume one of two isoenergetic diets for 24 wk: a low-dairy diet (< 1 serving/d) or a high-dairy diet (3 servings/d with at least one in the form of fluid milk). In both diets, macronutrient and fiber amounts were set to achieve values corresponding to the average consumption in the United States (values reported by authors ≈ 35% energy from fat, ≈ 49% energy from CHO, ≈ 16% energy from protein, and 8-12 g/d of fiber). Dairy products in the high-dairy diet were substituted for lean meats in the control diet. Plasma CRP concentrations significantly decreased by 11% ($P < 0.03$) and adiponectin concentrations significantly increased by 8% ($P = 0.003$) in subjects fed the high-dairy diet (post- vs. pre-diet values). These changes were observed concurrently to significant reductions in adiposity indices such as waist circumference,

trunk fat and body fat with the high-dairy diet (all $P < 0.01$). There was no significant change in CRP or adiponectin concentrations in subjects who consumed the low-dairy diet.

The second study (40) included 38 obese men and women who were randomized to one of two hypoenergetic diets providing a 500 kcal/d deficit over 12 wk: 1) a yogurt-enriched diet (3 daily six-ounce (i.e. 3 × 170 g) servings of a fat-free yogurt) or 2) a control diet (0-1 serving of dairy products/d, including 3 daily servings of a flavored gelatin dessert as a placebo). The placebo was sugar-free and calcium-free. Energy intake and macronutrient content of the two diets were similar, with values approximating the average consumption in the United States (values reported by authors ≈ 30% energy from fat, ≈ 52% energy from CHO, and ≈ 18% energy from protein). The primary outcomes of this study were changes in body weight and body fat. The yogurt-enriched diet induced significant reductions from baseline in CRP concentrations (-29%, $P < 0.01$) and significant increases in adiponectin concentrations (+18%, $P < 0.05$), whereas consumption of the control diet had no impact on CRP or adiponectin concentrations. Observed reductions in body weight, body fat and other adiposity indices were significantly greater in subjects fed the yogurt-enriched diet compared with subjects fed the control diet (all $P < 0.01$). Thus, the experimental design does not allow identifying yogurt intake *per se* as the real cause of the favorable changes observed in inflammatory biomarkers concentrations following the yogurt-enriched diet.

A parallel-group intervention study was conducted by Wennersberg *et al.* (35) to assess the impact of a 6-mo high-dairy diet on body composition and other factors related to the MetS in 121 overweight men and postmenopausal women characterized with at least two traits of the MetS (National Cholesterol Education Program Adult Treatment Panel III criteria (41)). The primary outcome of the study consisted of changes in waist circumference. Subjects were randomly assigned to a dairy-enriched diet (3-5 daily servings of dairy products of any kind, i.e. milk, yogurt, sour milk, cream, cheese, cottage cheese, butter, or ice cream occasionally) or a control diet (habitual diet without changing the intake of dairy products). Diets were not matched for energy and macronutrient intake. Energy intake tended to be higher in the dairy-enriched diet group compared with the control group ($P = 0.07$). Intakes of protein (g), total fat (g), SFA (g), sugar (g), cholesterol (mg) and calcium (mg) were higher while alcohol intake (%) was lower in the dairy-enriched diet group compared with

the control group ($P \leq 0.03$). Results showed that participants' baseline intake of dairy products approximated 200 g/d, representing between 0.8-1.1 servings of dairy/d. Dairy products consumption (mainly fluid milk and yogurt) increased by an average of 250 g/d in the dairy group, representing 1.0-1.4 servings of dairy/d, while there was no change in dairy intake in the control group ($P < 0.0001$ for the difference in dairy intake between the two groups). Changes from baseline to 6 mo in hs-CRP, IL-6, TNF-α and adiponectin concentrations were not significantly different between the milk and control groups (e.g. change in hs-CRP +2.9% vs. -3.3%, respectively, $P = 0.339$).

Using a parallel design, Rosado *et al.* (36) investigated the impact of three energy-restricted diets (500 kcal/d deficit) on anthropometric and biochemical variables in 139 obese Mexican women. Women consumed one of the following diets for 16 wk: a low-fat milk diet (250 mL servings, 3 times daily), a "low-fat milk with added micronutrients" diet (250 mL servings, 3 times daily), or a control diet (no intake of milk or any other dairy product). Milk was provided to participants in the two milk diets and was the only dairy product allowed. Meal plans that included specific quantities of foods regularly consumed in this population were provided to women in the control diet to achieve similar CHO (≈ 55% of energy), protein (≈ 20% of energy), fat (≈ 25% of energy) and fiber intake in all study groups. The primary outcomes of the study were changes in body weight and body composition. Results showed that inclusion of low-fat milk to the women's diet had no additional effect on body weight or body fat reduction compared with the energy-restricted control diet. Also, changes from baseline to 16 wk in hs-CRP concentrations were not significantly different between groups (+3.6%, -8.9%, and -16.4%, respectively, $P = 0.534$, in the low-fat milk, "low-fat milk + micronutrients" and control diets). Other inflammatory biomarkers were not assessed in this study.

Using a crossover design, Zemel *et al.* (37) compared in the context of isoenergetic weight-maintenance diets the impact of consuming 3 daily servings of dairy-based smoothies (made with non-fat dry milk) with soy-based placebo smoothies on inflammatory biomarkers in 10 overweight (mean BMI: 28.0 ± 1.0 kg/m^2) and 10 mildly obese (mean BMI: 32.5 ± 1.1 kg/m^2) subjects. Each intervention lasted 28 d and was separated by a 28-d washout. The two diets were designed to provide similar amounts of macronutrient and

fiber, which were set to the average consumption in the United States (values reported by authors ≈ 35% energy from fat, ≈ 49% energy from CHO, ≈ 16% energy from protein, and 8-12 g/d of fiber). Dietary calcium intake differed by approximately 600 mg between the two diets. Consumption of the dairy-based smoothies led to significant reductions from baseline in CRP (-57%, $P < 0.05$), IL-6 (-13%, $P < 0.01$), TNF-α (-15%, $P < 0.002$), and MCP-1 (-20%, $P < 0.0006$) concentrations as well as significant increases in adiponectin (+20%, $P < 0.002$), whereas the soy-based control intervention had no effect or even increased inflammatory biomarkers concentrations compared to baseline values. Most of the effects were evident after 7 d of dietary change and increased in magnitude at the end of the 28 d. There was no significant change in the anti-inflammatory cytokine IL-15 with any of the two diets. There was also no significant change in body weight, body fat, trunk fat, or lean mass during either dietary treatment. Authors indicated in their paper that their objective was to assess the impact of a dairy-rich diet on oxidative and inflammatory stress. However, it was not indicated if the study was designed using inflammation as the primary outcome and there was also significant discordance between the study design described in the publication and information found in ClinicalTrials.gov for this study. Thus, we were not able to determine if diet-induced change in the inflammatory profile was the primary outcome of this study.

Finally, van Meijl and Mensink (38) compared the impact of the consumption of low-fat dairy products (500 mL low-fat [1.5% w/w] milk + 150 g low-fat [1.5% w/w] yogurt daily) with CHO-rich control products (600 mL fruit juice with 3 fruit biscuits) on inflammatory biomarkers of 40 overweight or obese subjects in a crossover study. A 2-wk washout period separated the two 8-wk interventions. Subjects maintained their habitual background diet during the entire study. Dietary intakes estimated from a food frequency questionnaire, presented in another paper describing this study (42), showed that total energy intake was similar between diets. However, % energy from protein and fat (including SFA and MUFA) as well as intakes of cholesterol and calcium were higher while % energy from CHO and mono- and disaccharides as well as fiber intake were lower in the low-fat dairy intervention compared with the high-CHO control intervention (all $P < 0.05$). The primary outcome of this study was not clearly identified (38, 42). However, authors provided evidence of adequate statistical power to detect significant changes of 10% or more for

TNF-α, MCP-1, and soluble TNF-α receptors (s-TNFR) 1 and 2, but not IL-6. Plasma IL-6 and MCP-1 concentrations were not different between the interventions (both $P > 0.77$), while concentrations of TNF-α tended to be lower after the low-fat dairy intervention compared with the high-CHO intervention (-6.5%, $P = 0.07$). A significant 5.5% increase in s-TNFR-2 concentrations was observed after the low-fat dairy diet compared with the high-CHO diet ($P = 0.02$) and was considered by the authors as a beneficial effect of dairy. However, this change is small in magnitude and must be interpreted with caution since its clinical relevance is unknown. The low-fat dairy diet had no impact on hs-CRP concentrations compared with the high-CHO intervention ($P = 0.147$), as reported in the other paper describing this study (42).

Risk of bias within studies

As shown in **Table 2**, studies by Thompson *et al.* (33) and Rosado *et al.* (36) had the highest transparency in the report of items associated with the risk of biased results, i.e. the lowest presence of "unclear risk" statements. These were the only two studies to clearly describe both the methods used to randomize participants and methods used to conceal the allocation to the interventions prior to assignment. The majority of studies adequately reported the number of attrition and exclusions related to inflammatory biomarkers analyses, together with reasons (32-36, 38, 39). However, all eight trials provided insufficient details regarding the blinding of outcome assessors (laboratory staff that analyzed blood samples) to the test vs. control interventions. Blinding of participants and personnel to the test vs. control interventions was also impossible in most of the studies reviewed due to the nature of the interventions. Otherwise, the majority of studies provided insufficient information in order to evaluate the risk of selective outcome reporting, i.e. whether some inflammatory biomarkers were assessed but not reported or simply not assessed. Among crossover studies, a carry-over effect was evidenced for MCP-1 in the study by Zemel *et al.* (37). Regarding baseline imbalance in characteristics that are strongly related to inflammatory outcomes (i.e. age, sex, obesity indices, baseline inflammation), half of the studies (33, 36-38) were classified as presenting a low risk of bias. Indeed, in parallel-design studies by Thompson *et al.* (33) and Rosado *et al.* (36), the high-dairy and control groups showed similar baseline characteristics for age, sex, BMI and hs-CRP

concentrations. Baseline imbalance does not apply to the studies by Zemel *et al.* (37) and van Meijl and Mensink (38) due to their crossover design. Moreover, in Zemel *et al.* (37), baseline values for body weight, body fat, trunk fat, as well as CRP, IL-6, IL-15, TNF-α, MCP-1, and adiponectin concentrations were similar between the two dietary phases. The other four studies (32, 34, 35) are classified as presenting an unclear risk of bias regarding baseline imbalance. In the study by Stancliffe *et al.* (32) and in the hypoenergetic study reported by Zemel and Sun (34, 40), intervention groups had similar baseline characteristics including age, sex distribution, BMI, blood pressure and plasma lipids-lipoproteins concentrations. However, baseline CRP concentrations were not formally compared between intervention groups in these two studies and this represents a potential source of bias. In the isoenergetic study reported by Zemel and Sun (34, 39), baseline CRP concentrations were also not formally considered as a source of confounding. The risk of confounding in this study is, however, higher considering that the number of men and women was unbalanced between the low-dairy (8 men vs. 9 women) and high-dairy diets (3 men vs. 14 women). In the study by Wennersberg *et al.* (35), the two groups were comparable at baseline for most of the characteristics reported including age, sex distribution, BMI and hs-CRP concentrations, but adiponectin concentrations, which were an outcome of interest, were higher in the high-dairy group than in the control group at baseline ($P = 0.021$). Detailed justifications associated with the evaluation of the risk of bias within studies are provided in **Supplemental Table 1.**

DISCUSSION

The present paper reviewed the evidence from randomized controlled trials regarding the impact of dairy products consumption on circulating inflammatory biomarkers in adults. We first observed that this topic had been addressed in very few studies thus far, with only eight trials meeting the eligibility criteria for review. All of these eight studies were undertaken in overweight or obese men and women. Half of the studies point towards a potentially beneficial effect of dairy products consumption on low-grade systemic inflammation, while the other half suggests no effect. Several methodological factors and limitations in the analysis of these data need to be considered.

Whether inflammation was considered the primary outcome in studies reviewed is a crucial methodological factor that needs to be discussed. Only one trial had clearly identified change in the inflammatory profile as its primary outcome measure and provided sample size calculation based on the variability of CRP. Results indicated significant anti-inflammatory effects of consuming more than 3.5 servings/d of dairy products compared with less than 0.5 serving (32). It is stressed that the inflammation-lowering effects of dairy products may have been triggered, at least to some extent, by favorable changes in adiposity indices, as they are well-known correlates of inflammatory biomarkers concentrations (43, 44). The authors argued that the rapid onset of the effects of the high-dairy diet on inflammatory biomarkers, i.e. within 7 days of intervention, suggested an adiposity-independent effect as well. However, long-term analyses considering the potential impact of reduced adiposity on inflammatory biomarkers concentrations were not performed. On the other hand, between-diet differences in specific dietary components such as calcium and dairy proteins may be responsible, at least partly, for the inflammation-lowering effects of the high-dairy diet. Previous studies in mice models have suggested that dietary calcium reduces inflammatory cytokines expression in adipocytes through the suppression of calcitriol (34, 45). Milk-derived proteins such as lactoferrin may also exert anti-inflammatory effects (46) through the regulation of the recruitment and activation of cytokine-releasing immune cells (47). Finally, bioactive peptides from dairy have been shown to inhibit angiotensin-1-converting enzyme (48), thereby limiting the production of angiotensin II, which is known to induce the secretion of inflammatory cytokines in the vascular wall (49) and adipose tissue (50).

There is a strong possibility that the seven studies in which change in inflammatory biomarkers was a secondary or undefined outcome were not sufficiently powered to detect significant differences between high-dairy and control (low-dairy) interventions. This is particularly the case for three (33, 35, 36) out the five (33-36) studies that were definitely not designed *a priori* to assess inflammation-related outcomes, having provided sample size calculations for changes in body weight or waist circumference and having shown no impact of dairy consumption on key circulating inflammatory biomarkers (Table 1). Results from the other two studies, both reported in (34) as *a posteriori* analyses of studies (39) and (40), showed significant reductions from baseline in hs-CRP concentrations as

well as significant increases in adiponectin concentrations following the consumption of high-dairy diets. Despite the weight-maintenance context of the isoenergetic study (39), significant reductions were observed in waist circumference, trunk fat, and body fat after the high-dairy diet. In the energy-restricted study (40), reductions in body weight, body fat, and other adiposity indices were significantly greater in magnitude in subjects fed the yogurt-enriched diet compared with subjects fed the control diet. Therefore, it is likely that the favorable changes from baseline observed in the inflammatory profile after consumption of high-dairy diets in these two studies may have been confounded by concurrent favorable changes in adiposity indices.

Other major factors limiting the generalization of results based on the available randomized controlled trials are the heterogeneity of dairy products used and lack of detail regarding the type and fat content of these products. In five of the eight studies (32, 33, 35, 38, 39), test interventions consisted of a combination of different dairy products. In four of those studies (32, 33, 35, 39), the average fat content of dairy products that participants had added to their diet was not reported. We are therefore unable to distinguish the effects of specific dairy products (milk vs. yogurt vs. cheese or low-fat vs. high-fat dairy products) on inflammation. We are also unable based on the available information to evaluate the impact of other potentially important sources of variability on the inflammatory response to various dairy products, such as differences in sugar content (e.g. plain vs. aromatised products), protein content (e.g. regular vs. Greek-style yogurt) and fortification with prebiotics (e.g. inulin), probiotics, omega-3 fatty acids or phytosterols.

The difference in the amount of dairy products consumed by subjects between the test (high-dairy) and control (low-dairy) interventions is another factor that may contribute to heterogeneity among studies. In trials by Thompson *et al.* (33) and Wennersberg *et al.* (35), dairy products intake differed by less than 2 servings between the high-dairy and control groups, thereby limiting the capacity to observe changes in inflammatory biomarkers with dairy intake. In contrast, four (32, 34, 37) out of the six (32, 34, 36-38) studies in which dairy products intake differed by 3 or more daily servings between the high-dairy and control diets showed improved inflammatory biomarkers concentrations. Differences in the

nutritional content of the dairy and control diets were also important in many studies and this represents another factor contributing to heterogeneity among studies.

Additionally, differences in the nature of inflammatory biomarkers analyzed in each study need to be pointed out as a factor limiting generalization of results. CRP, IL-6, and TNF-α were analyzed concurrently in half of the studies together with at least one of the following markers, i.e. adiponectin, MCP-1, IL-15 or s-TNFR-1 and 2 (32, 35, 37, 38). On the other hand, two studies reported data on CRP concentrations only (33, 36), and two other assessed adiponectin in addition to CRP (34). Interpretation is obviously more limited in studies that assessed only one or two inflammatory biomarkers than in studies in which a more comprehensive assessment of the inflammatory profile was performed. The sensitivity of the assays used to assess changes in inflammatory biomarkers in response to dairy and their CVs are other potential sources of heterogeneity between studies. Most studies have used ELISA or high-sensitivity immunoturbidimetric tests to assess changes in biomarkers of inflammation with dairy intake. However, half of the studies (33, 34, 36) did not report the CV for theirs assays. Considering that precision metrics and CVs for the measurement of inflammatory biomarkers are not available in all included studies, it is not possible to establish the extent to which heterogeneity between studies is due to differences in methodologies used to assess inflammatory outcomes.

The evaluation of the risk of bias highlighted that the majority of studies did not clearly report how several items known to affect the validity of results were addressed, including detail on the randomization process, allocation concealment, blinding of outcome assessment, and selective outcome reporting. Although it is likely that investigators have dealt adequately with these methodological issues, we cannot exclude the possibility of biased results among studies included in the present review. Future studies obviously need to fill gaps in the report of items associated with the risk of biased results.

In conclusion, results from available randomized controlled trials conducted to date suggest that dairy products consumption has no adverse effect on low-grade systemic inflammation among overweight and obese adults. On the other hand, several methodological factors and limitations do not allow us to conclude if the impact of dairy products on inflammation is beneficial or simply neutral. Additional well-controlled and adequately powered nutritional

intervention studies specifically designed to assess the effects of dairy products on inflammatory biomarkers concentrations are therefore warranted. A better characterization of the type and amount of dairy products tested in each study will be needed in order to draw clear conclusions. In order to gain mechanistic knowledge about the effects of dairy products of inflammation, outcome measures in future studies should also include inflammation-related genes expression.

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AUTHORS' CONTRIBUTIONS

The authors' responsibilities were as follows: BL, PC, and SD designed the study; M-EL and CR conducted the search and performed analysis of the risk of bias within studies; M-EL extracted data and wrote the paper; BL had primary responsibility for the final content. All authors critically reviewed the manuscript and approved its final version.

REFERENCES

1. Packard RRS, Libby P. Inflammation in atherosclerosis: From vascular biology to biomarker discovery and risk prediction. Clin Chem 2008;54:24-38.

2. Tamakoshi K, Yatsuya H, Kondo T, Hori Y, Ishikawa M, Zhang H, Murata C, Otsuka R, Zhu S, Toyoshima H. The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. Int J Obes 2003;27:443-9.

3. Ford ES. The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. Atherosclerosis 2003;168:351-8.

4. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004;27:813-23.

5. Dehghan A, Kardys I, de Maat MPM, Uitterlinden AG, Sijbrands EJG, Bootsma AH, Stijnen T, Hofman A, Schram MT, Witteman JCM. Genetic variation, C-reactive protein levels, and incidence of diabetes. Diabetes 2007;56:872-8.

6. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327-34.

7. de Ferranti SD, Rifai N. C-reactive protein: a nontraditional serum marker of cardiovascular risk. Cardiovasc Pathol 2007;16:14-21.

8. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.

9. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557-65.

10. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men - Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999;99:237-42.

11. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A(2), high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Circulation 2004;109:837-42.

12. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767-72.

13. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation 2000;101:2149-53.

14. Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. Circ Res 2004;95:858-66.

15. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 2000;96:1723-32.

16. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochem Biophys Res Commun 2004;323:630-5.

17. Bruunsgaard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokines. Curr Opin Hematol 2001;8:131-6.

18. Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464-9.

19. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131-5.

20. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med 2003;138:891-7.

21. Albert MA, Glynn RJ, Ridker PM. Alcohol consumption and plasma concentration of C-reactive protein. Circulation 2003;107:443-7.

22. Abramson JL, Vaccarino V. Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. Arch Intern Med 2002;162:1286-92.

23. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, Esposito K, Jonsson LS, Kolb H, Lansink M et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr 2011;106:S5-78.

24. Bradley RL, Fisher FF, Maratos-Flier E. Dietary fatty acids differentially regulate production of TNF-alpha and IL-10 by murine 3T3-L1 adipocytes. Obesity (SilverSpring) 2008;16:938-44.

25. Bouwens M, van de Rest O, Dellschaft N, Bromhaar MG, de Groot LCPG, Geleijnse JM, Muller M, Afman LA. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr 2009;90:415-24.

26. North CJ, Venter CS, Jerling JC. The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. Eur J Clin Nutr 2009;63:921-33.

27. Esmaillzadeh A, Azadbakht L. Dairy consumption and circulating levels of inflammatory markers among Iranian women. Public Health Nutr 2010;13:1395-402.

28. Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy Products Consumption Is Associated with Decreased Levels of Inflammatory Markers Related to Cardiovascular Disease in Apparently Healthy Adults: The ATTICA Study. J Am Coll Nutr 2010;29:357-64.

29. Salas-Salvado J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M, Gomez-Gracia E, Vinoles E, Aros F, Herrera C et al. Components of the mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. Eur J Clin Nutr 2008;62:651-9.

30. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097. doi: 10.1371/journal.pmed.1000097.

31. Higgins JPT, Altman DG. Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S, eds. Cochrane handbook for systematic reviews of interventions version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2008. Internet: http://www.cochrane-handbook.org/ (accessed 26 April 2012).

32. Stancliffe RA, Thorpe T, Zemel MB. Dairy attentuates oxidative and inflammatory stress in metabolic syndrome. Am J Clin Nutr 2011;94:422-30.

33. Thompson WG, Holdman NR, Janzow DJ, Slezak JM, Morris KL, Zemel MB. Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. Obes Res 2005;13:1344-53.

34. Zemel MB, Sun XC. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. J Nutr 2008;138:1047-52.

35. Wennersberg MH, Smedman A, Turpeinen AM, Retterstol K, Tengblad S, Lipre E, Aro A, Mutanen P, Seljeflot I, Basu S et al. Dairy products and metabolic effects in

overweight men and women: results from a 6-mo intervention study. Am J Clin Nutr 2009;90:960-8.

36. Rosado JL, Garcia OP, Ronquillo D, Hervert-Hernandez D, Caamano MDC, Martinez G, Gutierrez J, Garcia S. Intake of Milk with Added Micronutrients Increases the Effectiveness of an Energy-Restricted Diet to Reduce Body Weight: A Randomized Controlled Clinical Trial in Mexican Women. J Am Diet Assoc 2011;111:1507-16.

37. Zemel MB, Sun XC, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. Am J Clin Nutr 2010;91:16-22.

38. van Meijl LEC, Mensink RP. Effects of low-fat dairy consumption on markers of low-grade systemic inflammation and endothelial function in overweight and obese subjects: an intervention study. Br J Nutr 2010;104:1523-7.

39. Zemel MB, Richards J, Milstead A, Campbell P. Effects of calcium and dairy on body composition and weight loss in African-American adults. Obes Res 2005;13:1218-25.

40. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E. Dairy augmentation of total and central fat loss in obese subjects. Int J Obes 2005;29:391-7.

41. NCEP Expert Panel. Executive Summary of the 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). JAMA 2001;285:2486-97.

42. van Meijl LEC, Mensink RP. Low-fat dairy consumption reduces systolic blood pressure, but does not improve other metabolic risk parameters in overweight and obese subjects. Nutr Metab Cardiovasc Dis 2011;21:355-61.

43. Neyestani TR, Salekzamani S, Kalayi A, Alavi-Majd H, Houshiarrad A, Nikooyeh B, Shariatzadeh N. Predictors of Serum Levels of High Sensitivity C-Reactive Protein and Systolic Blood Pressure in Overweight and Obese Nondiabetic Women in Tehran: A Cross-Sectional Study. Metab Syndr Relat Disord 2011;9:41-7.

44. Lemieux I, Pascot A, Prud'homme D, Almeras N, Bogaty P, Nadeau A, Bergeron J, Despres JP. Elevated C-reactive protein - Another component of the atherothrombotic profile of abdominal obesity. Arterioscler Thromb Vasc Biol 2001;21:961-7.

45. Sun X, Zemel MB. Calcium and 1,25-dihydroxyvitamin D-3 regulation of adipokine expression. Obesity 2007;15:340-8.

46. Bharadwaj S, Naidu TAG, Betageri GV, Prasadarao NV, Naidu AS. Inflammatory responses improve with milk ribonuclease-enriched lactoferrin supplementation in postmenopausal women. Inflamm Res 2010;59:971-8.

47. Legrand D. Lactoferrin, a key molecule in immune and inflammatory processes. Biochem Cell Biol 2012;90:252-68.

48. FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr 2000;84 Suppl 1:S33-7.

49. Savoia C, Burger D, Nishigaki N, Montezano A, Touyz RM. Angiotensin II and the vascular phenotype in hypertension. Expert Rev Mol Med 2011;13:1-25.

50. Kalupahana NS, Moustaid-Moussa N. The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. Obes Rev 2012;13:136-49.

TABLES

TABLE 1. Summary of randomized controlled trials that assessed the impact of dairy products consumption on circulating inflammatory biomarkers[1]

Study	Individuals (*n*)[2]	Sex (*n*)	Age (y)	Design	Interventions	Duration	Inflammation = primary outcome (yes/no/unclear)	Baseline CRP concentrations[3]	Observed changes in inflammatory biomarkers[4]		
									CRP	Cytokines	Other inflammatory markers
Stancliffe, 2011 (32)	40 overweight or obese subjects with MetS	M (19) / F (21)	Mean: 37.0±9.9	Parallel	2 weight-maintenance diets: - **adequate-dairy** (> 3.5 daily servings of dairy products with 2 as milk and/or yogurt) - **low-dairy** (< 0.5 daily serving)	12 wk	Yes	N/A	Within-diet changes: Adequate-dairy group: ↓	↓ TNF-α, IL-6 Low-dairy group: ↔	↓ MCP-1 ↑ adiponectin ↔ MCP-1, adiponectin
Thompson, 2005 (33)	90 obese subjects	M (13) / F (77)	25-70	Parallel	3 energy-restricted diets: - **high-dairy** (4 servings/d, with ≥ 2 as fluid milk) - **high-dairy/high-fiber** (4 servings/d, with ≥ 2 as fluid milk) - **standard diet** (2 servings/day)	48 wk	No	High-dairy group: 4.7 ± 3.8 mg/L High-dairy/high-fiber group: 5.6 ± 5.0 mg/L Standard diet group: 4.5 ± 3.5 mg/L	Within-diet changes (in all three diets): ↓ Between-diet differences: ↔		

Zemel and Sun, 2008 (34)[5]: *Isoenergetic study*	39 obese but otherwise healthy African-American subjects	M (11) / F (23)[6]	26-55	Parallel	2 isoenergetic diets: - **high-dairy** (3 servings/d with at least one as fluid milk) - **low-dairy** (< 1 serving/d)	24 wk	No	N/A	Within-diet changes: High-dairy group: ↓ ↑ adiponectin Low-dairy group: ↔ ↔ adiponectin
Hypo-energetic study	38 obese but otherwise healthy subjects	M (7) / F (27)[6]	18-50	Parallel	2 energy-restricted diets: - **yogurt-enriched diet** (3 six-ounce (3 × 170 g) servings/d of fat-free yogurt) - **control diet** (0-1 serving of dairy/d, with 3 servings of a flavored gelatin dessert as a placebo)	12 wk	No	N/A	Within-diet changes: Yogurt-enriched group: ↓ ↑ adiponectin Control group: ↔ ↔ adiponectin
Wennersberg, 2009 (35)	121 overweight subjects with MetS	M (41) / F (80)	30-65	Parallel	2 diets: - **dairy-enriched** (3-5 servings/d of dairy products of any kind) - **control group** (habitual diet without changing dairy products intake)	24 wk (6 mo)	No	Dairy-enriched group: 3.5 ± 3.3 mg/L Control group: 3.0 ± 3.0 mg/L	Between-diet differences in changes from baseline to 24 wk: ↔ ↔ IL-6, TNF-α ↔ adiponectin
Rosado, 2011 (36)	139 obese Mexican women	F	25-45	Parallel	3 energy-restricted diets including: - **low-fat milk** (750 mL/d) - **low-fat milk + micronutrients** (750 mL/d) - **control intervention** (no intake of milk)	16 wk	No	Low-fat milk group: 5.5 ± 3.0 mg/L Low-fat milk + micronutrients group: 7.9 ± 3.9 mg/L Control group: 6.7 ± 3.9 mg/L	Within- or between-diet differences: ↔

Zemel, 2010 (37)	20 overweight or mildly obese but otherwise healthy subjects	M (14) / F (6)	Mean: 31.0±10.3	Crossover	2 isoenergetic diets supplemented with: **- dairy-based smoothies** (3 servings/d; made with non-fat dry milk) **- soy-based placebo smoothies** (3 servings/d)	4 wk (28 d), with a 4-wk washout between phases	Unclear	Phase 1: 33.6 ± 12.5 μg/mL Phase 2: 26.2 ± 13.5 μg/mL	Within-diet changes: Dairy-based smoothies phase: ↓ ↓ TNF-α, IL-6 ↓ MCP-1 ↑ adiponectin ↔ IL-15 Soy-based placebo smoothies phase: ↔ ↑ TNF-α, IL-6, ↑ MCP-1 ↓ adiponectin ↔ IL-15
van Meijl and Mensink, 2010 (38)[7]	40 overweight or obese subjects without CVD	M (10) / F (30)	18-70	Crossover	2 diets supplemented with: **- low-fat dairy products** (500 mL low-fat milk/d and 150 g low-fat yogurt/d) **- CHO-rich control products** (600 mL fruit juice and 43 g of fruit biscuits)	8 wk, with a 2-wk washout between phases	Unclear[8]	N/A	Between-diet differences (low-fat dairy vs. control phase): ↔ ↓ TNF-α (trend) ↑ s-TNFR-1 (trend) and s-TNFR-2 (significant) ↔ IL-6 ↔ MCP-1

[1] CHO, carbohydrate; CRP, C-reactive protein; CVD, cardiovascular disease; hs-CRP, high-sensitivity CRP; MCP-1, monocyte chemoattractant protein 1; MetS, metabolic syndrome; s-TNFR, soluble TNF-α receptor.

[2] *n* = number of individuals enrolled in each study.

[3] Baseline CRP concentrations were not significantly different between groups, except for the low-fat milk and "low-fat milk + micronutrients" interventions in Rosado, 2011 (36). N/A = unreported baseline CRP concentrations.

[4] ↓ = statistically significant reduction; ↔ = no change; ↑ = statistically significant increase.

[5] Detailed descriptions of the isoenergetic and hypoenergetic studies are found in references (39) and (40), respectively.

[6] Separate numbers of men and women represent individuals who completed the study, as these data were not reported for individuals who were enrolled.

[7] Results for CRP are reported in another paper by van Meijl and Mensink (42).

[8] Unclear definition of the primary outcome, but statistical power shown to be sufficient for the assessment of TNF-α, MCP-1, and s-TNFR-1 and 2.

TABLE 2. Evaluation of the risk of bias in randomized controlled trials that assessed the impact of dairy products consumption on circulating inflammatory biomarkers

Study	Criteria							
	Random sequence generation	Allocation concealment[1]	Blinding of participants	Blinding of personnel	Blinding of outcome assessment[2]	Incomplete outcome data[3]	Selective reporting	Other possible bias[4]
Stancliffe, 2011 (32)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	High risk	Unclear risk
Thompson, 2005 (33)	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk
Zemel and Sun, 2008 (34)[5]: *Isoenergetic study*	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Unclear risk
Hypoenergetic study	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
Wennersberg, 2009 (35)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Unclear risk
Rosado, 2011 (36)	Low risk	Low risk	Unclear risk[6]	Unclear risk[6]	Unclear risk[7]	Low risk	Unclear risk	Low risk
Zemel, 2010 (37)	Unclear risk	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk for baseline imbalance, but high risk for carry-over effect
van Meijl and Mensink, 2010 (38)	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Low risk for baseline imbalance or carry-over effect

[1] Prior to assignment of participants to the interventions.

[2] Blinding of the staff that performed laboratory analyses.

[3] Incomplete report of the number of attrition and exclusions related to inflammatory biomarkers analyses, together with reasons.

[4] Baseline imbalance in factors strongly related to outcome measures or carry-over effect in crossover studies.

[5] Detailed descriptions of the isoenergetic and hypoenergetic studies are found in references (39) and (40), respectively.

[6] The "Unclear risk" statement applies to the comparison between the two milk interventions and the control intervention. Thus, participants and personnel were blinded to the low-fat milk vs. "low-fat milk + micronutrients" intervention.

[7] The "Unclear risk" statement applies to the comparison between the two milk interventions and the control intervention. Thus, outcome assessors were blinded to the low-fat milk vs. "low-fat milk + micronutrients" intervention.

FIGURES

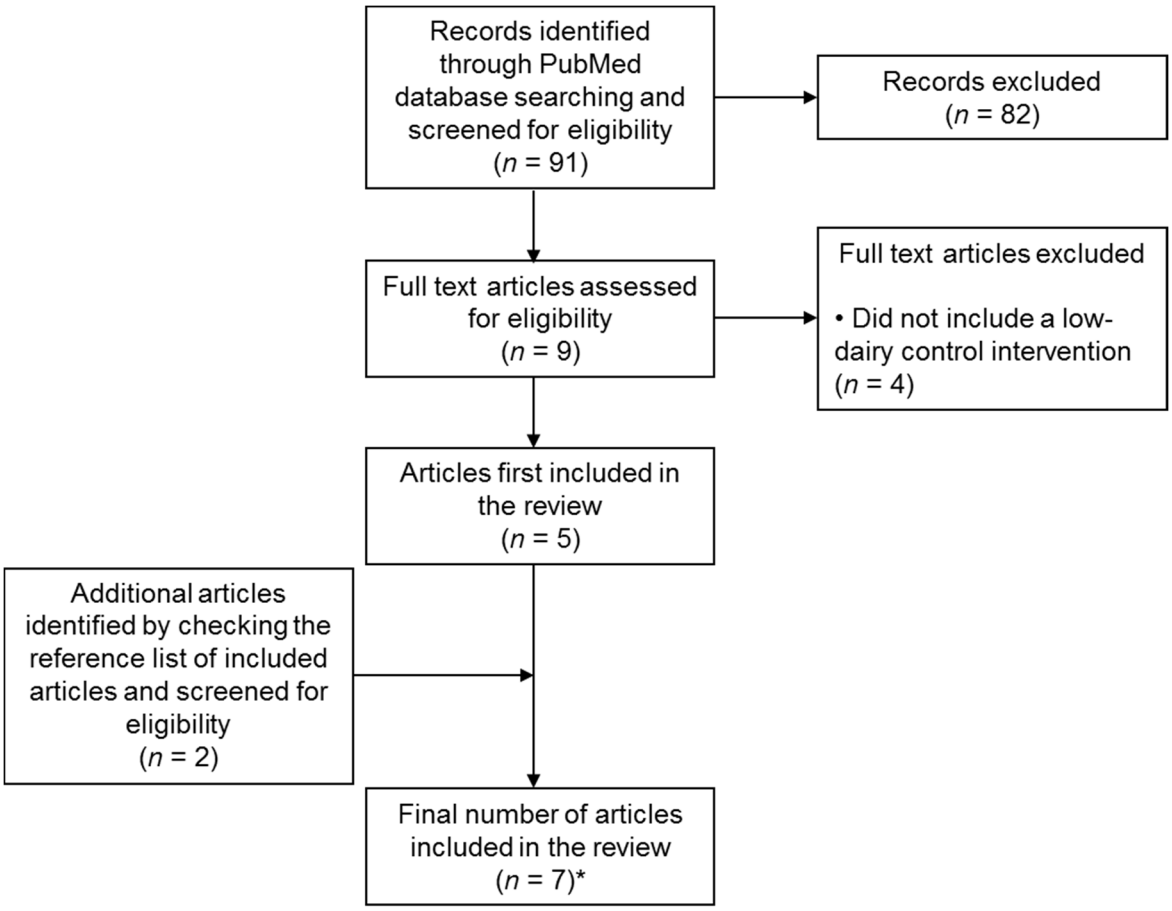

FIGURE 1. Flow diagram of study selection**.** *One out of the seven research articles described results pertaining to inflammatory biomarkers from two different randomized controlled nutritional intervention trials. Pubmed, www.ncbi.nlm.nih.gov/pubmed/.

SUPPLEMENTARY FILES

SUPPLEMENTAL TABLE 1. Details of the evaluation of the risk of bias in randomized controlled trials that assessed the impact of dairy products consumption on circulating inflammatory biomarkers[1]

Study	Criteria							
	Random sequence generation	Allocation concealment[2]	Blinding of participants	Blinding of personnel	Blinding of outcome assessment[3]	Incomplete outcome data[4]	Selective reporting	Other possible bias[5]
Stancliffe, 2011 (32)	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* All enrolled subjects completed the trial and were included in the analyses.	High risk *Justification:* According to information found in ClinicalTrials.gov for this study, not all of the study's pre-specified inflammatory stress outcomes have been reported in the published paper (i.e. missing results for IL-15).	Unclear risk *Justification:* No baseline differences were observed between groups for age, sex, BMI, blood pressure and plasma lipids-lipoproteins concentrations, but baseline inflammatory biomarkers concentrations were not formally compared.

Thompson, 2005 (33)	Low risk *Justification:* Computer randomization algorithm generated by a statistician.	Low risk *Justification:* Participants were assigned to their groups by the unit secretary of the General Clinical Research Center (otherwise uninvolved with the study). The sequence was concealed until the intervention was assigned.	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* Number of attrition and exclusions related to inflammatory biomarkers analyses was adequately reported, together with reasons. Intention-to-treat analyses were performed, and it was found that results were unchanged.	Unclear risk *Justification:* Insufficient information to permit clear judgement	Low risk *Justification:* No baseline differences between groups, including no difference in age, sex, BMI and CRP concentrations.
Zemel and Sun, 2008 (34)[6]:								
Isoenergetic study	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* Randomization performed at the end of the run-in period, i.e. after the enrollment of participants.	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* Number of attrition and exclusions related to inflammatory biomarkers analyses was adequately reported in the primary study (39), together with reasons. Those who did not complete the study did not have	Unclear risk *Justification:* Insufficient information to permit clear judgement	Unclear risk *Justification:* No baseline differences were observed between groups for age and BMI, but baseline inflammatory biomarkers concentrations were not formally compared. Also, the number of

			could have directly influenced inflammatory biomarkers concentrations.	could have directly influenced inflammatory biomarkers concentrations.		baseline characteristics significantly different than those who did complete the study.		men and women was unbalanced between the low-dairy (8 men vs. 9 women) and high-dairy diets (3 men vs. 14 women).
Hypoenergetic study	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* Randomization performed at the end of the run-in period, i.e. after the enrollment of participants.	Low risk *Justification:* Use of a flavored gelatin dessert as a placebo.	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Number of attrition (drop-out) was provided together with reasons in the primary study (40). However, in subsequent analyses of CRP and adiponectin (34), two subjects appeared to have been excluded from the yogurt-enriched group and reasons for these exclusions are not reported (n =16 instead of n =18).	Unclear risk *Justification:* Insufficient information to permit clear judgement	Unclear risk *Justification:* No baseline differences were observed between groups for age, sex, BMI, blood pressure and plasma lipids-lipoproteins concentrations, but baseline inflammatory biomarkers concentrations were not formally compared.

Wennersberg, 2009 (35)	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* Number of attrition and exclusions related to inflammatory biomarkers analyses was adequately reported, together with reasons.	Unclear risk *Justification:* Insufficient information to permit clear judgement	Unclear risk *Justification:* Most of the clinical and biochemical characteristics including age, sex, BMI and hs-CRP concentrations were similar between groups at baseline, but adiponectin, which was an outcome of interest, was higher (P = 0.021) and HDL-C was lower (P = 0.019) at baseline in the dairy group vs. control group. Thus, it is unclear whether the risk of baseline imbalance should be classified as high or not.
Rosado, 2011 (36)	Low risk *Justification:* Use of computer randomization.	Low risk *Justification:* Participants assigned to their groups by a	Unclear risk[7] *Justification:* There was no blinding and this would have	Unclear risk[7] *Justification:* There was no blinding and this would have	Unclear risk[8] *Justification:* Unspecified in the paper	Low risk *Justification:* Number of attrition and exclusions related	Unclear risk *Justification:* Insufficient information to permit clear	Low risk *Justification:* CRP concentrations were similar at

		researcher who had no direct contact with participants. A treatment code was also assigned to participants.	normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.		to inflammatory biomarkers analyses was adequately reported, together with reasons.	judgement	baseline between each of the milk interventions and the control intervention. CRP was significantly higher in the "low-fat milk + micronutrients" vs. low-fat milk group, but this was not the comparison of interest here. Other baseline characteristics including age, sex and BMI were similar between groups.
Zemel, 2010 (37)	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* Use of fruit-flavored soy-based smoothies as a placebo.	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Data from at least one subject are missing in inflammatory biomarkers analyses in each phase (dairy and soy-based smoothies), but reasons for these exclusions are not reported.	Unclear risk *Justification:* Insufficient information obtained from the published paper as well as ClinicalTrials.gov to permit clear judgement	Low risk for baseline imbalance, but high risk for carry-over effect *Justification:* No significant difference between baseline values of the two dietary phases for obesity indices and inflammatory biomarkers. Moreover, due

								to the nature of the study design (crossover), subjects served as their own control. A carry-over effect was present for MCP-1: Subjects who received dairy before soy exhibited a significant decrease, whereas those who received soy before dairy exhibited an increase from baseline in MCP-1 concentrations (*P* for treatment order = 0.0173).
van Meijl and Mensink, 2010 (38)	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* Unspecified in the paper	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention	Unclear risk *Justification:* There was no blinding and this would have normally been associated with a high risk of bias. However, blinding was impossible due to the nature of the intervention	Unclear risk *Justification:* Unspecified in the paper	Low risk *Justification:* Number of attrition and exclusions related to inflammatory biomarkers analyses was adequately reported, together with reasons.	Unclear risk *Justification:* Insufficient information to permit clear judgement	Low risk for baseline imbalance or carry-over effect *Justification:* Baseline values at the beginning of each dietary phase are not reported, but due to the nature of the

		and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.	and it is unclear whether this lack of blinding could have directly influenced inflammatory biomarkers concentrations.			study design (crossover), subjects served as their own control. No time or sequence effects were observed in performed analyses (no carry-over effect).

[1] CRP, C-reactive protein; HDL-C, HDL-cholesterol; MCP-1, monocyte chemoattractant protein 1.

[2] Prior to assignment of participants to the interventions.

[3] Blinding of the staff that performed laboratory analyses.

[4] Incomplete report of the number of attrition and exclusions related to inflammatory biomarkers analyses, together with reasons.

[5] Risk of bias related to baseline imbalance in factors strongly related to outcome measures or carry-over effect in crossover studies.

[6] Detailed descriptions of the isoenergetic and hypoenergetic studies are found in references (39) and (40), respectively.

[7] The "Unclear risk" statement applies to the comparison between the two milk interventions and the control intervention. Thus, participants and personnel were blinded to the low-fat milk vs. "low-fat milk + micronutrients" intervention.

[8] The "Unclear risk" statement applies to the comparison between the two milk interventions and the control intervention. Thus, outcome assessors were blinded to the low-fat milk vs. "low-fat milk + micronutrients" intervention.

CHAPITRE 9 :

LA CONSOMMATION DE PRODUITS LAITIERS N'A PAS D'IMPACT SUR LES BIOMARQUEURS INFLAMMATOIRES CHEZ DES HOMMES ET DES FEMMES AVEC UNE INFLAMMATION SYSTÉMIQUE DE FAIBLE INTENSITÉ

Labonté ME, Cyr A, Abdullah MM, Lépine MC, Vohl MC, Jones P, Couture P, Lamarche B.

Dairy product consumption has no impact on biomarkers of inflammation among men and women with low-grade systemic inflammation

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http://jn.nutrition.org/content/144/11/1760.long

RÉSUMÉ

Nous avons évalué l'impact de la consommation de produits laitiers sur l'inflammation chez des hommes et des femmes présentant une inflammation systémique de faible intensité. Cette étude multicentrique randomisée, en chassé-croisé, incluait 112 hommes et femmes adultes avec des concentrations de la hs-CRP au-dessus de 1 mg/L. Les participants ont consommé 3 portions/jour de produits laitiers (375 mL de lait à 1% de M.G. ; 175 g de yogourt à 1,5% de M.G. ; 30 g de fromage cheddar à 34% de M.G.) ou des produits « témoins » équivalents en énergie (jus de fruits, jus de légumes, noix de cajou, biscuit) dans le cadre de diètes « prudentes » d'une durée de 4 semaines chacune. Chaque phase (diète) était séparée d'une période de repos (« *washout* ») de 4 à 8 semaines. Les concentrations sériques de biomarqueurs inflammatoires ont été mesurées au début et à la fin de chaque diète. L'expression de gènes inflammatoires et de facteurs de transcription dans les cellules sanguines complètes a été mesurée à la fin de chaque diète par amplification en chaîne par polymérase dans un sous-groupe aléatoire de 53 sujets. L'analyse des changements à l'intérieur de chacune des diètes (valeurs post- moins pré-diète) a montré une réduction des concentrations de la hs-CRP suite à la diète témoin (-11,7%, $P = 0,05$), mais aucun changement suite à la diète riche en produits laitiers (-7,3%, $P = 0,47$). Ainsi, les variations de la hs-CRP différaient entre les deux diètes ($P = 0,04$). Les diètes témoin et riche en produits laitiers ont réduit de façon similaire les concentrations de l'IL-6 comparativement aux valeurs initiales spécifiques à chacune (-17,6% et -19,9%, respectivement, $P < 0,0001$ pour les deux, $P = 0,77$ pour la comparaison entre les diètes). Aucune différence à l'intérieur des diètes ou entre celles-ci n'a été observée dans les concentrations d'adiponectine. Il n'y avait également aucune différence entre les diètes dans l'expression de gènes inflammatoires et de facteurs de transcription. En accord avec les résultats des études précédentes, ces résultats suggèrent que la consommation à court terme d'une combinaison de produits laitiers faibles et riches en matières grasses dans le cadre d'une alimentation saine n'exerce aucun effet néfaste sur l'inflammation.

TITLE PAGE

TITLE

Dairy product consumption has no impact on biomarkers of inflammation among men and women with low-grade systemic inflammation

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RUNNING TITLE: Dairy consumption and inflammation

ABBREVIATIONS: 25(OH)D, 25-hydroxyvitamin D; *CCL2*, chemokine (C-C motif) ligand 2; CHO, carbohydrate; CVD, cardiovascular disease; FSH, follicle-stimulating hormone; *G6PD*, glucose-6-phosphate dehydrogenase; hs-CRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein 1; *NFKB1*, NF-κB subunit 1; *NPR3*, natriuretic peptide receptor C; *PPARA*, PPAR alpha; *SREBF2*, sterol regulatory element binding transcription factor 2; *TRAF3*, TNF receptor-associated factor 3

ABSTRACT

Background: Randomized controlled trials specifically designed to assess inflammation-related outcomes in response to dairy consumption are lacking. **Objective:** We investigated the impact of dairy food consumption on biomarkers of inflammation in healthy men and women with low-grade systemic inflammation. **Methods:** In a multicenter randomized crossover study, 112 adult men and women with high-sensitivity C-reactive protein (hs-CRP) values > 1 mg/L consumed 3 servings/d of DAIRY (375 mL low-fat milk, 175 g low-fat yogurt and 30 g regular fat cheddar cheese) or energy-matched CONTROL products (fruit juice, vegetable juice, cashews and one cookie) as part of prudent 4-wk diets, each separated by a 4-8 wk washout period. Serum concentrations of inflammation biomarkers were measured at the beginning and end of each dietary phase. Expression levels of key inflammatory genes and transcription factors in whole blood cells were assessed at the end of each diet by real-time polymerase chain reaction in a random subset of 53 subjects. **Results:** Analysis of within-diet changes (post- vs. pre-diet values) showed a significant reduction in hs-CRP concentrations after CONTROL (-11.7%, P=0.05), but no change after DAIRY (-7.3%, P=0.47). As a result, changes in hs-CRP differed between DAIRY and CONTROL (P=0.04). Both the CONTROL and DAIRY diets similarly reduced interleukin (IL)-6 concentrations compared with diet-specific baseline values (-17.6 and -19.9%, respectively, P<0.0001 for both, P=0.77 for between-diet comparison). No between- nor within-diet difference was observed in adiponectin concentrations and there was also no between-diet difference in the expression of inflammatory genes and transcription factors. **Conclusions:** Consistent with data from previous work, these results suggest that short-term consumption of a combination of low- and high-fat dairy products as part of a healthy diet has no adverse effects on inflammation (ClinicalTrials.gov Identifier: NCT01444326).

KEYWORDS: C-reactive protein, dairy, inflammatory markers, randomized controlled trial, gene expression, adults with low-grade systemic inflammation

INTRODUCTION

A growing body of evidence from epidemiological studies suggests that milk and dairy product consumption is associated with a modest but significant reduction in the risk of cardiovascular disease (CVD) (1), coronary heart disease (2-4) and type 2 diabetes (2-8). Dairy product consumption may also be protective against metabolic syndrome, although this evidence remains equivocal (9). The presence of an underlying pro-inflammatory state, characterized by increased circulating concentrations of C-reactive protein (CRP) and inflammatory cytokines (e.g. IL-6 and TNF-α) as well as decreased concentrations of anti-inflammatory mediators such as adiponectin, plays a central role in the pathophysiology of several disorders, including metabolic syndrome and CVD (10-12).

Conflicting results emerge regarding the impact of dairy products on inflammation. Earlier cell culture studies have shown that incubating mononuclear cells with lactic acid bacteria stimulates the production of pro-inflammatory cytokines (13-15). Yet in humans, cross-sectional studies have quite consistently showed that consumption of dairy, particularly low-fat dairy products, as part of a healthy diet was associated with less systemic inflammation (16-19). We have recently shown in a systematic review of randomized controlled nutritional intervention studies that dairy product consumption did not promote a pro-inflammatory state in overweight and obese adults (20). However, definite conclusions on a beneficial impact of dairy products on inflammation could not be drawn. Our systematic review stressed the need for additional studies on this topic, with particular emphasis on considering inflammation as a primary study outcome.

The primary objective of this randomized nutritional intervention study was to investigate the impact of dairy food consumption on biomarkers of inflammation in healthy men and women with low-grade systemic inflammation. Based on the apparent cardioprotective effects of dairy products, we hypothesized that dairy food consumption has favorable effects on the inflammatory profile. We also investigated how dairy food consumption modifies the expression of inflammatory genes in whole blood cells.

MATERIALS AND METHODS

Participants

This research was undertaken as a multicenter study involving two academic Canadian research centers, the Institute of Nutrition and Functional Foods (INAF) in Quebec City and the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) in Winnipeg. Men and women were recruited between January and September 2011 through the media and electronic newsletter to participate in the study. For eligibility, participants had to be between 18-70 y of age with sub-clinical inflammation as assessed by two consecutive measurements of plasma high-sensitivity (hs)-CRP concentrations above 1.0 and below 10.0 mg/L. The *a priori* defined lower limit of 2.0 mg/L for hs-CRP was changed to 1.0 mg/L during the course of the study to facilitate recruitment. There was no eligibility criterion based on usual dairy intake but participants were asked to reduce their dairy intake to 2 servings/d or less during the run-in period. Exclusion criteria included: smoking, body weight variation over the last 6 mo greater than 10%; a BMI over 35 kg/m^2; a previous history of CVD, type 2 diabetes, monogenic dyslipidemia, or any diagnosed endocrine or gastrointestinal disorder; the use of anti-hypertensive, anti-inflammatory or lipid-lowering medication (i.e. statins); allergies and intolerance to dairy foods; aversion to foods provided during the study and clinical use of vitamin D and calcium supplements. The *a priori* defined cutoff of 35 kg/m^2 for BMI was modified during the recruitment phase to include two participants (one in each center) with a BMI of 36.3 kg/m^2. Among premenopausal women, only those with a regular menstrual cycle (25–35 d) for the past 3 mo were included. All postmenopausal women were eligible irrespective of their hormone supplementation status as long as it remained constant throughout the study. Follicle-stimulating hormone (FSH) measurements were performed to confirm the premenopausal or postmenopausal status (FSH < 20 IU/L or FSH > 25 IU/L). Women who had initiated hormone replacement therapy within 6 mo of study onset were not eligible. Women who used contraceptive agents were not excluded from the study. Participants were enrolled by MCL and AC at INAF and by MMA at RCFFN. The study protocol was fully explained to all participants, who gave written informed consent at the time of enrollment. The study protocol was in accordance with the ethical standards of each institution involved. A total

of 212 men and women were found to be eligible (**Figure 1**), among whom 137 were randomized to one of the two intervention sequences. Seven participants never started the intervention and six dropped out due to the incapacity to commit to the demanding nature of the protocol. Of the remaining 124 participants who completed the two dietary phases, twelve were excluded from analyses because of a post-diet value of hs-CRP above 10 mg/L in at least one of the two dietary phases, which is an indication of an acute inflammatory response (21). Retaining these participants in the analyses did not materially alter the results (not shown).

Study design and diets composition

The study was undertaken using a randomized, crossover design with two 4-wk treatments, DAIRY and CONTROL. During a 2-wk run-in period, enrolled participants received dietary advice by a registered dietitian on how to adapt their diet to a prudent dietary pattern characterized by low intakes of SFA (< 10% energy), *trans* fat (< 1% energy), dietary cholesterol (< 200 mg/d) and sodium (< 2300 mg/d) (22). The total amount of fat was not restricted, with recommendations ranging from 25-35% of total energy. Substituting saturated by unsaturated fat was advocated. Advice on how to restrict the consumption of processed and energy-dense foods were provided, with also a specific recommendation to consume a maximum of 2 servings of dairy products per d during the run-in. No food was provided to participants during this 2-wk run-in period. Randomization of participants to one of the two diet sequences (DAIRY/CONTROL or CONTROL/DAIRY) was computer-generated after the run-in period by a member of the research team at INAF without any knowledge of participants' status and eligibility. The sequence for each participant was thereafter passed on to the study coordinators at both centers. Randomization to diet sequences within each center was further stratified for baseline hs-CRP concentrations (1-2 mg/L and 2-10 mg/L based on the screening results) and sex. The backbone of the CONTROL and DAIRY diets corresponded to the prudent dietary pattern recommended during the run-in period. However, during the DAIRY diet, participants had to specifically incorporate 3 servings of dairy products into their everyday diet. The following combination of low- and high-fat dairy products was provided to participants on a weekly basis by the research team: 375 mL/d of low-fat milk (1% M.F.),

175 g/d of low-fat yogurt (1.5% M.F.) and 30 g/d of regular fat cheddar cheese (34% M.F.). During the CONTROL diet, participants were provided with the following energy-equivalent control products on a weekly basis: 290 mL/d of 100% fruit juice (Oasis® Classic line), 156 mL/d of vegetable juice (V8® Original Vegetable Cocktail) (23), 20 g/d of cashews and one cookie (39 g/d) prepared in the metabolic kitchen of each research center using a standardized recipe. Available fruit juice flavours were orange, apple or wildberry. Orange and apple were the most popular flavours among participants. The nutritional composition of all three flavours was similar (24). Consumption of dairy products during the CONTROL diet was forbidden. The composition of DAIRY and CONTROL foods is presented in **Table 1**. The two diets were separated by a 4- to 8-wk washout period during which participants were also asked to comply with the prudent dietary pattern described above. Participants were asked to maintain their body weight and usual levels of physical activity constant throughout the duration of the study. They were specifically told that this was not a weight loss study. A 3-d physical activity journal was collected at the end of each dietary phase. Consumption of tea and coffee (without milk or cream) was allowed within a limit of 2 cups (500 mL)/d. Alcohol was restricted to 1 drink/d, up to 5 per wk (1 drink was considered as a normal size bottle of beer [340 ml], a glass of wine [150 ml] or a glass of liquor [45 ml]). Supplementation with vitamins and natural health products was strictly forbidden throughout the study. Anti-inflammatory medication such as ibuprofen was also prohibited during the intervention. Study coordinators and participants were not blinded due to the nature of the interventions.

Dietary assessment

Dietary intake was assessed at screening and at the end of each dietary phase using a validated and highly reproducible web-based self-administered FFQ (25), which justifies its use in nutrition intervention studies assessing dietary intakes of participants over time, as is the case in the present crossover study. Intakes of specific food groups in terms of servings/d obtained from the web-FFQ were calculated based on servings of Canada's Food Guide (26).

Compliance

Compliance to the dietary recommendations was assessed using data from the web-FFQ and a checklist provided each wk to identify DAIRY or CONTROL foods that were consumed or not. This list also provided space to indicate alcohol, tea and coffee consumption as well as medication use. Participants had to notify the physician in charge of the clinical aspects of the study before initiating any medication. Behavioral and psychological counseling was offered to participants to maximize the success of the nutritional changes. Compliance was also assessed by measuring serum 25-hydroxyvitamin D (25(OH)D) concentrations after the DAIRY and CONTROL dietary phases. The concentration of 25(OH)D is expected to increase when dietary intake of vitamin D is increased (27).

Clinical measures at screening

Systolic and diastolic blood pressures at screening were averaged from 3 measurements taken after a 10-min rest in the sitting position with an automated blood pressure monitor (BPM 300-BpTRU model; Vital Signs Monitor). Serum lipid profile at screening was performed according to methods described previously (28-31). hs-CRP concentrations at screening were measured by nephelometry as previously described (32). The CV of this assay is <1% for both low and high hs-CRP concentrations (32).

Anthropometric measures

Body weight, waist and hip circumferences were measured according to standardized procedures at screening as well as at the beginning and at the end of each dietary phase (33). Body weight was specifically measured twice within the last wk of each diet. The mean of the two post-diet values of body weight was used for the calculation of BMI post-diet. Lean and fat mass were measured by the DXA technique (GE Healthcare, Madison, WI) once at the end of each dietary phase.

Inflammatory biomarkers

Fasting blood samples (12-h) were collected from an antecubital vein once at the beginning and twice within the last wk of each dietary phase, 2-5 d apart. The mean of the two post-diet values was used in analyses of hs-CRP, IL-6 and adiponectin in order to minimize intra-individual variability. Serum hs-CRP concentrations were measured using a commercial ELISA kit for the human form (BioCheck Inc, Foster City, CA). Serum IL-6 (R&D Systems Europe, Inc.) and adiponectin concentrations (B-Bridge International, Inc.) were also measured using commercial ELISA kits. The intra-assay CV were 4.4%, 7.4% and 5.2% for hs-CRP, IL-6 and adiponectin, respectively. The laboratory staff was blinded to the dietary interventions.

Inflammatory gene expression analysis

Samples were collected using PAXGene Blood RNA tubes (Qiagen, Valencia, CA) at the end of each dietary phase for the direct measurement of inflammatory gene expression in whole blood cells in a random subsample of 53 participants. Total RNA was first isolated from 2.5 mL of whole blood samples using the PAXGene Blood RNA purification kit (Qiagen, Valencia, CA) according to manufacturer's instructions. RNA was thereafter quantified using NanoDrop (Thermo Fisher, Wilmington, DE). cDNA was synthesized from 1 µg of RNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies). Gene expression was measured by qRT-PCR using TaqMan® OpenArray® Real-Time PCR Plates with Inventoried Gene Expression Assays (Applied Biosystems, Foster City, CA) and the QuantStudio 12K Flex Software (Applied Biosystems, Foster City, CA). GenBank and Life Technologies numbers of the analyzed genes are provided in **Supplemental Table 1**. Each sample was analyzed in triplicate. Expression of the target genes was normalized to the expression of the internal control gene glucose-6-phosphate dehydrogenase (*G6PD*) using the formula ΔCt = (Ct value of the target gene – Ct value of the internal control gene) (34). Ct values used in the formula consisted of the mean of the triplicates' individual Ct values, unless the SD of a triplicate was > 0.5, in which case the outlier value was excluded from the calculation of the mean. Expression of the following 10 inflammation-related genes and transcription factors was measured: chemokine (C-C motif) ligand 2 (*CCL2*), *IL18*, *IL6*, *IL1B*, nuclear factor kappa-B subunit 1 (*NFKB1*),

natriuretic peptide receptor C (*NPR3*), peroxisome proliferator-activated receptor alpha (*PPARA*), sterol regulatory element-binding transcription factor 2 (*SREBF2*), *TNF*, and TNF receptor-associated factor 3 (*TRAF3*). *CCL2*, *IL6* and *NPR3* were not retained in the analyses since their level of expression was too low to be detected in approximately half of the samples and thus did not allow reliable data to be obtained. Normalization of the target genes to the expression of the internal control gene *GAPDH* was also considered and led to similar results, but *G6PD* showed the smallest variation in expression after the DAIRY vs. CONTROL diet (fold change -1.01 for *G6PD* vs. 1.05 for *GAPDH*).

Sample size calculation

Sample size estimates were calculated based on data from Zemel *et al.* (35). When combining the various groups in that study (overweight, obese, all) and the 2 time points during the intervention (7 and 28 d), the SD of the change in plasma hs-CRP in response to dairy was on average 300% (3-fold) greater than the actual change in mg/L in that study (35). Our anticipated dropout rate was set at 15%. Based on these considerations and on the assumption that the average hs-CRP concentration at baseline would be around 3 mg/L as per our inclusion criteria, we have calculated that the study with 120 subjects completing the two phases of the intervention would allow us to detect a clinically meaningful 0.8 mg/L (25%) difference in hs-CRP concentrations between the DAIRY and CONTROL diets, with a power of 82% and a type 1 error (α) of 5%. Between-diet differences in other markers of inflammation, anthropometry indices and relative expression levels of inflammatory genes represented secondary study outcomes.

Statistical analyses

The pre-specified primary analysis was undertaken using a *per protocol* approach. This analysis consisted of assessing within-diet changes in study outcomes (i.e. delta scores calculated as post-diet [wk 4] minus pre-diet [wk 0] values in the DAIRY and CONTROL diets) as well as between-diet (DAIRY vs. CONTROL) differences in delta scores using the MIXED procedure for repeated measures in SAS (version 9.2; SAS Institute, Cary, NC), with diet as a fixed effect and subject as a random effect. The significance of within-diet changes was determined using the least-square means statistic in the MIXED procedure.

The pre-specified secondary analysis consisted of comparing post-diet values of outcome variables between the DAIRY and CONTROL diets also using the MIXED procedure, with diet as a fixed effect and subject as a random effect. The structure of the covariance matrix for each variable was taken into account in all analyses to ensure the most adequate statistical fit of the model to the experimental data. Pre-specified potential confounders of the inflammatory markers' response to diet, namely sex, study center, and pre-diet inflammatory status, were included as covariates if they were found to be significant at $P < 0.05$ in a selected model. Further adjustment for anthropometry indices (i.e. waist circumference or BMI) had no impact on the results and thus was not retained in any of the analyses. The interaction of the above mentioned potential confounders as well as the interaction of age and menopausal status with the main treatment effect was also tested in all analyses using appropriate interaction terms and multivariate modeling. No such interactions were found. Analyses also showed no evidence of a carry-over effect of the dietary treatments on inflammatory outcomes (not shown), except for the relative *IL1B* gene expression levels (described in the Results section). Univariate correlations among key outcomes were assessed using Spearman correlation coefficients. Baseline characteristics of the random subsample of 53 subjects used for gene expression analyses were compared with those of the whole study sample using the Student's unpaired t-test. Variables with a normal distribution are reported in the text as means ± SD. Abnormally distributed variables were natural log-transformed before statistical analysis and are reported in the text as geometric means (95% CI), except data pertaining to dietary intakes. Indeed, dietary intakes were analyzed using non-parametric tests (Friedman test or Wilcoxon matched-pairs signed-rank test) and data are therefore reported as medians (IQR). Frequencies are reported in the text as % with number of participants in parentheses. Differences at $P \leq 0.05$ were considered significant.

RESULTS

The 112 subjects who were included in the final *per protocol* analysis had a mean (± SD) age of 40.1 ± 16.7 y and were primarily women (66.1%, *n*=74) and Caucasian (66.7%, *n*=74) (**Table 2**). They were in good health with average blood pressure and cholesterol concentrations within acceptable ranges (36). The mean concentration of hs-CRP at

screening was 2.51 mg/L (geometric mean, 95% CI: 2.29-2.74). Among women, 35.1% (*n*=26) were postmenopausal and 6.8% (*n*=5) had been using hormone replacement therapy for more than 6 mo. Among premenopausal women, 43.8% (*n*=21) were using oral contraceptives. Participants' intake of dairy products was 2.0 servings/d (median, IQR: 1.9, **Table 3**) at screening. We calculated based on checklists that 98.5 ± 2.8% and 96.6 ± 5.9% (means ± SD) of the DAIRY and CONTROL products were consumed by participants during the intervention periods. Self-reported intakes of the test foods during each phase also matched very closely the number of servings provided to participants (Table 3). Serum concentrations of 25(OH)D were significantly higher after DAIRY (76.4 ± 27.8 nmol/L) than after CONTROL (68.2 ± 29.0 nmol/L, $P = 0.0004$).

Intakes of grain products and animal proteins in terms of servings per day did not differ between DAIRY and CONTROL (Table 3). However, intakes of dairy products, fruits and vegetables, meat and alternatives, and vegetable proteins all differed between the two diets (all $P \leq 0.0006$). In terms of nutrient intake, participants during the DAIRY diet consumed more energy, protein, SFA, dietary cholesterol, calcium and vitamin D, but less carbohydrate, fiber, MUFA and PUFA than they did during the CONTROL diet (all $P < 0.05$) (**Supplemental Table 2**). However, after having removed food items provided to participants during the CONTROL and DAIRY diets from the self-reported dietary intake data, the remainder of these diets was very similar (**Supplemental Table 3**). The main residual differences in participants' background diet were a higher consumption of fruits (mostly from fruit juice), energy and carbohydrate and a lower consumption of protein in DAIRY vs. CONTROL (Supplemental Table 3).

There was a small increase in BMI after DAIRY (within-diet change of 0.4%, $P = 0.001$, **Table 4**), which remained significant after adjustment for energy intake. Within-diet changes in BMI as well as post-diet values of BMI also differed between CONTROL and DAIRY ($P = 0.0007$ for both), but again differences were very small and remained significant after adjustment for energy intake. However, differences in BMI between diets were not correlated with differences in hs-CRP, IL-6 or adiponectin ($r = 0.04$, $P = 0.67$; $r = 0.03$, $P = 0.73$; $r = -0.004$, $P = 0.97$, respectively). Variations in BMI within the DAIRY or CONTROL diet were also not significantly correlated with variations in hs-CRP, IL-6 or

adiponectin (-0.09 < r < 0.18). The CONTROL and DAIRY diets similarly increased waist circumference compared with pre-diet values (0.9% and 1.4%, respectively, within-diet $P \leq$ 0.01 for both, between-diet P = 0.56 for the comparison of delta scores). Post-diet waist circumference, abdominal fat mass and total body fat mass did not differ between the CONTROL and DAIRY diets ($P \geq$ 0.32).

The two measures of hs-CRP post-diet were strongly correlated after both the CONTROL and DAIRY diets (r = 0.89 and 0.88, respectively, both P < 0.0001) (**Supplemental Figure 1)**. The change vs. baseline in hs-CRP differed between DAIRY and CONTROL (P = 0.04, Table 4). Specifically, hs-CRP concentrations were reduced after CONTROL vs. diet-specific baseline values (-11.7%, within-diet P = 0.05, Table 4), while they were unchanged after DAIRY (-7.3%, P = 0.47). Both the CONTROL and DAIRY diets reduced IL-6 concentrations compared with diet-specific baseline values (-17.6 and -19.9%, respectively, within-diet P < 0.0001 for both) but these changes in IL-6 as well as post-diet IL-6 concentrations did not differ between the two diets ($P \geq$ 0.70). There was no between- nor within-diet difference in adiponectin concentrations (all $P \geq$ 0.23).

Expression of key inflammatory genes and transcription factors in whole blood cells in response to DAIRY vs. CONTROL was examined in a random subsample of 53 subjects having participated in both centers. Baseline characteristics of these 53 subjects did not differ from characteristics of the whole sample of 112 participants (P > 0.25, not shown). Between-diet differences in inflammatory biomarkers in this subgroup of subjects also reflected differences seen in the whole study sample (not shown). Post-diet expression levels of *IL18*, *IL1B*, *NFKB1*, *PPARA*, *SREBF2*, *TNF* and *TRAF3* did not differ between CONTROL and DAIRY (all $P \geq$ 0.12) (**Supplemental Table 4**). However, there was an effect of the diet sequence on the relative expression level of *IL1B* (P = 0.02 for the interaction; not shown): this level was higher after DAIRY in participants who consumed the CONTROL diet first (P = 0.02, not shown), while it remained unchanged in participants who consumed the DAIRY diet first (P = 0.30, not shown).

DISCUSSION

The present randomized crossover study assessed the impact of dairy consumption as part of a prudent diet on inflammation using hs-CRP as a primary outcome and other recognized inflammatory biomarkers as secondary outcomes. We also explored the impact of dairy consumption on the expression levels of genes acting as transcription factors and involved in the inflammatory response. Compared with diet-specific baseline values, short-term consumption of approximately 3 servings/d of commercially available dairy products had no impact on hs-CRP or adiponectin concentrations, but reduced IL-6 concentrations. Consumption of a control dairy-free diet reduced hs-CRP and IL-6 concentrations compared with diet-specific baseline values, but had no impact on adiponectin concentrations. As a result, the reduction in hs-CRP after the CONTROL diet was significantly greater than the small non-significant variation seen after the DAIRY diet, while variations in IL-6 and adiponectin concentrations were comparable between the two diets. These observations are unlikely to have been confounded by changes in obesity indices, although the absence of pre-diet data for abdominal fat mass and total body fat in the present study limits our analysis and interpretation of potential variation in body fat distribution during either diet.

Results from available studies that have documented the impact of dairy consumption on inflammatory biomarkers have been inconsistent. In a recent systematic review of the literature (20), we have highlighted that only one of eight clinical studies so far was *a priori* designed for that purpose. This one study reported favorable effects of adequate dairy consumption over 12 wk (> 3.5 daily servings vs. < 0.5 daily servings) on oxidative and inflammatory stress in individuals with metabolic syndrome (37). The biomarkers of inflammation that were favourably modified after dairy consumption in that study were hs-CRP, IL-6, TNF-α, monocyte chemoattractant protein 1 (MCP-1) and adiponectin. In the seven other randomized trials discussed in the systematic review, change in the inflammatory profile was assessed as a secondary or unclearly defined outcome (35, 38-42). Three of these seven studies reported inflammation-lowering effects of dairy consumption (35, 39). In the two studies reported by Zemel *et al.* in reference (39), concomitant reductions in adiposity indices such as total body fat and trunk fat after

consumption of dairy products are likely to have confounded their impact on the inflammatory profile (43, 44). In the other study, Zemel *et al.* (35) documented the short-term effects of a dairy-rich diet compared with soy products on inflammatory stress in overweight and obese subjects (n=20) in the absence of adiposity changes. The dairy-based diet had significant anti-inflammatory effects as evidenced by reductions in hs-CRP, IL-6, TNF-α and MCP-1 concentrations as well as an increase in adiponectin concentrations. These effects were evident by d 7 of the dairy-based diet and magnified at the end of the 28-d treatment period. The study length and crossover design were similar to features of the present study. However, the dairy-rich diet in Zemel *et al.* (35) incorporated smoothies formulated with non-fat dry milk, which does not reflect consumption of commercially available dairy products as per our own study. The extent to which differences in dairy product formulation between the two studies may have influenced the bioavailability of nutrients in dairy and, consequently, their impact on inflammation is unknown.

The purported benefit of dairy consumption on inflammation has not been observed consistently (38, 40-42). Van Meijl and Mensink (42, 45) reported no effect of an 8-wk low-fat dairy diet vs. a control diet on CRP and IL-6 among 40 overweight or obese subjects. This study shares several features with our study, including the crossover design, the use of CHO-rich control products (fruit juice and biscuits) and the relatively short-term duration of the diets. The lack of effect of dairy consumption on hs-CRP is also consistent with our findings.

Exploratory analyses in a subsample of study participants showed that consumption of 3 servings of dairy products per d had no impact on expression levels of key inflammatory genes and transcription factors in whole blood cells compared with a dairy-free diet. Consistent with our results, in a randomized parallel controlled feeding study, Van Loan *et al.* (46) showed no change in relative transcript abundances of inflammatory genes such as TNF-α, IL-6, MCP-1 (also known as CCL2), and IL-1β in subcutaneous adipose tissue of overweight and obese adults consuming energy-restricted diets combined with either adequate dairy intake (3-4 servings/d) or low dairy intake (≤ 1 serving/d). They also showed no between-diet difference in circulating concentrations of inflammatory mediators including pro-inflammatory cytokines, hs-CRP and adiponectin. To the best of our

knowledge, this is the only other report having assessed inflammatory gene expression levels following dairy consumption.

Limitations and strengths of this intervention trial need to be pointed out. First, in contrast with some of the previous studies, which matched macronutrient proportions between experimental diets (35, 37, 39, 41), the present study had by design inevitable but important differences in macronutrient proportions between the CONTROL and DAIRY diets. However, analyses of self-reported nutritional intakes showed that differences in global dietary intakes between the CONTROL and DAIRY diets were almost entirely attributable to foods provided during each phase of the intervention rather than to differences in participants' background diet between each phase (Supplemental Table 3). The higher consumption of "fruits" in DAIRY vs. CONTROL represented the major difference in participants' background diet, but it was mostly explained by a slightly higher intake of fruit juice while on DAIRY (not shown). The lack of diet-specific baseline data on food intake limits analysis and interpretation of dietary change when participants were on the CONTROL and DAIRY diets. This is also the case for gene expression levels, which were not measured at baseline of each dietary phase. It is stressed that the CONTROL diet had a beneficial impact on pro-inflammatory markers compared with diet-specific baseline values. A possible explanation for this effect is that control foods may have provided bioactive ingredients such as vitamins and phytochemical compounds, which have been suggested to exert potential anti-inflammatory effects in some (47-49), but not all studies (50-52). However, we argue that the use of commercially-available control products was appropriate and justified, each representing a plausible alternative to dairy products when transposed into a non-experimental, real-life setting. Both diets diet were consumed over a 4-wk period. This relatively short time frame was sufficient to observe significant reductions in IL-6 concentrations from baseline values. It is also stressed that two randomized controlled trials previously reported significant changes in inflammatory biomarkers even after shorter periods, i.e. after only one week of intervention with dairy products (35, 37). However, whether longer-term dairy consumption triggers a different anti-inflammatory response remains to be addressed. Key strengths of the present study include the randomized crossover and multicenter design, the large number of subjects enrolled, the fact that participants were specifically selected to have subclinical

inflammation, as well as the high compliance of subjects to the experimental procedures. The fact that inflammatory biomarkers concentrations were measured twice at the end of each diet to minimize intra-individual variations is also a significant strength. This is the first diet study, to the best of our knowledge, having used such an approach. The free-living nature of the protocol and the use of commercially available products make it relevant to everyday clinical practice.

In conclusion, we showed that short-term dairy consumption in the context of a prudent diet has no significant impact on hs-CRP or adiponectin concentrations, but reduces circulating levels of the pro-inflammatory cytokine IL-6 from baseline values. Our study was not able to relate any of these changes to variations in expression levels of key inflammatory genes in whole blood cells compared to a dairy-free diet in healthy men and women with low-grade systemic inflammation. Short-term dairy consumption was also associated with a slight increase in BMI, but this did not correlate with variations in the inflammatory profile. Combined with data from previous work, these results confirm that consumption of a combination of low- and high-fat dairy products as part of a healthy diet has no adverse effects on inflammation (20). Whether dairy consumption exerts anti-inflammatory effects is currently not fully substantiated by existing studies on this topic and requires further demonstration through well-designed and adequately powered studies of perhaps longer duration as well.

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CONFLICT OF INTEREST STATEMENT

Conflict of interest statement is as follows: MCV has received funding in the past from Danone Inc. PJ has received funding from the Dairy Farmers of Canada and Danone Inc. PC and BL have received funding in the past from the Dairy Farmers of Canada, Dairy Australia and Agriculture and Agri-Food Canada. BL also has received funding from Danone Institute and he is the Chair of the *Expert Scientific Advisory Committee* (ESAC) that reviews funding applications as part of the peer-reviewed research funding program of Dairy Farmers of Canada. MEL, AC, MMA, and MCL have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

The authors' responsibilities were as follows: BL, PC and PJ designed the study. PC was responsible for the screening and medical supervision of the study participants. MCV contributed her expertise for the gene expression studies. MCL and MMA coordinated the

study with the collaboration of AC. MEL and AC performed statistical analyses, interpreted the data, and wrote the manuscript. BL had primary responsibility for final content. All authors critically reviewed the manuscript and approved its final version.

REFERENCES

1. Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, Hu FB, Engberink MF, Willett WC, Geleijnse JM. Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. Am J Clin Nutr. 2011;93:158-71.

2. Rice BH, Quann EE, Miller GD. Meeting and exceeding dairy recommendations: effects of dairy consumption on nutrient intakes and risk of chronic disease. Nutr Rev. 2013;71:209-23.

3. Chrysant SG, Chrysant GS. An update on the cardiovascular pleiotropic effects of milk and milk products. J Clin Hypertens (Greenwich). 2013;15:503-10.

4. Elwood PC, Pickering JE, Givens DI, Gallacher JE. The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. Lipids. 2010;45:925-39.

5. Kalergis M, Leung Yinko SS, Nedelcu R. Dairy products and prevention of type 2 diabetes: implications for research and practice. Front Endocrinol (Lausanne). 2013;4:90.

6. Aune D, Norat T, Romundstad P, Vatten LJ. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. Am J Clin Nutr. 2013;98:1066-83.

7. Gao D, Ning N, Wang C, Wang Y, Li Q, Meng Z, Liu Y, Li Q. Dairy products consumption and risk of type 2 diabetes: systematic review and dose-response meta-analysis. PLoS One. 2013;8:e73965.

8. Tong X, Dong JY, Wu ZW, Li W, Qin LQ. Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. Eur J Clin Nutr. 2011;65:1027-31.

9. Crichton GE, Bryan J, Buckley J, Murphy KJ. Dairy consumption and metabolic syndrome: a systematic review of findings and methodological issues. Obes Rev. 2011;12:e190-201.

10. Scrivo R, Vasile M, Bartosiewicz I, Valesini G. Inflammation as "common soil" of the multifactorial diseases. Autoimmun Rev. 2011;10:369-74.

11. Pradhan A. Obesity, metabolic syndrome, and type 2 diabetes: inflammatory basis of glucose metabolic disorders. Nutr Rev. 2007;65:S152-6.

12. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care. 2004;27:813-23.

13. Solis-Pereyra B, Aattouri N, Lemonnier D. Role of food in the stimulation of cytokine production. Am J Clin Nutr. 1997;66:521S-5S.

14. Miettinen M, Matikainen S, Vuopio-Varkila J, Pirhonen J, Varkila K, Kurimoto M, Julkunen I. Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. Infect Immun. 1998;66:6058-62.

15. Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect Immun. 1996;64:5403-5.

16. Esmaillzadeh A, Azadbakht L. Dairy consumption and circulating levels of inflammatory markers among Iranian women. Public Health Nutr. 2010;13:1395-402.

17. Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy products consumption is associated with decreased levels of inflammatory markers related to cardiovascular disease in apparently healthy adults: the ATTICA study. J Am Coll Nutr. 2010;29:357-64.

18. Salas-Salvado J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M, Gomez-Gracia E, Vinoles E, Aros F, et al. Components of the mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. Eur J Clin Nutr. 2008;62:651-9.

19. Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. Metabolism. 2008;57:824-30.

20. Labonte ME, Couture P, Richard C, Desroches S, Lamarche B. Impact of dairy products on biomarkers of inflammation: a systematic review of randomized controlled nutritional intervention studies in overweight and obese adults. Am J Clin Nutr. 2013;97:706-17.

21. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003;107:363-9.

22. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation. 2006;114:82-96.

23. Campbell Company of Canada. V8® Original Vegetable Cocktail 156 mL. Nutrition Information. [cited 22 May 2014]; Available from: http://www.campbellsoup.ca/en-ca/products/v8/v8-original-vegetable-cocktail-156-ml

24. Industries Lassonde Inc. Oasis® Classic. [cited 22 May 2014]; Available from: http://www.oasis.ca/en/products/oasis/

25. Labonte ME, Cyr A, Baril-Gravel L, Royer MM, Lamarche B. Validity and reproducibility of a web-based, self-administered food frequency questionnaire. Eur J Clin Nutr. 2012;66:166-73.

26. Health Canada. Eating Well with Canada's Food Guide. 2007 [cited 5 December 2013]; Available from: http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php

27. Bertrand KA, Giovannucci E, Liu Y, Malspeis S, Eliassen AH, Wu K, Holmes MD, Laden F, Feskanich D. Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three US cohorts. Br J Nutr. 2012;108:1889-96.

28. Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L, Breckenridge C, Chow A, Kuba K, et al. Multi-laboratory comparison of three heparin-Mn2+ precipitation procedures for estimating cholesterol in high-density lipoprotein. Clin Chem. 1978;24:853-6.

29. 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.

30. Moorjani S, Dupont A, Labrie F, Lupien PJ, Brun D, Gagne C, Giguere M, Belanger A. Increase in plasma high-density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. Metabolism. 1987;36:244-50.

31. Couillard C, Despres JP, Lamarche B, Bergeron J, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. Arterioscler Thromb Vasc Biol. 2001;21:1226-32.

32. Pirro M, Bergeron J, Dagenais GR, Bernard PM, Cantin B, Despres JP, Lamarche B. Age and duration of follow-up as modulators of the risk for ischemic heart

disease associated with high plasma C-reactive protein levels in men. Arch Intern Med. 2001;161:2474-80.

33. Lohman TG, Roche A, Martorel R. The Airlie (VA) consensus conference. In: Lohman TG, Roche AF, Martorell R, eds. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics, 1988:39-80.

34. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8.

35. Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. Am J Clin Nutr. 2010;91:16-22.

36. Anderson TJ, Gregoire J, Hegele RA, Couture P, Mancini GB, McPherson R, Francis GA, Poirier P, Lau DC, 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;29:151-67.

37. Stancliffe RA, Thorpe T, Zemel MB. Dairy attentuates oxidative and inflammatory stress in metabolic syndrome. Am J Clin Nutr. 2011;94:422-30.

38. Thompson WG, Rostad Holdman N, Janzow DJ, Slezak JM, Morris KL, Zemel MB. Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. Obes Res. 2005;13:1344-53.

39. Zemel MB, Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. J Nutr. 2008;138:1047-52.

40. Wennersberg MH, Smedman A, Turpeinen AM, Retterstol K, Tengblad S, Lipre E, Aro A, Mutanen P, Seljeflot I, et al. Dairy products and metabolic effects in overweight men and women: results from a 6-mo intervention study. Am J Clin Nutr. 2009;90:960-8.

41. Rosado JL, Garcia OP, Ronquillo D, Hervert-Hernandez D, Caamano Mdel C, Martinez G, Gutierrez J, Garcia S. Intake of milk with added micronutrients increases the effectiveness of an energy-restricted diet to reduce body weight: a randomized controlled clinical trial in Mexican women. J Am Diet Assoc. 2011;111:1507-16.

42. van Meijl LE, Mensink RP. Effects of low-fat dairy consumption on markers of low-grade systemic inflammation and endothelial function in overweight and obese subjects: an intervention study. Br J Nutr. 2010;104:1523-7.

43. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E. Dairy augmentation of total and central fat loss in obese subjects. Int J Obes (Lond). 2005;29:391-7.

44. Zemel MB, Richards J, Milstead A, Campbell P. Effects of calcium and dairy on body composition and weight loss in African-American adults. Obes Res. 2005;13:1218-25.

45. van Meijl LE, Mensink RP. Low-fat dairy consumption reduces systolic blood pressure, but does not improve other metabolic risk parameters in overweight and obese subjects. Nutr Metab Cardiovasc Dis. 2011;21:355-61.

46. Van Loan MD, Keim NL, Adams SH, Souza E, Woodhouse LR, Thomas A, Witbracht M, Gertz ER, Piccolo B, et al. Dairy Foods in a Moderate Energy Restricted Diet Do Not Enhance Central Fat, Weight, and Intra-Abdominal Adipose Tissue Losses nor Reduce Adipocyte Size or Inflammatory Markers in Overweight and Obese Adults: A Controlled Feeding Study. J Obes. 2011;2011:989657.

47. Soriano-Maldonado A, Hidalgo M, Arteaga P, de Pascual-Teresa S, Nova E. Effects of regular consumption of vitamin C-rich or polyphenol-rich apple juice on cardiometabolic markers in healthy adults: a randomized crossover trial. Eur J Nutr. 2014. [in press].

48. Coelho RC, Hermsdorff HH, Bressan J. Anti-inflammatory properties of orange juice: possible favorable molecular and metabolic effects. Plant Foods Hum Nutr. 2013;68:1-10.

49. Ghavipour M, Saedisomeolia A, Djalali M, Sotoudeh G, Eshraghyan MR, Moghadam AM, Wood LG. Tomato juice consumption reduces systemic inflammation in overweight and obese females. Br J Nutr. 2013;109:2031-5.

50. Ravn-Haren G, Dragsted LO, Buch-Andersen T, Jensen EN, Jensen RI, Nemeth-Balogh M, Paulovicsova B, Bergstrom A, Wilcks A, et al. Intake of whole apples or clear apple juice has contrasting effects on plasma lipids in healthy volunteers. Eur J Nutr. 2013;52:1875-89.

51. Deopurkar R, Ghanim H, Friedman J, Abuaysheh S, Sia CL, Mohanty P, Viswanathan P, Chaudhuri A, Dandona P. Differential effects of cream, glucose, and orange juice on inflammation, endotoxin, and the expression of Toll-like receptor-4 and suppressor of cytokine signaling-3. Diabetes Care. 2010;33:991-7.

52. Thies F, Masson LF, Rudd A, Vaughan N, Tsang C, Brittenden J, Simpson WG, Duthie S, Horgan GW, Duthie G. Effect of a tomato-rich diet on markers of cardiovascular disease risk in moderately overweight, disease-free, middle-aged adults: a randomized controlled trial. Am J Clin Nutr. 2012;95:1013-22.

TABLES

TABLE 1. Average nutritional composition of foods provided as part of the CONTROL and DAIRY diets

	CONTROL	DAIRY
Nutrient	Fruit juice, 290 ml; Vegetable juice, 156 ml; Cashews, 20 g; Cookie, 39 g	Low fat milk, 375 ml; Yogurt, 175 g; Cheese, 30 g
Energy, *kJ*	1833	1807
Total fat, *g*	17.1	16.3
SFA, *g*	7.6	8.7
MUFA, *g*	6.6	4.1
PUFA, *g*	1.91	0.42
TFA[1], *g*	0.01	0.08
Protein, *g*	7.7	26.0
Carbohydrate, *g*	63.3	45.0
Fiber, *g*	3.2	0
Sodium, *mg*	459	465
Calcium, *mg*	84	913
Vitamin D, *μg*	0	5.2

[1] TFA, *trans* fatty acids.

TABLE 2. Characteristics of the 112 men and women with low grade inflammation at screening[1]

	Mean ± SD[2]	(min-max)
Women, *n (%)*	74 (66)	
Postmenopausal women, *n (% of women)*	26 (35)	
Ethnicity[3], *n (%)*		
Caucasian	74 (67)	
Asian	28 (25)	
African	3 (3)	
Hispanic	3 (3)	
Other	3 (3)	
Age, *y*	40.1 ± 16.7	(18-69)
Body weight, *kg*	72.2 ± 15.6	(46.3-122.4)
BMI, *kg/m²*	25.8 ± 4.3	(17.4-36.3)
Waist circumference, *cm*	87.5 ± 13.6	(61.5-118.0)
hs-CRP[4], *mg/L*	2.51 [2.29-2.74]	(1.06-8.47)
Blood pressure[5], *mm Hg*		
Systolic	114.2 ± 12.4	(94.7-148.0)
Diastolic	67.6 ± 7.3	(53.3-84.0)
Plasma lipids[6], *mmol/L*		
Total-C	5.3 ± 1.0	(3.1-7.9)
LDL-C	3.0 ± 1.0	(1.2-5.3)
HDL-C	1.7 ± 0.4	(0.9-2.8)
TG	1.4 ± 0.6	(0.6-3.2)
Total-C/HDL-C ratio[6]	3.3 ± 1.0	(1.8-7.0)

[1] HDL-C, HDL-cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, LDL-cholesterol; Total-C, total cholesterol

[2] Values are means ± SD unless indicated otherwise, i.e. frequency (%) or geometric mean [95% CI].

[3] Percent values based on $n = 111$ as data on ethnicity was missing for one participant.

[4] Value is the geometric mean [95% CI].

[5] Values at screening available for only 52 participants because they were not measured in one of the two centers.

[6] Values at screening available for only 51 participants because they were not measured in one of the two centers.

TABLE 3. Self-reported intakes by food groups of participants' usual diet as well as during the CONTROL and DAIRY 4-wk diets in men and women with low grade inflammation[1]

Food groups	USUAL	CONTROL	DAIRY	P^2
		servings/d		
Dairy products	2.0 (1.9)	0.0 (0.3)[a]	3.5 (1.0)[a,b]	<0.0001
Milk	0.6 (1.3)	0.0 (0.0)[a]	1.6 (0.5)[a,b]	<0.0001
Yogurt	0.2 (0.3)	0.0 (0.0)[a]	0.9 (0.0)[a,b]	<0.0001
Cheese	0.3 (0.9)	0.0 (0.0)[a]	0.6 (0.0)[a,b]	<0.0001
Fruits and vegetables	5.5 (3.9)	8.7 (5.1)[a]	5.5 (5.1)[b]	<0.0001
Fruit juice	0.3 (0.9)	2.0 (1.4)[a]	0.2 (1.0)[b]	<0.0001
Vegetable juice	0.0 (0.2)	1.3 (0.0)[a]	0.0 (0.2)[a,b]	<0.0001
Grain products	4.5 (3.1)	3.7 (2.8)[a]	4.1 (2.5)[a]	<0.0001
Meat and alternatives	2.2 (1.8)	2.5 (1.8)[a]	1.9 (1.8)[a,b]	<0.0001
Animal protein	1.5 (1.2)	1.5 (1.2)	1.5 (1.2)[a]	0.01
Vegetable protein	0.4 (0.6)	0.9 (0.8)[a]	0.4 (0.6)[b]	<0.0001
Nuts	0.1 (0.2)	0.6 (0.1)[a]	0.1 (0.2)[b]	<0.0001
Other foods				
Cookies	0.2 (0.4)	1.0 (0.7)[a]	0.2 (0.4)[b]	<0.0001

[1] Values are medians (IQR). n = 108 for the USUAL intakes (i.e. measured at screening) as dietary data for 4 participants were excluded because of non-plausible energy intakes (< 2092 or > 14 644 kJ [< 500 or > 3500 kcal] in women and < 3347 or > 17 573 kJ [< 800 or > 4200 kcal] in men); n =106 in the CONTROL diet as dietary data from the FFQ were missing for 3 participants and 3 participants were excluded because of non-plausible energy intakes; n = 104 in the DAIRY diet as dietary data were missing for 6 participants and 2 participants were excluded because of non-plausible energy intakes.

[2] P values for differences between measurements, as determined by the Friedman test. Pairwise comparisons were performed using rank transformation of the data followed by the general linear model procedure and the Tukey adjustment for multiple comparisons. [a] = significantly different from USUAL ($P < 0.05$); [b] = significantly different from CONTROL ($P < 0.05$).

TABLE 4. Effects of the CONTROL and DAIRY diets on anthropometry indices and inflammatory biomarkers in the 112 men and women with low grade inflammation[1]

Variable	CONTROL				DAIRY				Between-diet *P*	
	Pre	Post	Δ (95% CI)[2]	%	Pre	Post	Δ (95% CI)[2]	%	Δ vs. Δ[3]	Post vs. Post[4]
Anthropometry										
BMI[5], *kg/m²*	25.8 ± 4.3	25.8 ± 4.3	-0.04 (-0.10, 0.02)	-0.1	25.8 ± 4.3	25.9 ± 4.4	0.10 (0.04, 0.16)*	0.4	0.0007	0.0007
Waist circumference[6], *cm*	86.4 ± 14.1	87.2 ± 13.9	0.84 (0.24, 1.43)*	0.9	86.6 ± 14.4	87.8 ± 14.1	1.06 (0.55, 1.56)*	1.4	0.56	0.54
Android fat mass, *kg*	N/A	2.26 ± 1.12	-	-	N/A	2.26 ± 1.14	-	-	-	0.80
Total body fat mass, *kg*	N/A	24.1 ± 9.9	-	-	N/A	24.2 ± 10.0	-	-	-	0.32
Inflammatory biomarkers										
hs-CRP[7], *mg/L*	2.89 ± 2.29	2.55 ± 1.96	-0.34 (-0.68, 0.00)*	-11.7	3.08 ± 3.07	2.85 ± 2.14	-0.23 (-0.74, 0.29)	-7.3	0.04	0.06
IL-6[7], *pg/mL*	1.65 ± 1.10	1.36 ± 0.73	-0.29 (-0.43, -0.15)*	-17.6	1.73 ± 1.08	1.39 ± 0.68	-0.34 (-0.50, -0.19)*	-19.9	0.77	0.70
Adiponectin[7], *μg/mL*	8.98 ± 4.71	8.97 ± 4.36	0.00 (-0.38, 0.37)	0.0	8.91 ± 4.61	8.81 ± 4.23	-0.09 (-0.41, 0.22)	-1.0	0.37	0.49

[1] Values are means ± SD or mean Δ post- vs. pre-diet (95% CI) with the corresponding % change. hs-CRP, high-sensitivity C-reactive protein; N/A, unavailable data

[2] * = Significant within-diet effects ($P \leq 0.05$), as determined by the least-square means statistic in MIXED models performed on delta scores representing post- vs. pre-diet variations.

[3] *P* values for between-diet effects, as determined by MIXED models performed on delta scores representing post- vs. pre-diet variations. Adjustment for potential covariates (sex, centre, and/or pre-diet values of the selected variable) was considered only when they were found to be significant at $P < 0.05$ in the models.

[4] *P* values for between-diet effects, as determined by MIXED models performed on post-diet values (end-point to end-point). Adjustment for potential covariates (sex, centre, and/or pre-diet values of the selected variable) was considered only when they were found to be significant at $P < 0.05$ in the models.

[5] Analysis of post-diet values was adjusted for pre-diet values of BMI.

[6] *n*=111 post-diet in both dietary interventions as data were missing for one participant in each diet. Analysis of delta scores was adjusted for centre only. Analysis of post-diet values was further adjusted for pre-diet values of waist circumference.

[7] Analyses of delta scores were performed on post- vs. pre-diet variations of log-transformed data since post- vs. pre-diet variations of crude data were not normally distributed. Analyses of post-diet values were performed on log-transformed data. All analyses were adjusted for pre-diet values of the selected inflammatory biomarker. Analyses of delta scores and post-diet values of hs-CRP were further adjusted for study center.

FIGURES

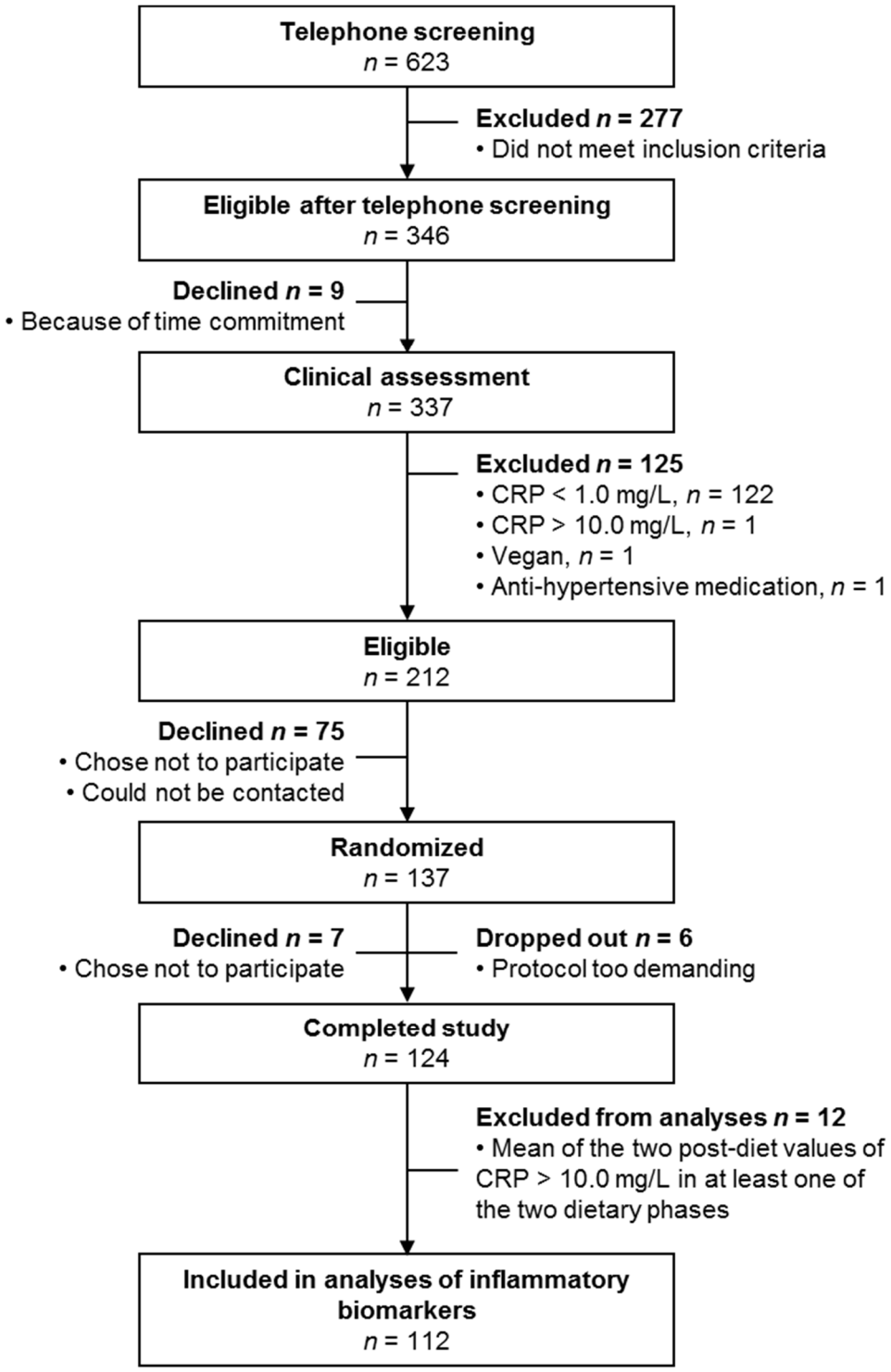


FIGURE 1. Flow of participating men and women with low grade inflammation throughout the study**.**

SUPPLEMENTARY FILES

SUPPLEMENTAL TABLE 1. GenBank and Life Technologies numbers of the analyzed genes[1]

	# GenBank[2]	# Life Technologies[3]
Target genes		
CCL2	NM_002982	Hs00234140_m1
IL18	NM_001562	Hs01038788_m1
IL6	NM_00600	Hs00985639_m1
IL1B	NM_000576	Hs01555410_m1
NFKB1	NM_003998	Hs00765730_m1
NPR3	-	Hs01099013_m1
PPARA	NM_005036	Hs00947536_m1
SREBF2	NM_004599	Hs01081784_m1
TNF	NM_000594	Hs01113624_g1
TRAF3	NM_145725	Hs00936781_m1
Control genes		
G6PD	-	Hs00166169_m1
GAPDH	NM_002046	Hs00266705_g1

[1] *G6PD*, glucose-6-phosphate dehydrogenase; *NPR3*, natriuretic peptide receptor C

[2] National Center for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/genbank/)

[3] Life Technologies Genome Database (http://www.lifetechnologies.com/order/genome-database/)

SUPPLEMENTAL TABLE 2. Energy and nutrient intakes prior to (USUAL) as well as during the CONTROL and DAIRY dietary interventions in men and women with low grade inflammation[1]

Nutrient	USUAL	CONTROL	DAIRY	P^2
Energy, *kJ*	8056 (3840)	7321 (3506)[a]	7856 (3048)[b]	0.0002
Alcohol, *%*	1.0 (2.3)	0.8 (1.8)	0.7 (1.7)[a]	<0.0001
Lipids, *%*	33.9 (6.3)	33.0 (7.7)	30.3 (6.1)[a]	0.0004
SFA, *%*	11.0 (3.1)	8.7 (2.4)[a]	10.7 (2.3)[b]	<0.0001
MUFA, *%*	13.1 (3.3)	14.1 (3.4)[a]	11.8 (3.0)[a,b]	<0.0001
PUFA, *%*	6.2 (2.3)	7.2 (1.7)[a]	5.2 (1.8)[a,b]	<0.0001
TFA, *%*	1.3 (0.5)	1.1 (0.6)	1.1 (0.5)[a]	0.008
Dietary cholesterol, *mg*	242 (128)	174 (124)[a]	209 (130)[a,b]	<0.0001
Protein, *%*	17.5 (4.3)	14.0 (3.5)[a]	18.5 (3.1)[a,b]	<0.0001
Carbohydrate, *%*	49.8 (7.7)	55.1 (7.9)[a]	52.3 (8.4)[a,b]	<0.0001
Fiber, *g*	22.0 (12.2)	22.6 (14.5)	21.0 (13.8)[a,b]	0.02
Sodium, *mg*	2665 (1273)	2239 (973)[a]	2357 (1189)[a]	<0.0001
Calcium, *mg*	1009 (769)	437 (249)[a]	1411 (398)[a,b]	<0.0001
Vitamin D, *μg*	7.7 (6.0)	3.7 (4.7)[a]	8.3 (5.5)[a,b]	<0.0001

[1] Values are medians (IQR). n = 108 for the USUAL intakes (i.e. measured at screening) as dietary data for 4 participants were excluded because of non-plausible energy intakes (< 2092 or > 14 644 kJ [< 500 or > 3500 kcal] in women and < 3347 or > 17 573 kJ [< 800 or > 4200 kcal] in men); n =106 in the CONTROL diet as dietary data from the FFQ were missing for 3 participants and 3 participants were excluded because of non-plausible energy intakes; n = 104 in the DAIRY diet as dietary data were missing for 6 participants and 2 participants were excluded because of non-plausible energy intakes.

[2] P values for differences between measurements, as determined by the Friedman test. Pairwise comparisons were performed using rank transformation of the data followed by the general linear model procedure and the Tukey adjustment for multiple comparisons. [a] = significantly different from USUAL ($P < 0.05$); [b] = significantly different from CONTROL ($P < 0.05$).

SUPPLEMENTAL TABLE 3. Nutritional intakes during the CONTROL and DAIRY dietary interventions in men and women with low grade inflammation after exclusion of foods provided to participants during each phase[1]

	CONTROL	DAIRY	P^2
Food groups, *servings/d*			
Dairy products	0.0 (0.3)	0.1 (0.2)	0.72
Fruits and vegetables	4.8 (4.2)	5.5 (5.1)	0.0003
Fruits	1.6 (2.2)	2.4 (3.0)	0.0002
Vegetables	2.7 (2.8)	2.7 (3.0)	0.10
Grain products	3.7 (2.8)	4.1 (2.5)	0.28
Meat and alternatives	2.0 (1.6)	1.9 (1.8)	0.69
Animal protein	1.5 (1.2)	1.5 (1.2)	0.17
Vegetable protein	0.3 (0.6)	0.4 (0.6)	0.11
Nutrient			
Energy, *kJ*	5237 (3117)	5903 (2974)	0.009
Alcohol, *%*	0.9 (2.4)	0.9 (2.4)	0.17
Lipids, *%*	32.8 (8.3)	30.8 (8.1)	0.48
SFA, *%*	8.0 (2.6)	8.1 (2.5)	0.36
MUFA, *%*	13.9 (5.2)	12.8 (4.2)	0.15
PUFA, *%*	6.9 (2.3)	6.7 (2.3)	0.54
TFA, *%*	1.2 (0.7)	1.2 (0.6)	0.76
Dietary cholesterol, *mg*	164 (126)	161 (120)	0.26
Protein, *%*	16.2 (4.1)	15.4 (3.6)	0.01
Carbohydrate, *%*	51.3 (10.3)	53.9 (10.9)	0.03
Fiber, *g*	18.4 (13.4)	20.4 (13.6)	0.06
Sodium, *mg*	1652 (914)	1845 (1187)	0.051
Calcium, *mg*	343 (208)	373 (236)	0.09
Vitamin D, *μg*	3.7 (4.7)	2.8 (4.3)	0.26

[1] Values are medians (IQR). Data represent nutritional intakes without the contribution of fruit juice, vegetable juice, nuts and cookies during the CONTROL diet and without the contribution of milk, yogurt and cheese during the DAIRY diet. *n* =106 in the CONTROL

diet as dietary data from the FFQ were missing for 3 participants and 3 participants were excluded because of non-plausible energy intakes (< 2092 or > 14 644 kJ [< 500 or > 3500 kcal] in women and < 3347 or > 17 573 kJ [< 800 or > 4200 kcal] in men); n = 104 in the DAIRY diet as dietary data were missing for 6 participants and 2 participants were excluded because of non-plausible energy intakes.

[2] P values obtained by the Wilcoxon matched-pairs signed-rank test.

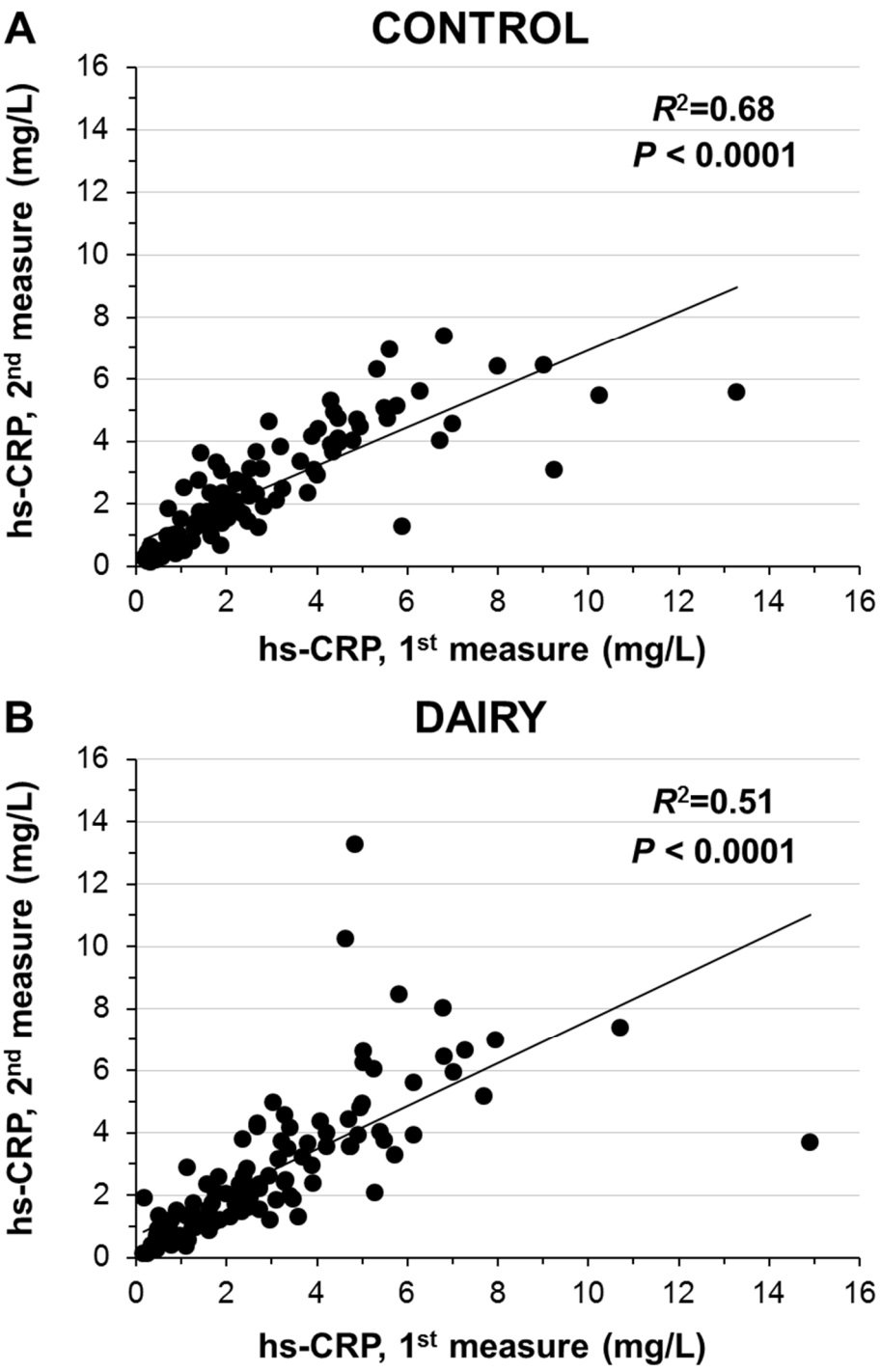
SUPPLEMENTAL TABLE 4. Mean fold change in expression levels of key inflammatory genes and transcription factors after the DAIRY compared with the CONTROL diet in a random subset of 53 men and women with low grade inflammation[1]

Genes	Fold change (DAIRY vs. CONTROL)[2]	*P*[3]
IL18	1.09	0.20
IL1B	1.04	0.34
NFKB1	1.05	0.12
PPARA	1.04	0.46
SREBF2	1.05	0.36
TNF	1.00	0.98
TRAF3	1.05	0.32

[1] *G6PD*, glucose-6-phosphate dehydrogenase; *IL18*, interleukin 18; *IL1B*, interleukin 1-beta; *NFKB1*, nuclear factor kappa-B subunit 1; *PPARA*, peroxisome proliferator-activated receptor alpha; *SREBF2*, sterol regulatory element binding transcription factor 2; *TNF*, tumor necrosis factor; *TRAF3*, TNF receptor-associated factor 3

[2] Values are fold change in gene expression calculated using the $2^{-\Delta\Delta Ct}$ method, i.e. 2^(mean ΔCt from the DAIRY diet – mean ΔCt from the CONTROL diet). ΔCt were calculated as Ct values of a selected target gene – Ct values of the internal control gene *G6PD*. Ct values used in the formula consisted of the mean of the triplicates' individual Ct values, unless the SD of a triplicate was > 0.5, in which case the outlier value was excluded from the calculation of the mean.

[3] *P* values for between-diet effects, as determined by MIXED models performed on post-diet gene expression levels normalized to the expression of the reference gene *G6PD*. Adjustment for potential covariates including sex, age, and study center did not change the results.



SUPPLEMENTAL FIGURE 1. Correlation analysis between the two post-diet measures of high-sensitivity C-reactive protein (hs-CRP) in the CONTROL (A) and DAIRY (B) diets.

CHAPITRE 10 : ÉTUDE DE L'IMPACT DES PRODUITS LAITIERS SUR L'INFLAMMATION – CONCLUSION

Ce deuxième volet de mon projet de doctorat visait globalement à faire la lumière sur la controverse entourant l'impact de la consommation de produits laitiers sur l'inflammation. Comme l'indique la conclusion du chapitre précédent, deux principaux constats dérivent de notre revue systématique des études d'intervention nutritionnelle randomisées contrôlées et du projet PLI : 1) Il n'est pas possible de déterminer, à l'heure actuelle, si la consommation de produits laitiers exerce des *effets bénéfiques* sur le profil inflammatoire d'individus principalement en surpoids ou obèses, donc davantage « à risque » d'inflammation chronique de faible intensité ; 2) Il est cependant tout à fait possible de conclure que la consommation de produits laitiers dans le cadre d'une alimentation saine n'exerce *aucun effet néfaste* sur le profil inflammatoire de ces individus.

En plus des nombreux éléments de discussion qui ont déjà été soulevés aux chapitres 8 et 9, d'autres facteurs sont à prendre en considération en lien avec la revue systématique et/ou le projet PLI. Premièrement, prenons connaissance d'une limite inhérente à la revue systématique elle-même. Même si notre recherche dans la littérature scientifique a été soigneusement effectuée à deux reprises, par deux personnes différentes, en utilisant des critères bien définis et en s'attardant à trois bases de données (*PubMed*, *Embase* et *Cochrane Library*), il est possible que nous n'ayons pas pu identifier toutes les études pertinentes sur le sujet. D'ailleurs, l'étude de Van Loan *et al.* (207), dont il fut question dans la discussion des résultats du projet PLI au chapitre 9, aurait très bien pu se qualifier pour faire partie de la revue systématique. Cette étude a été publiée en 2011. Cependant, la stratégie de recherche utilisée n'avait pas permis de l'identifier en avril ou en juin 2012 et nous l'avons donc découverte « par hasard » sur *PubMed* après coup. Il est tout de même intéressant de noter que nos conclusions de la revue systématique seraient demeurées les mêmes si cette étude avait été incluse dès le départ.

Deuxièmement, nos constats ci-haut demeurent une fois de plus les mêmes si nous prenons en considération les résultats des études d'intervention nutritionnelle randomisées contrôlées qui ont été publiées depuis la réalisation de la revue systématique (208, 209).

Brièvement, dans le cadre d'une étude conçue selon un devis en parallèle, Jones *et al.* (208) ont comparé l'impact d'une diète riche en produits laitiers faibles en matières grasses (3 à 4 portions/jour de lait ou de yogourt à 0 ou 1% de M.G., combinées à un supplément de 350 mg de calcium) et d'une diète témoin (1 portion/jour de produits laitiers similaires, sans le supplément de calcium) sur différents biomarqueurs pro-inflammatoires chez 49 individus en surpoids ou obèses. Aucune différence significative n'a été observée dans les concentrations de l'IL-1β, de l'IL-6, de MCP-1 ou de TNF-α à l'intérieur de chacune des diètes (variations « post- moins pré-diète ») ou entre celles-ci. Par contre, comme pour la plupart des études sur le sujet, l'inflammation n'était pas la mesure principale de cette étude. D'un point de vue davantage « métabolique/mécanistique », nous avons récemment découvert qu'une autre étude que PLI et que celle de Van Loan *et al.* (207) a aussi évalué l'expression de gènes inflammatoires en réponse à la consommation de produits laitiers. En utilisant un devis en parallèle, Serra *et al.* (209) ont démontré que l'expression de gènes pro-inflammatoires incluant le TNF-α, l'IL-1β et l'IL-6 dans le muscle squelettique ne différait pas suite à la consommation de 3 portions/jour de lait réduit en matières grasses (pourcentage non spécifié) comparativement à la consommation de 3 portions/jour de boisson de soya à la vanille pendant 28 jours chez 31 femmes post-ménopausées.

Troisièmement, il importe d'insister sur le fait que les résultats de la revue systématique ne peuvent pas être généralisés aux populations de poids normal puisque toutes les études incluses ont été réalisées chez des individus en surpoids ou obèses. C'est aussi le cas dans les études de Van Loan *et al.* (207) et Jones *et al.* (208). Dans l'étude de Serra *et al.* (209), l'IMC pouvait varier entre 19 et 35 kg/m^2, mais les femmes étaient tout de même globalement en situation de surpoids (moyennes ± écart type de 26,3 ± 4,0 et 25,4 ± 4,1 kg/m^2 dans les groupes « lait » et « boisson de soya », respectivement). De façon similaire, dans le projet PLI, l'IMC des participants variait entre un poids insuffisant (17,4 kg/m^2) et l'obésité de classe II (36,3 kg/m^2). Cependant, en tant que groupe, les participants étaient en léger surpoids (moyenne de 25,8 ± 4,3 kg/m^2). Même si les études actuelles ne peuvent pas s'appliquer aux individus de poids normal, il semble peu probable que la consommation de produits laitiers ait un impact significatif sur les processus inflammatoires chez ceux-ci puisqu'ils sont normalement moins prédisposés que les

individus en surpoids ou obèses à présenter un profil pro-inflammatoire (voir section 1.2 du chapitre 1).

Quatrièmement, je rappelle qu'un des buts du projet PLI était d'évaluer la consommation de produits laitiers dans un contexte reflétant le plus possible la vie réelle en combinant à la fois des produits laitiers faibles et riches en matières grasses. Pour le futur, il serait malgré tout intéressant de vérifier les effets sur l'inflammation de produits laitiers faibles en matières grasses comparativement à des produits laitiers riches en matières grasses ainsi que comparativement à des produits témoins à l'intérieur d'une même étude et idéalement selon un devis en chassé-croisé, où chaque participant est comparé à lui-même. Ceci permettrait entre autres d'obtenir des réponses quant à l'impact des produits laitiers riches en matières grasses sur l'inflammation, car aucune des études d'intervention dont il a été question précédemment ne s'est *spécifiquement* attardée à ce type de produits laitiers. Il est intéressant de noter qu'une récente étude en chassé-croisé de Nestel *et al.* (210) a démontré que la consommation à court terme (3 semaines) de produits laitiers riches en matières grasses fermentés (yogourt riche en M.G. et fromage cheddar) ou non fermentés (beurre, crème, crème glacée) n'avait globalement pas d'impact sur le profil inflammatoire (hs-CRP, MCP-1, IL-1β, IL-6, TNF-α) comparativement à la consommation de produits laitiers faibles en matières grasses (lait écrémé et yogourt 1%). Toutefois, cette étude incluait peu de sujets (n = 12) et ne comprenait pas de diète témoin contenant peu ou pas de produits laitiers, ce qui limite la portée des résultats.

Cinquièmement, pour faire suite au paragraphe précédent, on doit garder en tête que le choix d'une diète témoin adéquate représente toujours un défi dans les études d'intervention nutritionnelle sur les produits laitiers. Il est très rarement possible de tester des produits témoins similaires aux produits laitiers, mais sans les ingrédients actifs, particulièrement dans des situations comme celle du projet PLI où l'on vise à refléter la vie réelle. Ceci explique potentiellement pourquoi les interventions « témoins » diffèrent grandement entre les études d'intervention dont il a été question jusqu'à maintenant.

Sixièmement, il faut retenir que l'absence d'effets néfastes de la consommation de produits laitiers sur l'inflammation tel que suggéré par les études actuelles sur le sujet ne s'applique pas nécessairement aux individus souffrant d'inflammation chronique dite « franche »

(maladies inflammatoires chroniques comme l'arthrite rhumatoïde ou les maladies inflammatoires de l'intestin) (voir section 1.1 du chapitre 1). En effet, le but poursuivi dans le présent volet de mon projet de doctorat était seulement de mettre l'accent sur les études conduites dans le contexte de l'inflammation chronique de faible intensité reliée au risque cardiovasculaire, et non pas de s'attarder aux conditions reliées à l'inflammation chronique d'intensité plus élevée.

Pour terminer, bien qu'on ne puisse pas dire actuellement si les produits laitiers exercent un impact tout simplement neutre ou bien bénéfique sur l'inflammation, il est intéressant de noter que les études qui ont mis l'accent sur des nutriments spécifiques contenus dans les produits laitiers semblent supporter la présence d'effets anti-inflammatoires. Tel que souligné brièvement dans la discussion de l'article au chapitre 8, ces nutriments incluent le calcium (211, 212), la vitamine D (213) et certains peptides bioactifs (3, 214).

En somme, le volet « Impact des produits laitiers sur l'inflammation » de mon projet de doctorat représente, à mon avis, une de mes meilleures contributions en recherche à l'heure actuelle. J'ai la conviction que mes travaux peuvent servir de guide pour l'élaboration de futures études sur le sujet. Des études d'intervention nutritionnelle randomisées contrôlées additionnelles sont sans aucun doute nécessaires pour déterminer les effets précis des produits laitiers sur l'inflammation, que ce soit en termes de consommation totale, de produits faibles en matières grasses, de produits riches en matières grasses ou bien de produits individuels (lait / yogourt / fromage). Malgré tout, les évidences actuelles tendent vers l'absence d'effets néfastes dans le cadre d'une alimentation saine chez des individus en surpoids ou obèses. Ceci soulève la possibilité de relayer au statut de mythe la croyance populaire comme quoi les produits laitiers seraient des aliments pro-inflammatoires. Il s'agit d'une bonne nouvelle considérant que ces aliments de grande valeur nutritive sont plus ou moins bien vus aux yeux de la population générale, ce qui se reflète dans un faible taux de consommation chez les Canadiens. Par exemple, seulement 28% des femmes et 35% des hommes de 31 à 50 ans consommeraient suffisamment de produits laitiers chaque jour (215).

CHAPITRE 11 : ÉTUDE DE L'IMPACT DES ACIDES GRAS ALIMENTAIRES SUR L'INFLAMMATION – PROBLÉMATIQUE

Il a été introduit au chapitre 1 (section 1.5.2) que des nutriments particulièrement d'intérêt en lien avec l'inflammation sont les acides gras alimentaires, à cause de leur potentiel d'activation ou d'inhibition de certains processus inflammatoires. Toutefois, la littérature scientifique sur le sujet est relativement vaste et complexe. La mise en commun des résultats des études épidémiologiques, cliniques et mécanistiques sème parfois la confusion quant aux effets de différents acides gras sur les processus pro- et anti-inflammatoires, sans compter que plusieurs questions clés demeurent non résolues. Avant d'introduire les travaux réalisés dans le cadre du troisième et dernier volet de mon projet de doctorat, voici un survol de la littérature actuelle sur le sujet des acides gras et de l'inflammation. Notez que je discuterai davantage des acides gras oméga-3 d'origine marine, puisqu'ils sont ceux ayant reçu le plus d'attention en lien avec l'inflammation dans la communauté scientifique ainsi que dans les travaux du présent volet.

11.1 Acides gras alimentaires et inflammation : état des connaissances

11.1.1 Acides gras saturés et trans

Dans leur vaste revue de la littérature sur l'alimentation et l'inflammation publiée en 2011, Calder *et al.* (3) suggèrent assez explicitement, sur la base d'études de nature observationnelle, clinique et mécanistique, que la consommation d'AGS et d'AGT est associée à des effets pro-inflammatoires. Cependant, en jetant un coup d'œil un peu plus approfondi à la littérature scientifique, on se rend compte que les AGS et les AGT ne sont peut-être pas aussi *clairement* associés à des effets pro-inflammatoires que Calder *et al.* (3) le prétendent.

En effet, une récente revue systématique de la littérature suggère la présence d'une association potentiellement positive entre les AGS et la hs-CRP, mais pas d'association avec les cytokines (IL-6, TNF-α) ni les adipokines (dont fait partie l'adiponectine) (216). Même s'il faut reconnaître que 12 des 15 études incluses dans cette revue systématique

étaient de nature transversale, ce qui peut limiter la portée des résultats, il semble qu'une association positive entre les AGS et l'inflammation ne soit pas observée « hors de tout doute ».

Du côté des AGT, certaines études d'intervention (217-220), mais pas toutes (221-225), ont rapporté que leur consommation entraînait des effets pro-inflammatoires. Ce constat peut donc nous amener à se questionner concernant quelles caractéristiques (ex : état de santé des sujets, doses d'AGT, sources d'AGT naturelles ou industrielles, biomarqueurs inflammatoires évalués, puissance statistique de l'étude) influencent les résultats des études en question. Puisque les AGS et les AGT n'ont pas spécifiquement fait l'objet de mes travaux, je n'en discuterai pas davantage dans le présent chapitre.

11.1.2 Acides gras monoinsaturés

Le principal acide gras de la famille des AGMI est l'acide oléique (OA, « *oleic acid* », C18:1 n-9), retrouvé principalement dans les produits d'origine végétale comme les huiles d'olive et de canola, les noix (amandes, pacanes) et les avocats.

Une étude transversale réalisée chez 3017 hommes et femmes japonais a démontré que les apports en acide oléique tels que mesurés par un questionnaire sur l'histoire alimentaire étaient inversement associés à la hs-CRP après ajustement pour diverses variables potentiellement confondantes (âge, IMC, tabagisme, etc.), particulièrement lorsque les individus se situaient dans le tertile intermédiaire des apports en EPA et DHA (226).

En ce qui concerne les études d'intervention, l'isolement des effets des AGMI/acide oléique sur l'inflammation est parfois difficile considérant qu'ils sont souvent étudiés dans le cadre de la consommation d'aliments spécifiques (ex : amandes (227)) ou de diètes particulières (ex : diètes méditerranéennes riches en huile olive (228, 229)). Ainsi, les effets anti-inflammatoires observés dans ces études peuvent être attribuables, du moins partiellement, à d'autres facteurs nutritionnels entrant dans la composition des aliments ou diètes évalués (ex : composés phénoliques dans l'huile d'olive extra-vierge (230)) plutôt qu'aux AGMI.

De ce fait, il est intéressant de noter qu'une revue systématique et méta-analyse d'études randomisées contrôlées a démontré que la consommation de diètes enrichies en AGMI (> 12% de l'énergie) n'avait aucun impact sur la hs-CRP (-0,07 mg/dL ; IC 95% : -0,41 à 0,27) comparativement à des diètes faibles en AGMI (≤ 12% de l'énergie ; soit des diètes faibles en gras [≤ 30% de l'énergie en lipides totaux], des diètes avec un indice glycémique faible ou élevé ou encore des diètes avec un contenu élevé en protéines) (231). Les calculs réalisés étaient basés sur 5 études conçues selon un devis en parallèle d'une durée de plus de 6 mois. Dans le même ordre d'idées, des études d'intervention de durée plus courte, mais réalisées selon un devis chassé-croisé, n'ont observé aucune différence dans les concentrations de biomarqueurs inflammatoires incluant la hs-CRP, l'IL-6 et/ou l'adiponectine en comparant des diètes riches en AGMI (≥ 19% de l'énergie) à des diètes qualifiées en tant que typiquement « américaines », riches en AGS, riches en AGPI ou bien riches en glucides (232-234).

Contrairement aux études sur les biomarqueurs inflammatoires circulants, les études de nature mécanistique tendent beaucoup plus vers l'observation d'effets anti-inflammatoires suite à la consommation d'AGMI. Des études dans des modèles animaux et cellulaires suggèrent que les AGMI/acide oléique pourraient diminuer la sécrétion d'IL-6 et augmenter l'expression du gène de l'adiponectine dans les adipocytes (235, 236). En accord avec ces résultats, une étude d'intervention en parallèle réalisée chez 20 individus avec obésité abdominale a permis de démontrer que la consommation d'une diète enrichie en AGMI (20% de l'énergie ; AGS = 11%) induisait un profil général d'expression de gènes davantage anti-inflammatoire dans le tissu adipeux sous-cutané comparativement à une diète riche en AGS (19% de l'énergie ; AGMI = 11%) (237). En somme, on doit retenir que les effets exercés par les AGMI sur l'inflammation semblent différer selon le type d'approche expérimentale utilisé et qu'ils ne peuvent pas toujours être isolés.

11.1.3 Acides gras polyinsaturés oméga-6

L'AGPI le plus abondant dans l'alimentation est l'acide linoléique (LA, « *linoleic acid* », C18:2 n-6), un acide gras essentiel retrouvé principalement dans les huiles végétales (ex : huiles de tournesol, de carthame, de maïs, de canola et de soya) ainsi que dans les noix et les graines (ex : noix de pin, noix de Grenoble, graines de tournesol).

D'un point de vue strictement mécanistique, l'acide linoléique apparaît comme un acide gras pro-inflammatoire. En effet, cet acide gras partage la même voie métabolique qu'un autre acide gras essentiel, l'acide alpha-linolénique (ALA, « *alpha-linolenic acid* », C18:3 n-3), le précurseur de la famille des oméga-3 (238) (**Figure 11.1**). L'acide linoléique peut donc entrer en compétition avec l'ALA pour l'enzyme Δ-6 désaturase, aussi connue sous le nom de FADS2 (« *fatty acid desaturase 2* ») (239). Cette compétition limiterait la conversion de l'ALA en acide gras oméga-3 à plus longue chaîne et, subséquemment, la formation d'eicosanoïdes anti-inflammatoires (prostaglandines et thromboxanes de série 3

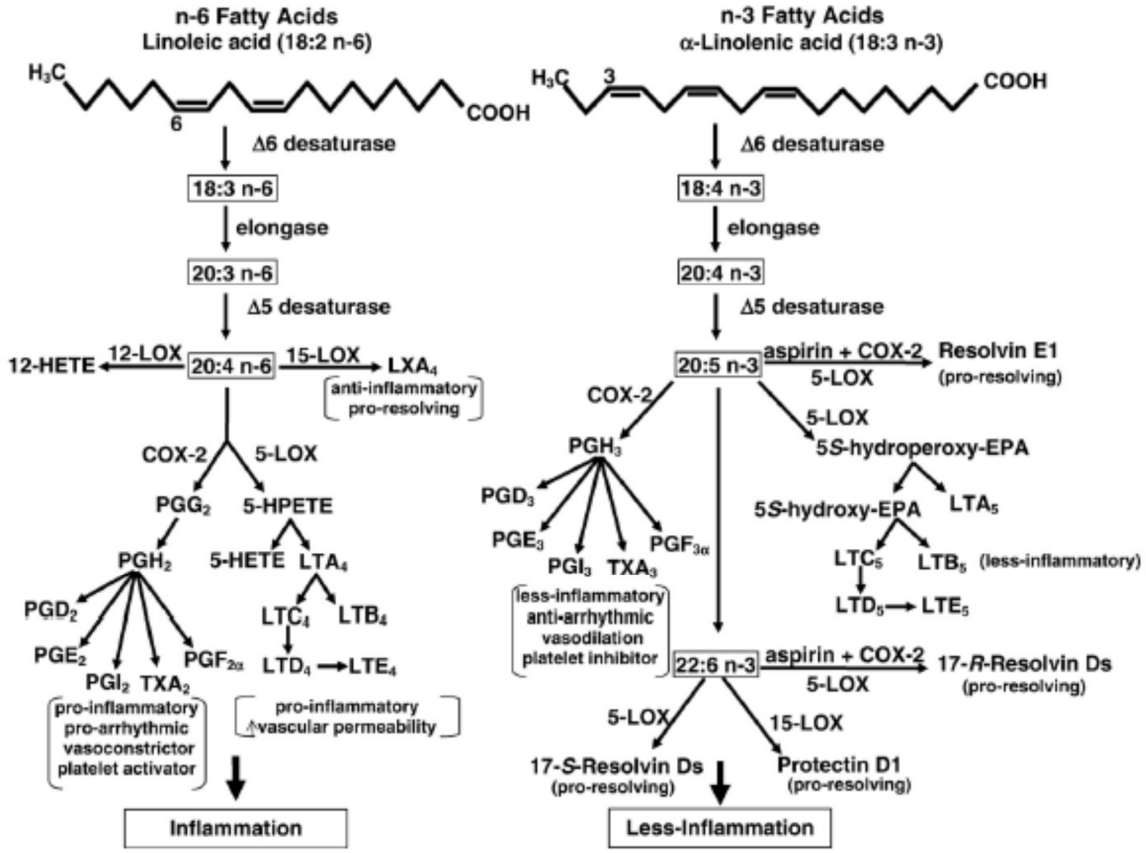


Figure 11.1 : Illustration du métabolisme des acides gras polyinsaturés oméga-6 et oméga-3 à partir des acides gras essentiels respectifs à chacune de ces familles d'acides gras, soit l'acide linoléique et l'acide alpha-linolénique. Figure reproduite avec la permission de Elsevier, provenant de la publication par Yuriko Adkins et Darshan S. Kelley. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *J Nutr Biochem*. Septembre 2010, Volume 21, Issue 9, Pages 781-792. http://dx.doi.org/10.1016/j.jnutbio.2009.12.004. Copyright 2010 Elsevier Inc.

et leucotriènes de série 5) ainsi que de médiateurs lipidiques pro-résolutifs (résolvines, protectines) (238, 240) (Figure 11.1). D'autre part, l'acide linoléique est le précurseur de l'acide arachidonique (AA, « *arachidonic acid* », C20:4 n-6), qui à son tour sert de substrat pour les enzymes cyclooxygénases et lipoxygénases menant à la production d'eicosanoïdes pro-inflammatoires (prostaglandines et thromboxanes de série 2 et leucotriènes de série 4) (238, 239). Toutefois, il est de plus en plus reconnu que l'AA et ses dérivés pourraient également exercer des effets anti-inflammatoires (238, 241). Entre autres, les prostaglandines E2 pourraient inhiber la production de TNF-α et de l'IL-1β dans les monocytes et les macrophages (242).

En ce sens, plutôt que de rapporter des associations positives, plusieurs études observationnelles supportent la présence d'associations inverses ou bien nulles entre les apports alimentaires ou les concentrations plasmatiques d'acide linoléique et l'inflammation (243-246). Dans le même ordre d'idées, une récente revue systématique regroupant 15 études d'intervention randomisées contrôlées suggère que des apports augmentés en acide linoléique, comparativement à une diète témoin, n'auraient pas d'effet sur les concentrations de différents biomarqueurs inflammatoires (hs-CRP, fibrinogène, inhibiteur de l'activateur du plasminogène 1 [PAI-1], cytokines) chez les individus en santé âgés de plus d'un an (247). Il est intéressant de noter que dans les études d'intervention retenues, le seul acide gras autre que l'acide linoléique qui pouvait différer de façon substantielle entre les diètes expérimentales et « témoins » était l'acide oléique. Aucune des études incluses n'a rapporté de résultats concernant l'adiponectine tandis qu'elle faisait partie des termes de recherche utilisés lors de la réalisation de la revue systématique. Les auteurs soulignent par ailleurs que des études additionnelles seront nécessaires pour confirmer leurs observations, entre autres avec de plus grands échantillons de participants. Les 15 études retenues comprenaient en effet un petit nombre de sujets (nombre maximum ayant complété l'intervention ≤ 60), ce qui limite la puissance statistique particulièrement en ce qui concerne les études conçues selon un devis en parallèle.

Ensuite, peu d'études semblent s'être intéressées à l'expression de gènes impliqués dans les processus inflammatoires en réponse à la consommation d'oméga-6 chez l'humain. Brièvement, une étude randomisée en parallèle de Bjermo *et al.* (248) a démontré que

l'expression du facteur de transcription PPAR-γ (« *peroxisome proliferator-activated receptor gamma* »), de MCP-1, de l'adiponectine, de TNF-α et de l'IL-6 dans le tissu adipeux sous-cutané ne différait pas suite à la consommation d'une diète enrichie en acide linoléique (15% de l'énergie) comparativement à une diète riche en AGS (beurre) durant 10 semaines chez 67 sujets avec obésité abdominale.

11.1.4. Acides gras polyinsaturés oméga-3 d'origine végétale

L'ALA est un oméga-3 de source végétale provenant principalement des huiles de lin et de canola, des graines de lin, des graines de chia et des noix de Grenoble. Les évidences de nature observationnelle suggèrent la présence d'une association inverse entre les apports en ALA et les concentrations sanguines de biomarqueurs inflammatoires, dont principalement la hs-CRP (226, 245, 249, 250).

Du côté des études d'intervention nutritionnelle, les résultats relatifs à l'adiponectine ainsi qu'aux biomarqueurs pro-inflammatoires sont globalement non concluants (251-255). En effet, une étude chez des hommes hypercholestérolémiques et une autre chez des hommes et des femmes en santé ont démontré que les concentrations d'adiponectine n'étaient pas influencées par la consommation d'une diète riche en ALA (environ 8 g/jour) comparativement à la consommation d'une diète « témoin » riche en acide oléique ou riche en acide linoléique (251, 252). Malgré tout, dans l'étude réalisée chez les sujets en santé (251), l'augmentation des concentrations érythrocytaires d'ALA corrélait positivement avec l'augmentation des concentrations sériques d'adiponectine lors de la consommation de la diète enrichie en ALA (r = 0,34 ; P = 0,007). À l'opposé, chez des adultes avec obésité abdominale, il a été démontré qu'une supplémentation en ALA (11 g/jour) sous forme de capsules d'huile de lin entraînait une *réduction* des concentrations d'adiponectine, indépendamment de polymorphismes dans le gène de l'adiponectine (253).

Dans le cas des biomarqueurs pro-inflammatoires, même si les résultats sont globalement contradictoires (254, 255), on constate que certaines tendances semblent vouloir se dessiner quant à l'efficacité des interventions avec l'ALA. Parmi un certain nombre d'études ayant évalué la production de cytokines par les cellules immunitaires sanguines telles que les PBMC (256-260), seulement celles dans lesquelles des doses d'ALA d'au moins 13,7

g/jour ont été fournies aux participants ont rapporté des effets anti-inflammatoires significatifs (256, 257). Dans le même ordre d'idées, la consommation d'ALA à des doses de 5 g/jour ou plus entraînerait, chez les individus dyslipidémiques, une réduction des concentrations circulantes de la hs-CRP et de l'IL-6 comparativement à la consommation d'une diète « témoin » ayant un faible contenu en ALA (diète « américaine » ou riche en acide linoléique, dépendamment de l'étude) (261-265). Au contraire, une supplémentation modérée en ALA correspondant à 3,8 g/jour n'aurait aucun effet sur les concentrations circulantes de la hs-CRP et de l'IL-6 chez les individus dyslipidémiques (266).

Certaines évidences suggèrent plutôt que les effets anti-inflammatoires de l'ALA pourraient davantage dépendre du statut inflammatoire initial des participants que de la dose d'ALA consommée. Sur la base d'une hypothèse soulevée pour la première fois en 2007 par Nelson *et al.* (267), il semble que l'ALA, peu importe la dose consommée (≈ 1 g/jour jusqu'à 11,6 g/jour, dépendamment de l'étude), n'influencerait pas le profil inflammatoire (hs-CRP, IL-6, TNF-α, SAA et/ou MCP-1) lorsque les participants présentent des concentrations initiales de biomarqueurs pro-inflammatoires « relativement faibles » (232, 251, 267-269). De telles concentrations initiales correspondraient, de façon plus précise, à des valeurs moyennes de la hs-CRP en-dessous de 3 mg/L et à des valeurs moyennes de l'IL-6 en-dessous de 2 pg/mL. Ce constat s'appliquerait peu importe les caractéristiques des individus, c'est-à-dire autant chez de jeunes hommes et femmes en santé et de poids normal (251, 268) que chez des sujets avec obésité abdominale (267), hypercholestérolémiques (232) ou bien atteints du syndrome métabolique (269).

Les caractéristiques de la diète sous-jacente (« *background diet* ») pourraient également influencer l'efficacité des interventions avec l'ALA. Par exemple, une supplémentation en ALA entraînerait des réductions plus prononcées de la hs-CRP, de l'IL-6 et de la SAA lorsque l'alimentation sous-jacente est riche en AGS et pauvre en AGMI comparativement à l'inverse (alimentation pauvre en AGS et riche en AGMI) (265).

D'un point de vue mécanistique, une étude *in vitro* a montré une diminution de l'expression des gènes IL-6, IL-1β et TNF-α lorsque des monocytes humains étaient incubés avec de l'ALA comparativement à de l'acide palmitique (270). À ma connaissance, les seules études récentes ayant évalué l'expression de gènes inflammatoires *in vivo* en réponse à la

consommation d'ALA dans différents tissus ont été réalisées dans des modèles animaux (rat, souris, chien) (271-273) plutôt que chez l'humain.

En somme, les facteurs qui influencent possiblement l'efficacité des interventions avec l'ALA sur le profil inflammatoire devront être confirmés dans de futures études. Il s'avèrerait aussi pertinent de combler les lacunes dans les connaissances scientifiques actuelles quant à l'impact de l'ALA sur l'expression de gènes inflammatoires *in vivo* chez l'humain.

11.1.5 Acides gras polyinsaturés oméga-3 d'origine marine

Tel que souligné dans l'introduction de l'article scientifique constituant le chapitre 4 (étude chez les Cris de la Baie-James), de nombreuses études de nature transversale ont observé que des apports augmentés en acides gras oméga-3 d'origine marine (EPA, DHA) ou en poisson étaient associés à un profil inflammatoire davantage favorable (243, 249, 274-280).

Du côté des études d'intervention nutritionnelle, les résultats diffèrent dépendamment du type de biomarqueurs inflammatoires évalué (biomarqueurs circulants anti- ou pro-inflammatoires). D'abord, les résultats semblent assez prometteurs en ce qui concerne l'adiponectine. Gray *et al.* (17) suggèrent, dans revue non-systématique des études réalisées chez l'animal (rongeurs) et chez l'humain, que la consommation d'oméga-3 d'origine marine engendre clairement une augmentation des concentrations d'adiponectine. Les effets seraient particulièrement notables lorsque la dose d'oméga-3 dépasse 1 g/jour (17). En accord avec Gray *et al.* (17), une récente méta-analyse de 14 études d'intervention randomisées contrôlées a démontré que la supplémentation en huile de poisson (dose médiane de 1,3 g/jour et durée médiane de 8 semaines) augmentait modérément, mais significativement les concentrations d'adiponectine (0,37 µg/mL, IC 95% : 0,07 à 0,67) (281). Toutefois, une grande hétérogénéité était présente entre les études incluses dans la méta-analyse. D'après les auteurs, cela signifie que la supplémentation en huile de poisson pourrait être davantage efficace dans certaines populations comparativement à d'autres. Les auteurs ne sont toutefois pas parvenus à identifier les facteurs d'influence potentiels.

Ensuite, plusieurs revues de la littérature publiées au cours des dernières années se sont attardées aux biomarqueurs pro-inflammatoires (206, 255, 282, 283). Ces revues de la littérature, systématiques ou non, incluaient majoritairement des études d'intervention nutritionnelle randomisées contrôlées. Leur principal constat est qu'aucune conclusion ferme ne peut être émise concernant l'impact des oméga-3 d'origine marine sur les concentrations sanguines de biomarqueurs pro-inflammatoires. Par exemple, Myhrstad *et al.* (206) ont indiqué qu'autant chez les sujets en santé, chez ceux à haut risque de MCV, que chez ceux atteints de MCV, au moins la moitié des interventions avec des oméga-3 d'origine marine (sous forme d'huile de poisson ou de poisson) n'ont engendré aucun changement significatif dans les concentrations sanguines de biomarqueurs inflammatoires comparativement à la consommation de produits alimentaires « témoins » (majoritairement des huiles de tournesol, d'olive et de maïs). Robinson et Mazurak (255) soulignent par ailleurs que des études partageant plusieurs similitudes (ex : devis, dose d'oméga-3 et durée d'intervention similaires) donnent parfois des résultats divergents.

Malgré les résultats contradictoires des études d'intervention qui ont spécifiquement étudié les concentrations circulantes de biomarqueurs pro-inflammatoires, d'autres études d'intervention ont montré une réduction de la production *ex vivo* de cytokines pro-inflammatoires (TNF-α, IL-6, IL-1β) par les cellules immunitaires sanguines de sujets en santé suite à la consommation de plus de 2 g/jour d'EPA et de DHA (256, 284-289).

Des revues de la littérature combinant les résultats d'études de nature mécanistique suggèrent également que les oméga-3 d'origine marine exerceraient clairement des effets anti-inflammatoires et pro-résolutifs par des mécanismes d'action directs et indirects (238, 240, 290, 291). Par exemple, dans les adipocytes et les macrophages, l'EPA et le DHA agiraient directement comme ligands endogènes de récepteurs nucléaires aux propriétés anti-inflammatoires tels que PPAR-α et PPAR-γ (292). De façon indirecte, tel que présenté précédemment à la figure 11.1, l'EPA et le DHA serviraient de substrats pour la formation d'eicosanoïdes anti-inflammatoires (séries 3 et 5) et de médiateurs lipidiques pro-résolutifs (résolvines, protectines, marésines) (238, 240, 290).

L'étude des mécanismes responsables des variations dans les concentrations sanguines de biomarqueurs inflammatoires suite à la consommation d'oméga-3 d'origine marine ne

saurait toutefois être complète sans la prise en compte des études sur l'expression de gènes inflammatoires *in vivo* chez l'humain. D'ailleurs, les travaux réalisés dans le cadre du volet « Impact des acides gras alimentaires sur l'inflammation » de mon projet de doctorat comprennent tous la mesure de l'expression de gènes inflammatoires en lien, du moins en partie, avec la consommation d'oméga-3 d'origine marine. Pour cette raison, j'ai prévu une sous-section distincte afin de traiter des connaissances actuelles sur le sujet.

Expression de gènes inflammatoires in vivo dans différents tissus en réponse à la consommation d'oméga-3 d'origine marine

Un certain nombre d'études chez l'humain se sont attardées à l'**expression de gènes inflammatoires dans le sang,** particulièrement dans les PBMC, suite à la consommation d'oméga-3 d'origine marine. Les résultats de ces études, de façon similaire aux études ayant évalué les biomarqueurs inflammatoires circulants, semblent globalement non concluants (293-297). Parmi les études n'ayant montré aucun effet significatif sur l'expression de gènes pro-inflammatoires tels que le TNF-α, l'IL-1β et l'IL-6 (296, 297), de Mello *et al.* (297) ont mentionné que la prise concomitante de statines par leurs participants avant et même pendant l'intervention avec des oméga-3 puisse expliquer le fait qu'ils n'aient observé aucun changement. En effet, il a déjà été démontré que les statines diminuent l'expression de l'IL-1β dans les PBMC (298). Parmi les études ayant démontré des effets bénéfiques significatifs sur l'expression de gènes impliqués dans les processus inflammatoires (293-295), une valant la peine d'être soulignée est celle de Bouwens *et al.* (293). Cette étude suggère en effet que la supplémentation en huile de poisson (1,8 g/jour d'EPA et de DHA durant 26 semaines) pourrait permettre de détecter des changements favorables dans l'expression de gènes reliés à l'inflammation (ex : synthèse des eicosanoïdes et voies de signalisation des interleukines et de NF-κB) en l'*absence* de variations significatives dans les concentrations plasmatiques de biomarqueurs inflammatoires (hs-CRP).

Quelques études se sont plutôt intéressées à l'**expression de gènes inflammatoires dans le tissu adipeux**, plus particulièrement le tissu adipeux sous-cutané, en réponse à la consommation d'oméga-3 d'origine marine. Chez 55 sujets sévèrement obèses, une supplémentation de 3,36 g/jour en EPA et DHA durant 8 semaines a influencé

favorablement l'expression de différents gènes reliés à l'inflammation dans le tissu adipeux sous-cutané (ex : MCP-1 ; protéine inflammatoire des macrophages 1α [MIP-1α, « *macrophage inflammatory protein 1α* »] ; CD40 [un marqueur des macrophages de type M1 pro-inflammatoires] ; IL-6 [tendance à $P < 0,10$] ; adiponectine [tendance à $P < 0,10$]) comparativement à la consommation de beurre comme témoin (299). Chez 24 sujets en surpoids ou modérément obèses (IMC entre 28 et 33 kg/m^2), des apports en EPA et DHA équivalant à 1,4 % de l'énergie (≈ 3,9 g/jour d'après l'estimation des apports énergétiques moyens) durant 14 semaines n'a toutefois eu aucun effet sur l'expression de 13 gènes inflammatoires dont MCP-1, TNF-α, IL-6, SAA et l'adiponectine comparativement à une diète ne comprenant pas d'EPA ni de DHA (300). Il est possible que l'observation d'effets bénéfiques dans la première étude comparativement à la seconde soit attribuable à un degré d'obésité plus sévère chez les participants, mais aussi à une plus grande puissance statistique. La première étude était spécifiquement conçue pour mesurer l'expression de gènes inflammatoires dans le tissu adipeux et son nombre de sujets était environ 2 fois plus élevé que dans la seconde. Chez des femmes postménopausées diabétiques, Kabir *et al.* (301) ont indiqué qu'une supplémentation de 3 g/jour en huile de poisson durant 2 mois n'avait aucun impact sur l'expression de l'adiponectine, mais réduisait l'expression de quelques marqueurs des macrophages (CD11b, CD18) dans le tissu adipeux sous-cutané comparativement à un placebo (huile de paraffine). Toutefois, les données sur l'expression des cytokines et des chimiokines les plus connues (IL-6, MCP-1, TNF-α) n'étaient pas rapportées, ce qui limite les comparaisons avec les autres études. De plus, seulement 7 participantes par groupe expérimental ont été retenues pour l'analyse de l'expression de gènes.

Enfin, une avenue relativement nouvelle dans l'étude des mécanismes inflammatoires en réponse à la consommation d'oméga-3 d'origine marine est la mesure de l'**expression de gènes inflammatoires dans le tractus gastro-intestinal**. Tel que mentionné au chapitre 1, l'importante contribution du tissu adipeux dans l'établissement d'un état inflammatoire chronique de faible intensité est bien établie. Sur la base d'études réalisées dans des modèles animaux, il est de plus en plus reconnu qu'une autre source de signaux inflammatoires serait le tractus gastro-intestinal, principalement en réponse à l'alimentation riche en lipides (« *high-fat diet* » incluant une importante proportion d'AGS à longue

chaîne [C14:0 et plus]) (302, 303). Une consommation élevée de matières grasses induirait entre autres la production de cytokines incluant le TNF-α et l'IL-6 par les cellules immunitaires intestinales de même que par les entérocytes (304, 305). Dans une revue de la littérature, Ding et Lund (306) soulignent que ces évidences en émergence dans les études chez l'animal sont particulièrement importantes puisque l'inflammation intestinale causée par l'alimentation (« *diet-induced inflammation* ») pourrait *précéder* le développement de l'obésité et de la résistance à l'insuline (302, 307, 308). Chez l'humain, de récentes évidences suggèrent également que l'obésité est associée à la présence d'inflammation intestinale (309). Ding et Lund (306) indiquent toutefois qu'encore très peu d'études ont démontré la présence d'inflammation intestinale chez les individus obèses et qu'elles ont investigué les concentrations de cytokines pro-inflammatoires ou les profils d'expression de gènes dans le côlon (gros intestin) plutôt que dans l'intestin grêle. En contrepartie, dans les modèles animaux, l'inflammation intestinale serait davantage notable dans l'intestin grêle que dans le côlon (306).

Fort possiblement à cause de leur grand potentiel anti-inflammatoire sur le plan mécanistique (238, 240, 290), certaines études ont évalué la mesure dans laquelle les oméga-3 d'origine marine pouvaient moduler l'expression de gènes impliqués dans l'inflammation intestinale chez l'animal. Dans l'intestin grêle de souris, de rats, de même que de porcelets, il est suggéré que les oméga-3 d'origine marine pourraient réduire l'inflammation en régulant à la hausse l'expression de PPAR-α et de PPAR-γ (310, 311), tout en régulant à la baisse l'expression de gènes impliqués dans des voies de signalisation aux effets pro-inflammatoires comme TLR4, NF-κB et JNK (« *c-Jun N-terminal kinase* ») (310, 312, 313). Par contre, apparemment aucune étude chez l'humain n'a évalué l'expression de gènes inflammatoires dans l'intestin grêle en réponse à une intervention nutritionnelle telle que la consommation d'oméga-3 d'origine marine.

Pour terminer ce survol de la littérature concernant les effets de différents acides gras sur l'inflammation, voici, à la page suivante, un tableau récapitulatif des pages précédentes.

Tableau 11.1 : Tableau récapitulatif des effets de différents acides gras sur l'inflammation d'après la littérature scientifique actuelle [a]

Acides gras	Effets sur l'inflammation
AG saturés et *trans*	**Selon divers types d'études :** -Effets pro-inflammatoires, qui demeurent à confirmer hors de tout doute
AGMI / acide oléique	**Études observationnelles :** -Associations apparemment inverses avec l'inflammation (1 seule étude répertoriée) **Études d'intervention :** -Résultats non concluants : absence d'effet ou effets anti-inflammatoires -Impact des AGMI parfois difficile à isoler **Études mécanistiques (expression de gènes inflammatoires) :** -Effets anti-inflammatoires potentiels, à confirmer chez l'humain
AGPI n-6 / acide linoléique	**Études observationnelles :** -Associations inverses ou nulles avec l'inflammation **Études d'intervention :** -Aucun effet **Études mécanistiques :** -Effets apparemment pro-inflammatoires d'un point de vue strictement mécanistique (métabolisme des AGPI n-6 et n-3) -Effets méconnus sur l'expression de gènes inflammatoires chez l'humain
AGPI n-3 d'origine végétale / ALA	**Études observationnelles :** -Associations inverses avec l'inflammation **Études d'intervention :** -Résultats globalement non concluants -Effets anti-inflammatoires potentiels selon certains facteurs précis (ex : état inflammatoire initial des participants, diète sous-jacente) **Études mécanistiques :** -Effets méconnus sur l'expression de gènes inflammatoires chez l'humain
AGPI n-3 d'origine marine / EPA et DHA	**Études observationnelles :** -Associations inverses avec l'inflammation **Études d'intervention :** -Augmentation de l'adiponectine circulante -Résultats non concluants pour les biomarqueurs pro-inflammatoires circulants -Réduction de la production *ex vivo* de cytokines pro-inflammatoires par les cellules immunitaires sanguines **Études mécanistiques :** -Modèles cellulaires et animaux : effets anti-inflammatoires -Expression de gènes inflammatoires *in vivo* chez l'humain : →Sang : Résultats non concluants →Tissu adipeux : Résultats non concluants →Tractus gastro-intestinal : Effets méconnus

[a] Abréviations : AG, acide gras ; AGMI, acide gras monoinsaturé ; AGPI, acide gras polyinsaturé ; ALA, acide alpha-linolénique ; DHA, acide docosahexaénoïque ; EPA, acide eicosapentaénoïque.

11.2. Avant-goût des travaux réalisés et énoncé des objectifs et hypothèses

11.2.1 Projet COMIT : Canola Oil Multicenter Intervention Trial

Sur la base des informations présentées précédemment, des études d'intervention nutritionnelle robustes étudiant simultanément les effets de divers acides gras sur les concentrations circulantes de biomarqueurs inflammatoires ainsi que sur l'expression des gènes inflammatoires dans un contexte d'alimentation contrôlée, où le « bruit » attribuable à la diète sous-jacente est éliminé, n'ont pas encore été réalisées. De telles études s'avèrent sans doute nécessaires afin de mieux caractériser les effets de chaque type d'acide gras sur le profil inflammatoire sanguin et sur les mécanismes responsables de ses variations. À ce sujet, nous avons récemment pris part à la réalisation d'un projet de grande envergure, le projet COMIT (*Canola Oil Multicenter Intervention Trial*), dont l'**objectif principal** était d'étudier l'impact de diverses formes d'huiles de canola et de lin sur la fonction endothéliale et les biomarqueurs du risque cardiovasculaire. En d'autres mots, l'idée derrière le projet COMIT était de comparer des huiles alimentaires exerçant potentiellement toutes des effets bénéfiques sur le plan cardiovasculaire en raison de leur faible contenu en AGS, mais comprenant différentes proportions d'acides gras insaturés (AGMI, AGPI n-6, AGPI n-3 d'origine végétale, AGPI n-3 d'origine marine).

L'objectif établi en lien avec mon **projet de doctorat** était :

Évaluer l'impact d'huiles alimentaires comprenant différents acides gras (acide oléique [OA], acide linoléique [LA], ALA, DHA) sur les concentrations sanguines de biomarqueurs inflammatoires ainsi que sur l'expression de gènes inflammatoires dans les cellules sanguines complètes.

Cet objectif est à la fois relié au **« contexte clinique »** et au **« contexte métabolique »** du troisième volet de mon projet de doctorat (**Figure 11.2**). Le prochain chapitre présente en détail la méthodologie du projet et les résultats obtenus, qui ont été publiés dans la revue *Nutrition Metabolism & Cardiovascular Diseases* (Baril-Gravel *et al.* 2015;25:52-59).

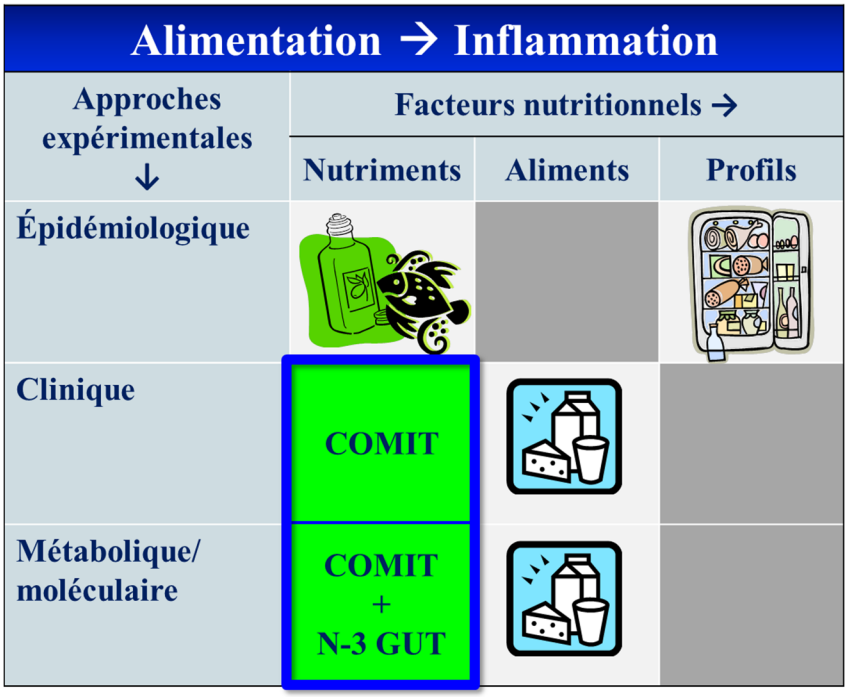


Figure 11.2 : Illustration mettant en lumière les travaux réalisés dans le cadre du **troisième volet** du présent projet de doctorat, dont le but était d'évaluer l'impact de la consommation de différents acides gras alimentaires sur l'inflammation dans un contexte clinique ainsi que métabolique (expression de gènes inflammatoires). Abréviations : COMIT, projet de recherche intitulé « Canola Oil Multicenter Intervention Trial » ; N-3 GUT, projet de recherche portant sur les oméga-3 d'origine marine et l'expression de gènes inflammatoires dans l'intestin grêle.

L'**hypothèse globale** émise en lien avec l'objectif ci-haut était :

1. L'huile enrichie en DHA (oméga-3 d'origine marine) exerce les plus grands effets anti-inflammatoires comparativement aux huiles enrichies en ALA (oméga-3 d'origine végétale), en LA (oméga-6) et en OA (AGMI).

Voici d'ailleurs quelques détails concernant les comparaisons qu'il était possible d'effectuer à l'intérieur du projet afin de vérifier cette hypothèse :

- La comparaison entre l'huile enrichie en ALA et l'huile enrichie en DHA permettait d'évaluer les effets sur l'inflammation des **oméga-3 d'origine végétale et marine à un ratio n-6 : n-3 similaire**.

- La comparaison entre l'huile enrichie en LA et l'huile enrichie en DHA permettait d'évaluer les effets sur l'inflammation de **remplacer des oméga-6 par des oméga-3 d'origine marine, mais aussi par de l'acide oléique**.
- La comparaison entre l'huile enrichie en OA (*CanolaOleic*) et l'huile enrichie en DHA permettait d'évaluer les effets sur l'inflammation de **remplacer directement des AGMI par des oméga-3 d'origine marine**.

Il était aussi possible de vérifier d'**autres hypothèses** telles que :

2. L'huile enrichie en ALA exerce des effets anti-inflammatoires comparativement à l'huile enrichie en LA.

- La comparaison entre l'huile enrichie en LA et l'huile enrichie en ALA permettait d'évaluer les effets sur l'inflammation de **remplacer des oméga-6 par des oméga-3 d'origine végétale à des quantités d'AGS, d'AGMI et d'AGPI totaux similaires**.

3. L'huile de canola régulière relativement riche en OA n'a pas d'effet sur l'inflammation comparativement aux huiles enrichies en ALA et en LA.

- La comparaison des huiles enrichies en ALA et en LA avec l'huile de canola régulière relativement riche en OA permettait d'évaluer les effets sur l'inflammation de **remplacer des AGPI d'origine végétale au profit d'AGMI tout en atteignant un ratio n-6 : n-3 relativement faible**.

Voici maintenant l'introduction des autres travaux réalisés dans le cadre de ce troisième et dernier volet de mon projet de doctorat.

11.2.2 Projet N-3 GUT : Oméga-3 et expression de gènes inflammatoires dans l'intestin grêle

Sur la base des informations présentées précédemment à la section 11.1.5, nous étions intéressés à combler certaines lacunes présentes dans la littérature scientifique en entreprenant, à notre connaissance, la première étude chez l'humain à avoir évalué l'expression de gènes inflammatoires dans l'intestin grêle en réponse à la consommation d'oméga-3 d'origine marine.

L'**objectif** établi dans le cadre de mon projet de doctorat était :

Évaluer l'impact d'une supplémentation en oméga-3 d'origine marine (EPA et DHA) sur l'expression de gènes pro-inflammatoires dans le duodénum d'hommes obèses atteints du diabète de type 2.

Cet objectif est entièrement relié au **« contexte métabolique »** du troisième volet de mon projet de doctorat (Figure 11.2). Sur la base des résultats des études réalisées chez l'animal, l'**hypothèse** émise en lien avec cet objectif était :

La supplémentation en EPA et DHA diminue l'expression de gènes pro-inflammatoires dans le duodénum comparativement à un placebo.

Le chapitre 13 présente en détail la méthodologie et les résultats du projet N-3 GUT, qui ont été publiés dans la revue *Nutrition Journal* (Labonté *et al.* 2013;12(1):98). Il est important de noter que l'objectif poursuivi ici était un objectif de nature principalement **exploratoire**, réalisé à l'intérieur d'un projet du Dr Patrick Couture dont l'objectif général était plutôt d'évaluer les mécanismes par lesquels les oméga-3 d'origine marine influencent le métabolisme des lipoprotéines intestinales chez des patients avec le diabète de type 2. La taille de l'échantillon a donc été déterminée en fonction du changement dans le taux de production de l'apolipoprotéine B48 enrichie en triglycérides en réponse à la supplémentation en EPA et DHA (données non publiées).

CHAPITRE 12 :

UNE HUILE DE CANOLA ENRICHIE EN ACIDE DOCOSAHEXAÉNOÏQUE AUGMENTE LES CONCENTRATIONS D'ADIPONECTINE : UNE ÉTUDE D'INTERVENTION NUTRITIONNELLE CONTRÔLÉE, RANDOMISÉE, EN CHASSÉ-CROISÉ

Baril-Gravel L, Labonté ME, Couture P, Vohl MC, Charest A, Guay V, Jenkins DA, Connelly PW, West S, Kris-Etherton PM, Jones PJ, Fleming JA, Lamarche B.

Docosahexaenoic acid-enriched canola oil increases adiponectin concentrations: A randomized crossover controlled intervention trial

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RÉSUMÉ

Nous avons évalué l'impact de 5 huiles comprenant différentes quantités d'acide alpha-linolénique (ALA), d'acide linoléique (LA), d'acide oléique (OA) et d'acide docosahexaénoïque (DHA) sur les concentrations plasmatiques de biomarqueurs inflammatoires et sur l'expression de gènes inflammatoires et de facteurs de transcription dans les cellules sanguines complètes. Dans le cadre d'une étude d'intervention nutritionnelle contrôlée, randomisée, en chassé-croisé, 114 hommes et femmes avec obésité abdominale et au moins une autre composante du syndrome métabolique ont consommé 5 diètes expérimentales isoénergétiques d'une durée de 4 semaines chacune et entrecoupées d'une période de repos (« *washout* ») de 4 semaines. Chaque diète fournissait 60 g/3000 kcal de différentes huiles : 1) mélange d'huile de maïs et de carthame considéré comme témoin (*CornSaff* ; riche en LA), 2) mélange d'huile de lin et de carthame (*FlaxSaff* ; riche en ALA), 3) huile de canola régulière (*Canola*, riche en OA), 4) huile de canola enrichie en OA (*CanolaOleic* ; contenu le plus élevé en OA), 5) huile de canola enrichie en OA et en DHA (*CanolaDHA*). L'expression de gènes dans les cellules sanguines complètes a été évaluée dans un sous-groupe de 62 sujets. La consommation de l'huile *CanolaDHA* a entraîné une augmentation des concentrations d'adiponectine comparativement à l'huile témoin *CornSaff* (+4,5% ; P = 0,04) et à l'huile *FlaxSaff* (+6,9% ; P = 0,0008). L'huile *CanolaDHA* a aussi réduit l'expression du gène *IL1B* comparativement aux huiles *CornSaff* et *Canola* (-11% et -13%, respectivement, P = 0,03). Les concentrations de la hs-CRP étaient plus faibles suite à la consommation de l'huile *Canola* comparativement à l'huile *FlaxSaff* (-17,8%, P = 0,047). Ces résultats suggèrent qu'une huile de canola enrichie en DHA exerce des effets anti-inflammatoires comparativement aux acides gras polyinsaturés de source végétale.

TITLE PAGE

TITLE

Docosahexaenoic acid-enriched canola oil increases adiponectin concentrations: A randomized crossover controlled intervention trial

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ACRONYMS: ALA, alpha-linolenic acid; BMI, body mass index; BP, blood pressure; *CCL2*, chemokine (C-C motif) ligand 2; cDNA, complementary deoxyribonucleic acid; COMIT, Canola Oil Multicenter Intervention Trial; CRP; C-reactive protein; CV, coefficient of variation; Ct, cycle threshold; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; *G6PD*, glucose-6-phosphate dehydrogenase; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; HDL-C, high-density lipoprotein cholesterol; hs-CRP; high-sensitivity CRP; IDF, International Diabetes Federation; IL, interleukin; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; MetSyn, metabolic syndrome; MUFA, monounsaturated fatty acids; *NFKB1*, nuclear factor kappa-B subunit 1; *NPR3*, natriuretic peptide receptor C; OA, oleic acid; PPAR, peroxisome proliferator-activated receptor; *PPARA*, PPAR alpha; PUFA, polyunsaturated fatty acids; RNA, ribonucleic acid; SD, standard deviation; SFA, saturated fatty acids; *SREBF2*, sterol regulatory element-binding transcription factor 2; TG, triglycerides; *TNF*, tumor necrosis factor; *TRAF3*, TNF receptor-associated factor 3

ABSTRACT

Background and Aims: Little is known about the effect of various dietary fatty acids on pro- and anti-inflammatory processes. We investigated the effect of 5 oils containing various amounts of alpha-linolenic acid (ALA), linoleic acid (LA), oleic acid (OA) and docosahexaenoic acid (DHA) on plasma inflammatory biomarkers and expression levels of key inflammatory genes and transcription factors in whole blood cells. **Methods and Results:** In a randomized, crossover controlled nutrition intervention, 114 adult men and women with abdominal obesity and at least one other criterion for the metabolic syndrome consumed 5 experimental isoenergetic diets for 4 weeks each, separated by 4-week washout periods. Each diet provided 60 g/3000 kcal of different oils: 1) control corn/safflower oil blend (CornSaff; LA-rich), 2) flax/safflower oil blend (FlaxSaff; ALA-rich), 3) conventional canola oil (Canola; OA-rich), 4) high oleic canola oil (CanolaOleic; highest OA content), 5) DHA-enriched high oleic canola oil (CanolaDHA; OA- and DHA-rich). Gene expression in whole blood cells was assessed in a subset of 62 subjects. CanolaDHA increased plasma adiponectin concentrations compared with the control CornSaff oil treatment (+4.5%, P=0.04) and FlaxSaff (+6.9%, P=0.0008). CanolaDHA also reduced relative expression levels of interleukin (*IL*)*1B* compared with CornSaff and Canola (-11% and -13%, respectively, both P=0.03). High-sensitivity C-reactive protein concentrations were lower after Canola than after FlaxSaff (-17.8%, P=0.047). **Conclusion:** DHA-enriched canola oil exerts anti-inflammatory effects compared with polyunsaturated fatty acids from plant sources.

INTRODUCTION

Pro-inflammatory biomarkers such as C-reactive protein (CRP) and interleukin (IL)-6 have been associated with an increased risk of all-cause mortality and cardiovascular events [1, 2]. Conversely, adiponectin is an adipose tissue-derived hormone recognized for its anti-atherosclerotic and anti-inflammatory properties [3].

Convincing evidence now suggests that diet significantly affects pro- and anti-inflammatory processes [4]. However, confusion remains about the effects that various dietary fatty acids have on inflammation. Saturated fatty acids are believed to have pro-inflammatory properties [4]. While observational studies have quite consistently reported inverse associations between consumption of n-3 polyunsaturated fatty acids (PUFA) from marine sources (eicosapentaenoic acid, EPA; docosahexaenoic, DHA) and inflammation [5, 6], results from randomized controlled trials have been inconsistent [5, 7]. Data from intervention studies on the effect of the plant-based essential n-3 PUFA alpha-linolenic acid (ALA) on inflammation are also conflicting [5, 8, 9]. Contrary to some beliefs, a recent systematic review of randomized controlled nutrition intervention studies has shown that consumption of the n-6 PUFA linoleic acid (LA) actually exerts no deleterious effect on inflammatory markers [10]. Finally, the effect of monounsaturated fatty acids (MUFA), specifically oleic acid (OA), on inflammation is poorly understood since most previous studies were conducted in the context of experimental diets that included changes beyond just MUFA, such as the Mediterranean diet or inclusion of MUFA-rich foods such as nuts [11, 12].

The objective of this study was to evaluate the effects of oils containing various amounts of ALA, LA, OA and DHA in the context of a low SFA diet [13, 14] on plasma high-sensitivity (hs)-CRP, IL-6, and adiponectin concentrations in subjects with abdominal obesity and at least one other criterion for the metabolic syndrome. Consistent with previous extensive review of the literature on diet and inflammation [4], we hypothesized that the oil containing DHA exerts the greatest benefit on inflammatory markers. In exploratory analyses, we also investigated how consumption of the different oil blends modified the expression of inflammatory genes and transcription factors in whole blood cells.

METHODS

Study population

COMIT (Canola Oil Multicenter Intervention Trial) was a multicenter trial designed to study the effect of various forms of canola and flax oils on vascular function and biomarkers of CVD risk. Methods have been described in detail elsewhere [13, 14]. Recruitment took place at the University of Manitoba in Winnipeg (Canada), Laval University in Québec City (Canada) and Pennsylvania State University (Penn State) in University Park, Pennsylvania (USA). Inclusion criteria were: age between 18-65 years, abdominal obesity defined by a waist circumference ≥ 94 cm for men, and ≥ 80 cm for women [15], and at least one of the four following metabolic abnormalities according to the International Diabetes Federation (IDF) criteria for metabolic syndrome [15]: fasting glucose ≥ 5.6 mmol/L, triglycerides (TG) ≥ 1.7 mmol/L, systolic and diastolic blood pressures ≥ 130/85 mm Hg, and high-density lipoprotein cholesterol (HDL-C) ≤ 1.0 mmol/L for men and ≤ 1.3 mmol/L for women. Otherwise subjects had to be healthy; exclusion criteria have been described previously [13, 14]. Written consent was obtained from all subjects at the beginning of the study. The protocol was approved by Institutional Ethics Boards at all centers.

Experimental design

The study was designed as a double-blind, randomized crossover intervention that utilized a controlled feeding design with five experimental 4-week phases each separated by a 2 to 4-week washout period [13, 14]. All foods were provided to participants during the five experimental dietary phases to maximize control over each experimental diet effect, as well as every subject's energy needs. Daily energy requirements were estimated at study onset using the Mifflin equation [16] and the Harris-Benedict equation [17] with adjustment for subjects' level of physical activity. Further details on the feeding protocol during the experimental phases are given in [13] and **Supplemental File 1**.

Diet and oils

Table 1 presents the fatty acid composition of the five oil blends. Experimental oils (60 g/3000 kcal) were incorporated into shakes made with non-fat milk, sorbet and fruits that were consumed twice a day, typically at breakfast and dinner. The 7-day cycle menu was identical for each of the five experimental diets at all centers and only the experimental oils provided in the shakes differed. The macronutrient content of the diets was 15% from protein, 50% from carbohydrate and 35% from lipids (**Table 2**).

Anthropometric measures and screening values

Details on the measurement of anthropometry indices and screening values are provided in **Supplemental File 2**.

Blood collection

Twelve-hour fasting blood samples were taken from an antecubital vein on two consecutive days at the beginning (days 1 and 2) and end (days 29 and 30) of each dietary phase as described previously [13].

Inflammatory biomarkers analyses

Plasma hs-CRP was analyzed by nephelometry at St-Michael's Hospital in Toronto (Behring BN ProSpec; Siemens, Mississauga, Canada) with an intra-assay coefficient of variation (CV) of 3.5% [18]. The average of the two post-treatment measures (days 29 and 30) was used in the analyses. Data from subjects with hs-CRP values > 10 mg/L at any point during the study were considered as missing values in the analyses. Plasma adiponectin (#K1001-1, B-Bridge International Inc., CV=5.2%) and IL-6 concentrations (Human IL-6 Immunoassay, #HS600B, R&D System., CV=7.4%) were measured at INAF in Québec City by commercially available ELISA kits on samples taken at day 30 of each dietary treatment.

Inflammatory gene expression analysis

Additional samples were collected using PAXGene Blood RNA tubes (Qiagen, Valencia, CA) on the last day of each dietary treatment for the measurement of inflammatory gene expression in whole blood cells in a subsample of 62 participants. Details on this analysis are provided in **Supplemental File 3**.

Statistical analyses

The COMIT study was designed to investigate the effects of different oil blends on vascular endothelial function as primary outcome [13, 14]. The data presented here are secondary analyses of outcomes related to inflammation. The *a priori* defined analysis consisted of comparing the difference in outcomes from the control CornSaff oil treatment (Δ vs. CornSaff) based on a *per protocol* approach using the least-square means statistic in the MIXED procedure for repeated measures in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), with treatment as a fixed effect and subject as a random effect. Experimental treatments were compared using the post-hoc Tukey-Kramer adjustment for multiple comparisons. The AR(1) covariance matrix structure was used in all cases except for sterol regulatory element-binding transcription factor 2 (*SREBF2*), for which UN was used. Pre-specified potential confounders such as age, sex, menopausal status, study center, and "baseline" inflammatory status (i.e. on the control CornSaff oil treatment) and their interaction with the main treatment effect were investigated in all models. Analyses showed no evidence of a carry-over effect of the dietary treatments on inflammatory markers. Variables with a skewed distribution were natural log-transformed before statistical analyses. Values are presented as untransformed means ± standard deviation (SD) unless stated otherwise. A P value < 0.05 was considered statistically significant.

RESULTS

Subject characteristics

A total of 151 men and women met the inclusion criteria used in this analysis (**Supplemental File 4**). The combined dropout and exclusion rate during the dietary intervention phases was 23.8% (n=36). Dropout/exclusion rate in each center is provided in

Supplemental File 5. Of the randomized subjects, 115 completed all five dietary phases, but one participant was excluded from analyses because of CRP values > 10 mg/L on all dietary treatments. Analyses are therefore based on a total of 114 subjects. Characteristics of these 114 subjects at screening are presented in **Table 3**. The sample comprised 53% women, among which 58% were postmenopausal. Forty-six percent of participants were obese (body mass index [BMI] ≥ 30 kg/m^2) and 46% had metabolic syndrome as defined by the IDF criteria [15]. As per the inclusion criteria, all subjects had a waist circumference ≥ 94 cm or ≥ 80 cm for men and women, respectively.

Anthropometric data

There was no between-treatment difference in body weight (maximum difference between any of the 5 treatments = 0.5 kg) or waist circumference (both $P > 0.06$ for the main treatment effect; data not shown). A significant treatment effect was observed for BMI (P=0.04; data not shown), but no between-treatment difference was evidenced based on the post-hoc Tukey-Kramer adjustment for multiple comparisons (all $P > 0.07$).

Inflammatory markers

Daily variation in hs-CRP concentrations (day 29 vs. day 30) was similar between treatments and averaged 13.7%. As indicated above, the primary analysis tested the effect of each experimental oil vs. the control CornSaff oil. Post-hoc comparison also allowed us to compare the difference from CornSaff between the various experimental oils (**Table 4** and **Supplemental File 6** for a graphic illustration of percent differences from CornSaff). A significant treatment effect was observed for plasma hs-CRP concentrations (P=0.04, Table 4). Compared with the control CornSaff oil, the reduction in hs-CRP concentrations after Canola was significantly greater than after FlaxSaff (Δ hs-CRP: -0.20 vs. +0.15 mg/L, respectively, P=0.047). There was no significant treatment effect on plasma IL-6 concentrations (Table 4). A significant treatment effect was observed for plasma adiponectin concentrations (P=0.002, Table 4). Plasma adiponectin concentrations were higher after CanolaDHA than after CornSaff (+4.5%, Δ adiponectin: +0.37 µg/L, P=0.04). The increase in plasma adiponectin concentrations with CanolaDHA vs. CornSaff was greater than with FlaxSaff (Δ adiponectin: +0.37 vs. -0.20 µg/L, respectively, P=0.0008).

The response of adiponectin to the experimental oils also varied according to menopausal status (P=0.02 for this interaction). There was no significant difference in adiponectin vs. CornSaff for any of the four experimental oils among premenopausal women (P=0.91) while a significant treatment effect was observed among postmenopausal women (P=0.001). As shown in **Supplemental File 7,** plasma adiponectin concentrations were significantly reduced after FlaxSaff vs. CornSaff in postmenopausal women (-6.8%, P=0.02) and this was significantly different than the change with CanolaDHA (Δ adiponectin: -0.77 vs. +0.61 μg/L, respectively, P=0.003). Sex, age, and values on the control CornSaff oil treatment (serving as "baseline" values) showed no significant interaction with treatment for any of the markers measured.

Inflammatory gene expression

Expression of key inflammatory genes and transcription factors in whole blood cells in response to the FlaxSaff, Canola, CanolaOleic and CanolaDHA oils compared with the control CornSaff oil was examined in a subsample of 62 subjects. The baseline characteristics of these 62 subjects did not differ from characteristics of the total sample of 114 participants ($P \geq 0.08$, **Supplemental File 8**). **Table 5** shows that there was no significant treatment effect on relative expression levels of interleukin-18 (*IL18*), nuclear factor kappa-B subunit 1 (*NFKB1*), peroxisome proliferator-activated receptor alpha (*PPARA*), *SREBF2*, tumor necrosis factor (*TNF*), and TNF receptor-associated factor 3 (*TRAF3*) (all $P \geq 0.45$). A significant treatment effect was observed for the relative expression level of *IL1B* (P=0.04). More specifically, *IL1B* expression was 11% lower after CanolaDHA than after CornSaff (fold change -1.11, P=0.03). This reduction in *IL1B* gene expression after CanolaDHA vs. CornSaff was also greater than the difference after Canola vs. CornSaff (fold change -1.11 vs. +1.02, respectively, P=0.03).

DISCUSSION

The experimental design of the present study allowed us to compare the effect of different oil blends in the context of a low SFA diet on biomarkers of inflammation, with emphasis on substitution of specific fatty acids by others in men and women with abdominal obesity and at least one other risk factor for the metabolic syndrome. Results showed that DHA-

enriched canola oil increased adiponectin concentrations and that OA-rich conventional canola oil reduced hs-CRP concentrations compared with oils rich in PUFA from plant sources.

The comparison of the control CornSaff and FlaxSaff oil treatments allowed us to evaluate the effect of replacing n-6 PUFA (primarily LA) with n-3 PUFA (primarily ALA) at similar SFA, MUFA and total PUFA intakes. Inflammatory biomarker concentrations did not differ after consumption of these two oil blends despite a marked difference in their n-6:n-3 ratio (208:1 vs. 1.2:1). These data are consistent with the emerging concept that a high intake of n-6 PUFA, including LA, may not promote inflammation as originally thought [4, 10].

We observed an increase in plasma adiponectin concentrations following consumption of the DHA-enriched high-oleic canola oil compared with the CornSaff oil blend high in n-6 PUFA and the flax-safflower oil blend rich in ALA. Among women, this effect was particularly strong after menopause, a period generally characterized by the presence of a pro-inflammatory state [19]. It is important to appreciate that this anti-inflammatory effect cannot be ascribed solely to a higher consumption of the marine-derived n-3 PUFA since MUFA (primary oleic acid) content was higher in the DHA-enriched oil treatment compared with the other two treatment oils. Nevertheless, a recent systematic review and meta-analysis of 14 randomized placebo-controlled trials by Wu et al. [20] showed that fish oil supplementation (median intake 1.3 g/d) increased circulating adiponectin levels by 0.37 μg/mL (95% CI 0.07-0.67), which is consistent with the increase seen in the present study. Such an increase in adiponectin concentrations with DHA predicts a 3% lower risk of incident type 2 diabetes [20, 21]. Treatment of murine and human adipocytes with *IL1B* has been shown to decrease the production of adiponectin in a cell culture model [22]. Based on this, and consistent with data from the present study, it appears that the increase in adiponectin concentrations with the DHA-enriched canola oil may be explained, at least in part, by down-regulation of *IL1B*. Additionally, the comparison of the CanolaDHA and FlaxSaff oil treatments allowed us to evaluate the effect of a marine and vegetable source of n-3 PUFA at a similar n-6:n-3 ratio. Consistent with our results, a study in a rat model showed that dietary supplementation for 12 weeks with n-3 PUFA derived from fish increased plasma adiponectin concentrations in a dose-dependent manner, while

supplementation with n-3 PUFA from vegetable sources had no effect [23]. This differential effect of marine vs. vegetable sources of n-3 PUFA on adiponectin concentrations may be explained, at least partly, by data suggesting that DHA is a potent ligand for PPAR-γ, a key transcription factor involved in the regulation of the adiponectin gene [24], whereas ALA is not [25].

Comparison of the regular canola oil treatment with the flax-safflower oil treatment showed that increasing MUFA (oleic acid) at the expense of n-6 and n-3 PUFA from plant sources reduces hs-CRP concentrations. This contrasts with results from previous crossover studies that showed no difference in hs-CRP when MUFA-enriched diets were compared with PUFA-enriched diets (n-6 and/or n-3 PUFA from plant sources) [26-28]. It is important to note, however, that these studies each included fewer than 40 subjects, thereby limiting their capacity to detect small yet significant changes in hs-CRP.

This study has strengths and limitations. The crossover randomized double-blind design of this study is a major strength, considering also that the treatment oils were evaluated under tightly controlled feeding conditions, a rare feature in such large clinical trials. The multicenter nature of the study also improves the generalization of the results. Plasma hs-CRP concentrations were measured twice after each treatment, consistent with current clinical guidelines for the use of CRP [29], and combined with the large sample size this provided more statistical power to detect small changes. The control corn-safflower oil treatment was low in SFA (≤ 7% of energy) and high in plant-derived n-6 PUFA (16%). Any difference in inflammatory markers compared to this heart healthy control diet, such as the one observed for the DHA-enriched canola oil, is therefore likely to be robust and of potential clinical significance. Experimental oils were consumed for short periods of time and therefore their longer-term effects need to be investigated. Average consumption of DHA was 2.9 ± 0.6 g/day during the CanolaDHA treatment (not shown). High doses of DHA have been shown to promote oxidative stress [30] but this was not measured in our study. The increase in adiponectin concentrations after the CanolaDHA treatment was observed despite this potential induction of oxidative stress. This needs further investigation. Finally, the dropout rate was relatively high, but this was anticipated given the amount of time and dedication required to complete the study.

In conclusion, our results suggest that in the context of a heart healthy diet with a favorable fatty acid profile including low SFA, DHA and oleic acid beneficially modify different biomarkers of inflammation compared with PUFAs of plant origin (n-6 and n-3). Our findings also suggest that consumption of n-6 PUFA compared with MUFA or n-3 PUFA from vegetable sources has no apparent deleterious effect on biomarkers of inflammation in men and women with abdominal obesity and other risk factors associated with metabolic syndrome.

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CONFLICT OF INTEREST STATEMENT

DAJ reported serving on the Scientific Advisory Board of Unilever, Sanitarium Company, California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and Canola Council of Canada; receiving honoraria for scientific advice from the Almond Board of California, International Tree Nut Council Nutrition Research and Education Foundation, Barilla, Unilever Canada, Solae, Oldways, Kellogg's, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, and Alpro Foundation; being on the speakers panel for the Almond Board of California; receiving research grants from Loblaw Brands Ltd, Unilever, Barilla, Almond Board of California, Solae, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council, and Peanut Institute; and

receiving travel support to meetings from the Almond Board of California, Unilever, Alpro Foundation, and International Tree Nut Council, Canadian Institutes of Health Research, Canada Foundation for Innovation, and the Ontario Research Fund. DAJ receives salary support as a Canada Research Chair from the federal government of Canada and his wife is a director of Glycemic Index Laboratories, Toronto, Ontario, Canada. SW served as a consultant and received travel funding from the Canola Council of Canada. PJJ reported receiving grants from Advanced Foods and Materials Network, Danone, Enzymotec, Unilever, the Canadian Institutes of Health Research and Canada Research Chair Endowment of the Federal Government of Canada. PJJ also serves as President of Nutritional Fundamentals for Health Inc., which markets plant sterols among other nutraceuticals. BL has received research funding from the Dairy Farmers of Canada, Dairy Australia, the Danone Institute and Atrium Innovations and honoraria from Unilever, Danone, and the Dairy Farmers of Canada. BL is Chair in Nutrition and Cardiovascular Health, supported in part by Provigo/Loblaws. LBG, MEL, PC, MCV, AC, VG, PWC, PMKE, JAF have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

PJ, PC, PWC, DAJ, SW, PMKE, and BL obtained funding for the study and collaborated to the study design. LBG, AC, VG and JAF contributed to study conduct and data entry. MCV contributed her expertise for the gene expression studies. MEL and LBG performed statistical analyses, interpreted the data, and wrote the manuscript. BL has primary responsibility for final content. All authors critically reviewed the manuscript and approved the final version.

REFERENCES

[1] Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem. 2001;47:403-11.

[2] Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000;101:1767-72.

[3] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev. 2005;26:439-51. doi: 10.1210/er.2005-0005.

[4] Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106 Suppl 3:S5-78. doi: 10.1017/S0007114511005460.

[5] Robinson LE, Mazurak VC. N-3 polyunsaturated fatty acids: relationship to inflammation in healthy adults and adults exhibiting features of metabolic syndrome. Lipids. 2013;48:319-32. doi: 10.1007/s11745-013-3774-6.

[6] Robinson LE, Buchholz AC, Mazurak VC. Inflammation, obesity, and fatty acid metabolism: influence of n-3 polyunsaturated fatty acids on factors contributing to metabolic syndrome. Appl Physiol Nutr Metab. 2007;32:1008-24. doi: 10.1139/H07-087.

[7] Myhrstad MC, Retterstol K, Telle-Hansen VH, Ottestad I, Halvorsen B, Holven KB, et al. Effect of marine n-3 fatty acids on circulating inflammatory markers in healthy subjects and subjects with cardiovascular risk factors. Inflamm Res. 2011;60:309-19. doi: 10.1007/s00011-010-0302-5.

[8] Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr. 2004;134:2991-7.

[9] Dewell A, Marvasti FF, Harris WS, Tsao P, Gardner CD. Low- and high-dose plant and marine (n-3) fatty acids do not affect plasma inflammatory markers in adults with metabolic syndrome. J Nutr. 2011;141:2166-71. doi: 10.3945/jn.111.142240.

[10] Johnson GH, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. J Acad Nutr Diet. 2012;112:1029-41, 41 e1-15. doi: 10.1016/j.jand.2012.03.029.

[11] Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA. 2004;292:1440-6. doi: 10.1001/jama.292.12.1440.

[12] Rajaram S, Connell KM, Sabate J. Effect of almond-enriched high-monounsaturated fat diet on selected markers of inflammation: a randomised, controlled, crossover study. Br J Nutr. 2010;103:907-12. doi: 10.1017/S0007114509992480.

[13] Senanayake VK, Pu S, Jenkins DA, Lamarche B, Kris-Etherton PM, West SG, et al. Plasma fatty acid changes following consumption of dietary oils containing n-3, n-6, and n-9 fatty acids at different proportions: preliminary findings of the Canola Oil Multicenter Intervention Trial (COMIT). Trials. 2014;15:136. doi: 10.1186/1745-6215-15-136.

[14] Jones PJ, Senanayake VK, Pu S, Jenkins DJ, Connelly PW, Lamarche B, et al. DHA-enriched high-oleic acid canola oil improves lipid profile and lowers predicted cardiovascular disease risk in the canola oil multicenter randomized controlled trial. Am J Clin Nutr. 2014. doi: 10.3945/ajcn.113.081133.

[15] Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366:1059-62. doi: 10.1016/S0140-6736(05)67402-8.

[16] Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr. 1990;51:241-7.

[17] Harris J, Benedict F. A biometric study of basal metabolism in man. Publication No 279. The Carnegie Institution of Washington. 1919.

[18] Jenkins DJ, Kendall CW, Marchie A, Faulkner DA, Josse AR, Wong JM, et al. Direct comparison of dietary portfolio vs statin on C-reactive protein. Eur J Clin Nutr. 2005;59:851-60. doi: 10.1038/sj.ejcn.1602152.

[19] Camilleri G, Borg M, Brincat S, Schembri-Wismayer P, Brincat M, Calleja-Agius J. The role of cytokines in cardiovascular disease in menopause. Climacteric. 2012;15:524-30. doi: 10.3109/13697137.2012.700743.

[20] Wu JH, Cahill LE, Mozaffarian D. Effect of fish oil on circulating adiponectin: a systematic review and meta-analysis of randomized controlled trials. J Clin Endocrinol Metab. 2013;98:2451-9. doi: 10.1210/jc.2012-3899.

[21] Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2009;302:179-88. doi: 10.1001/jama.2009.976.

[22] Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulange A, Capeau J, et al. Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. Diabetologia. 2006;49:2162-73. doi: 10.1007/s00125-006-0335-z.

[23] Duda MK, O'Shea KM, Tintinu A, Xu W, Khairallah RJ, Barrows BR, et al. Fish oil, but not flaxseed oil, decreases inflammation and prevents pressure overload-induced cardiac dysfunction. Cardiovasc Res. 2009;81:319-27. doi: 10.1093/cvr/cvn310.

[24] Oster RT, Tishinsky JM, Yuan Z, Robinson LE. Docosahexaenoic acid increases cellular adiponectin mRNA and secreted adiponectin protein, as well as PPARgamma mRNA, in 3T3-L1 adipocytes. Appl Physiol Nutr Metab. 2010;35:783-9. doi: 10.1139/H10-076.

[25] Paschos GK, Zampelas A, Panagiotakos DB, Katsiougiannis S, Griffin BA, Votteas V, et al. Effects of flaxseed oil supplementation on plasma adiponectin levels in dyslipidemic men. Eur J Nutr. 2007;46:315-20. doi: 10.1007/s00394-007-0668-5.

[26] Keogh JB, Grieger JA, Noakes M, Clifton PM. Flow-mediated dilatation is impaired by a high-saturated fat diet but not by a high-carbohydrate diet. Arterioscler Thromb Vasc Biol. 2005;25:1274-9. doi: 10.1161/01.ATV.0000163185.28245.a1.

[27] Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. J Nutr. 2007;137:945-52.

[28] Kontogianni MD, Vlassopoulos A, Gatzieva A, Farmaki AE, Katsiougiannis S, Panagiotakos DB, et al. Flaxseed oil does not affect inflammatory markers and lipid profile compared to olive oil, in young, healthy, normal weight adults. Metabolism. 2013;62:686-93. doi: 10.1016/j.metabol.2012.11.007.

[29] Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier A, et al. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult - 2009 recommendations. Can J Cardiol. 2009;25:567-79.

[30] Guillot N, Caillet E, Laville M, Calzada C, Lagarde M, Vericel E. Increasing intakes of the long-chain omega-3 docosahexaenoic acid: effects on platelet functions and redox status in healthy men. FASEB J. 2009;23:2909-16. doi: 10.1096/fj.09-133421.

TABLES

Table 1. Fatty acid profiles of the five experimental oils (g per 60 g oil)[a,b]

	CornSaff	FlaxSaff	Canola	CanolaOleic	CanolaDHA
SFA	**4.7**	**4.9**	**4.3**	**3.9**	**5.2**
C12:0	0	0	0.1	0	0
C14:0	0	0	0	0	0.5
C16:0	3.5	2.9	2.4	2.2	3.2
C17:0	0	0	0	0.1	0.1
C18:0	1.1	1.9	1.1	1.1	1.0
MUFA	**10.6**	**10.7**	**37.7**	**43.2**	**38.3**
C18:1 (OA)	10.6	10.7	35.2	42.9	37.9
PUFA	**41.8**	**41.7**	**17.6**	**10.3**	**14.0**
C18:2 (LA)	41.6	22.5	11.7	8.8	7.6
C18:3 (ALA)	0.2	19.2	5.9	1.4	1.2
C20:5 (EPA)	0	0	0	0	0.1
C22:5	0	0	0	0	1.4
C22:6 (DHA)	0	0	0	0	3.5
n-6:n-3	208:1	1.2:1	2:1	6.3:1	1.2:1

[a] Data in this table, except for the n-6:n-3 ratio, are reproduced from Table 2 of reference [13] (Senanayake VK, Pu S, Jenkins DA, Lamarche B, Kris-Etherton PM, West SG, et al. Plasma fatty acid changes following consumption of dietary oils containing n-3, n-6, and n-9 fatty acids at different proportions: preliminary findings of the Canola Oil Multicenter Intervention Trial (COMIT). Trials. 2014;15:136. doi: 10.1186/1745-6215-15-136. Copyright 2014 Senanayake *et al*, Publisher: BioMed Central). ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; OA, oleic acid; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

[b] CornSaff (considered as the control oil blend) was a 25% corn and 75% safflower oil blend rich in LA, bought from local grocery stores; FlaxSaff was a 60% flax and 40% safflower oil blend rich in ALA, provided by Shape Foods Inc., Brandon, MB, Canada;

Canola was a conventional canola oil; CanolaOleic was a proprietary canola oil enriched in oleic acid, provided by Richardson Oilseed Limited, Winnipeg, MB, Canada; and CanolaDHA was a proprietary canola oil enriched in oleic acid and DHA, provided by Martek Biosciences Corporation, Columbia, MD, USA. The three canola-based oil treatments were all high in OA, with CanolaOleic having the highest amount.

Table 2. Nutrient composition of the five experimental diets (% of energy)[a]

	CornSaff	FlaxSaff	Canola	CanolaOleic	CanolaDHA
Protein	15	15	15	15	15
Carbohydrate	50	50	50	50	50
Lipid	35	35	35	35	35
SFA	6.7	6.8	6.6	6.5	6.9
MUFA	9.5	9.6	17.6	19.3	17.8
PUFA	16.3	16.3	9.1	6.9	8.0
LA	15.4	9.7	6.5	5.6	5.2
ALA	<1	6.0	2.0	<1	<1
DHA	0	0	0	0	1.1
n-6:n-3	46.7:1	1.6:1	3.2:1	7.8:1	2.4:1
Total fiber (g/d)[b]	40	40	40	40	40
Cholesterol (mg/d)[b]	170	170	170	170	170

[a] Nutrient composition of the background diet combined with shakes containing experimental oils. % of energy from protein, carbohydrate, lipid, SFA, MUFA and PUFA are reproduced from Table 1 of reference [14] (Jones PJ, Senanayake VK, Pu S, Jenkins DJ, Connelly PW, Lamarche B, et al. DHA-enriched high-oleic acid canola oil improves lipid profile and lowers predicted cardiovascular disease risk in the canola oil multicenter randomized controlled trial. Am J Clin Nutr. 2014. doi: 10.3945/ajcn.113.081133. Printed in USA. Copyright 2014 American Society for Nutrition). ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; OA, oleic acid; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

[b] Estimated for a 3000 kcal/day intake.

Table 3. Subject characteristics at screening (*n*=114)[a]

	Screening	Range
Females (*n*, % of total *n*)	60 (53 %)	
Age (years)	47.5 ± 12.9	19 - 65
Weight (kg)	85.4 ± 15.1	54.3 - 133.5
BMI (kg/m^2)	29.9 ± 4.1	21.6 - 43.6
Waist circumference (cm)	101.6 ± 10.0	81.3 - 134.6
Systolic BP (mm Hg)[b]	125.2 ± 17.4	91 - 185
Diastolic BP (mm Hg)[b]	80.4 ± 12.2	54 - 117
Fasting glucose (mmol/L)	5.4 ± 1.2	1.6 - 13.9
TG (mmol/L)	1.8 ± 1.0	0.4 - 6.5
Cholesterol (mmol/L)	5.4 ± 1.1	3.1 - 8.7
HDL-C (mmol/L)	1.3 ± 0.3	0.7 - 2.6
LDL-C (mmol/L)[c]	3.3 ± 0.9	1.5 - 6.6
n with MetSyn[b,d]	52 (46 %)	

[a] Data are presented as means ± SD unless stated otherwise. BMI, body mass index; BP, blood pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MetSyn, metabolic syndrome; TG, triglycerides.

[b] *n*=112 as data were missing for 2 participants.

[c] *n*=113 as data were missing for 1 participant.

[d] Percent of subjects with metabolic syndrome based on the International Diabetes Federation criteria [15].

Table 4. Difference from the control CornSaff oil treatment in plasma concentrations of inflammatory biomarkers (n=114)[a]

		Δ vs. CornSaff				
	CornSaff	FlaxSaff	Canola	CanolaOleic	CanolaDHA	P[b]
hs-CRP (mg/L)[c,d]	1.97 ± 2.04	0.15 (-0.10, 0.40)	-0.20 (-0.42, 0.02)[e]	0.13 (-0.16, 0.41)	0.07 (-0.19, 0.32)	0.04
IL-6 (pg/mL)[c,f]	1.82 ± 3.32	-0.24 (-0.82, 0.34)	-0.35 (-0.94, 0.23)	-0.17 (-0.74, 0.41)	-0.44 (-0.96, 0.08)	0.06
Adiponectin (µg/L)[f]	8.19 ± 4.64	-0.20 (-0.52, 0.11)	0.12 (-0.18, 0.43)	0.05 (-0.20, 0.29)	0.37 (0.04, 0.69)[g]	0.002

[a] Values are unadjusted means ± SD for the control CornSaff oil treatment and unadjusted mean Δ vs. CornSaff (95% CI) for the FlaxSaff, Canola, CanolaOleic and CanolaDHA oil treatments. hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6.

[b] P values for the main treatment effect, as determined by MIXED models performed on delta scores representing variations from the control CornSaff oil treatment. Analyses of delta scores were performed on variations from the control CornSaff oil of log-transformed data since variations from the control CornSaff oil of crude data were not normally distributed. Adjustment for potential covariates (sex, centre, and/or values of the selected parameter on the control CornSaff oil treatment) was considered only when they were found to be significant at $P < 0.05$ in the mixed models.

[c] Analysis was adjusted for values on the control CornSaff oil treatment only.

[d] n=112 in the control CornSaff oil treatment since hs-CRP was over 10 mg/L post-treatment (days 29 and 30) for two participants. hs-CRP data on the control CornSaff oil treatment for these 2 participants were considered as missing values. Thus, the maximum possible n was 112 for all Δ vs. CornSaff in the analysis of hs-CRP. However, n=111 for the Δ FlaxSaff vs. CornSaff because one participant had hs-CRP values > 10 mg/L at days 29 and 30 in the FlaxSaff oil treatment and n=110 for the Δ Canola vs. CornSaff and for the Δ CanolaDHA vs. CornSaff because two participants had hs-CRP values > 10 mg/L at days 29 and 30 in the Canola and CanolaDHA oil treatments, respectively.

[e] Variation in hs-CRP vs. CornSaff differed between Canola and FlaxSaff (*P* Tukey-Kramer = 0.047).

[f] *n*=113 for the Δ CanolaOleic vs. CornSaff and for the Δ CanolaDHA vs. CornSaff due to missing data in one participant in the CanolaOleic and CanolaDHA oil treatments, respectively.

[g] Adiponectin concentrations after CanolaDHA differed from CornSaff (*P* least-square means = 0.04). Variation in adiponectin from CornSaff also differed between CanolaDHA and FlaxSaff (*P* Tukey-Kramer = 0.0008).

Table 5. Mean fold change in expression levels of key inflammatory genes[a] and transcription factors compared with the control CornSaff oil treatment in a subset of 62 participants

	Fold change vs. CornSaff[b]				
Genes	FlaxSaff	Canola	CanolaOleic	CanolaDHA	*P*[c]
IL18	1.18	1.16	1.16	1.10	0.86
IL1B	1.00	1.02	-1.02	-1.11[d]	0.04
NFKB1	1.02	1.01	1.00	-1.01	0.82
PPARA	1.05	1.05	1.03	1.07	0.93
SREBF2	1.05	1.00	1.06	1.00	0.45
TNF	-1.04	-1.02	-1.05	1.01	0.77
TRAF3	1.01	1.01	-1.01	1.00	0.99

[a] Ct, cycle threshold; *G6PD*, glucose-6-phosphate dehydrogenase; *IL*, interleukin; *NFKB1*, nuclear factor kappa-B 1; *PPARA*, peroxisome proliferator-activated receptor alpha; SD, standard deviation; *SREBF2*, sterol regulatory element binding transcription factor 2; *TNF*, tumor necrosis factor; *TRAF3*, TNF receptor-associated factor 3

[b] Fold change in gene expression calculated using the $2^{-\Delta\Delta Ct}$ method, i.e. 2^(mean ΔCt from the FlaxSaff, Canola, CanolaOleic or CanolaDHA oil treatments – mean ΔCt from the control CornSaff oil treatment). ΔCt were calculated as Ct values of a selected target gene – Ct values of the internal control gene *G6PD*. Ct values used in the formula consisted of the mean of the triplicates' individual Ct values, unless the SD of a triplicate was > 0.5, in which case the outlier value was excluded from the calculation of the mean.

[c] *P* values for the main treatment effect, as determined by MIXED models performed on ΔΔCt, i.e. gene expression levels at the end of the FlaxSaff, Canola, CanolaOleic or CanolaDHA oil treatments normalized to the expression of the reference gene *G6PD* and relative to gene expression on the control CornSaff oil treatment. Adjustment for potential covariates including sex, study center and gene expression levels on the control CornSaff oil treatment did not change the results.

[d] Relative *IL1B* gene expression levels after CanolaDHA differed from CornSaff (*P* least-square means = 0.03). Relative *IL1B* gene expression levels also differed between CanolaDHA and Canola (*P* Tukey-Kramer = 0.03).

SUPPLEMENTARY FILES

Supplemental File 1. Details on the feeding protocol during the experimental phases

On weekdays, participants were weighed daily and ate one meal under the supervision of one of the study coordinators, breakfast or lunch, at the Clinical Investigation Unit of each participating center. Other meals were provided to participants to eat at home or at a place of convenience. On Friday, foods for the weekend days were packed and taken away. The amount of food provided was adjusted (± 300 kcal; intakes between 1800 – 4500 kcal/day) according to fluctuations in body weight, when necessary. Use of anti-inflammatory drugs, natural health products, or dietary supplements was not allowed for the duration of study. Subjects were asked to eat all foods provided, to avoid eating any other food, limit alcohol consumption to a maximum of 2 drinks/week, and limit coffee and tea to a maximum of 5 cups/day. Subjects were also asked to maintain their usual level of physical activity throughout the study, and were instructed not to lose weight. Questionnaires were used daily and/or weekly to assess compliance to the diet and to record protocol violation, use of medication(s), adverse events or any other relevant information. Compliance was also assessed by measuring changes in plasma fatty acid profile at the end of each dietary period, as reported by Senanayake et al. [1].

References

[1] Senanayake VK, Pu S, Jenkins DA, Lamarche B, Kris-Etherton PM, West SG, et al. Plasma fatty acid changes following consumption of dietary oils containing n-3, n-6, and n-9 fatty acids at different proportions: preliminary findings of the Canola Oil Multicenter Intervention Trial (COMIT). Trials. 2014;15:136. doi: 10.1186/1745-6215-15-136.

Supplemental File 2. Details on the measurement of anthropometry indices and screening values

Anthropometric measures

Participants were weighed each weekday during all experimental phases without shoes wearing only light clothes. Waist circumference was measured once at the beginning and end of each of the five experimental phases. Anthropometric measurements were obtained using standardized procedures [1].

Screening values

Cardiovascular risk profile factors were assessed at screening for clinical assessment of potential participants. Systolic and diastolic blood pressures were taken after a 10 min rest and averaged from 2 or 3 measures using automated apparel (HEM-773 model, Omron; Baumanometer Standby Model, W.A. Baum Co. Inc.). The lipid/lipoprotein profile at the screening visit was assessed at each center as described previously [2-5].

References

[1] Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988.

[2] 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;24:853-6.

[3] Couillard C, Despres JP, Lamarche B, Bergeron J, Gagnon J, Leon AS, et al. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. Arterioscler Thromb Vasc Biol. 2001;21:1226-32.

[4] Skulas-Ray AC, Kris-Etherton PM, Harris WS, Vanden Heuvel JP, Wagner PR, West SG. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and

endothelial function in healthy persons with moderate hypertriglyceridemia. Am J Clin Nutr. 2011;93:243-52. doi: 10.3945/ajcn.110.003871.

[5] Gillingham LG, Gustafson JA, Han SY, Jassal DS, Jones PJ. High-oleic rapeseed (canola) and flaxseed oils modulate serum lipids and inflammatory biomarkers in hypercholesterolaemic subjects. Br J Nutr. 2011;105:417-27. doi: 10.1017/S0007114510003697.

Supplemental File 3. Details on inflammatory gene expression analysis

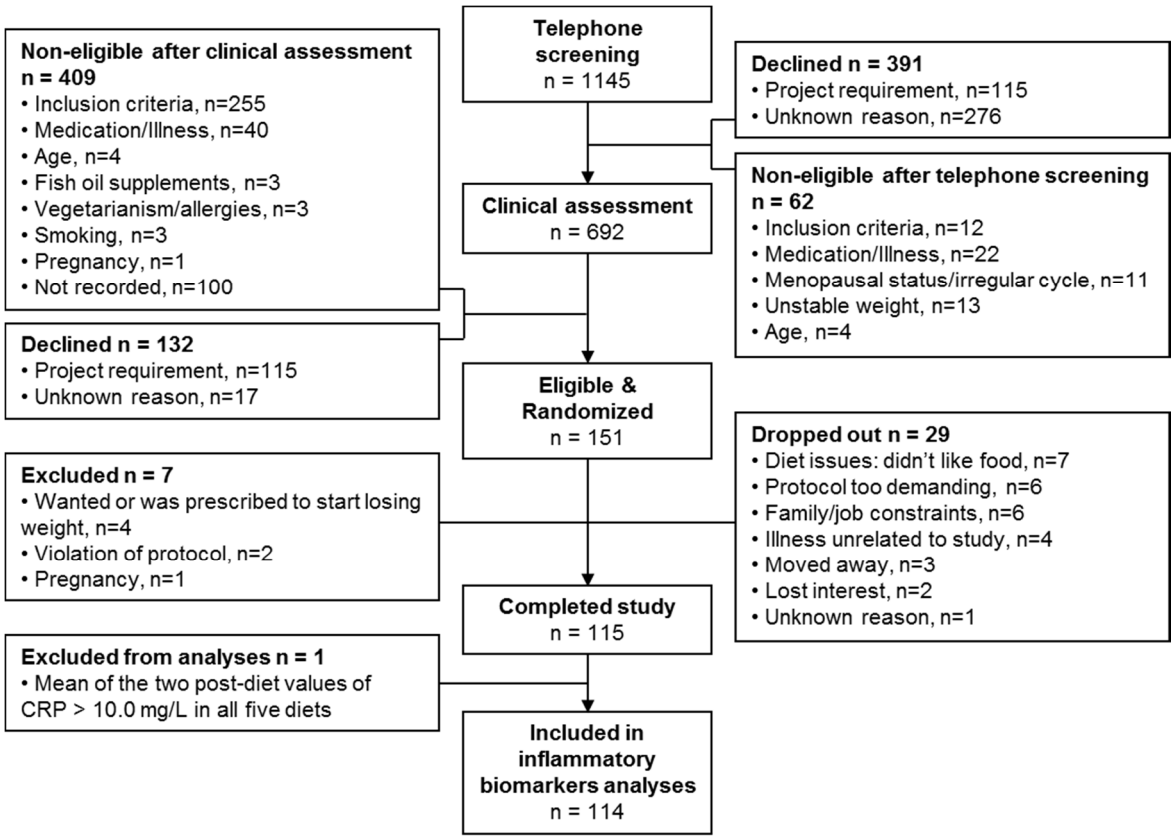
The subsample of 62 participants included in the inflammatory gene expression analysis represents the maximum number of participants for whom PaxGene Blood RNA tubes were available for all 5 treatments and unaltered during transport to the research center responsible for this analysis. Total ribonucleic acid (RNA) was first isolated from 2.5 ml of whole blood samples using the PAXGene Blood RNA purification kit (Qiagen, Valencia, CA) according to manufacturer's instructions. RNA was thereafter quantified using NanoDrop (Thermo Fisher, Wilmington, DE). Complementary deoxyribonucleic acid (cDNA) was synthesized from 1 μg of RNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies). Gene expression was measured by quantitative real time polymerase chain reaction using TaqMan® OpenArray® Real-Time PCR Plates with Inventoried Gene Expression Assays (Applied Biosystems, Foster City, CA) and the QuantStudio 12K Flex Software (Applied Biosystems, Foster City, CA). GenBank and Life Technologies numbers of the analyzed genes are provided in the Table below. Each sample was analyzed in triplicate. Expression of the target genes was normalized to the expression of the internal control gene glucose-6-phosphate dehydrogenase (*G6PD*) using the formula ΔCt = (cycle threshold (Ct) value of the target gene – Ct value of the internal control gene) [1]. Ct values used in the formula consisted of the mean of the triplicates' individual Ct values, unless the standard deviation (SD) of a triplicate was > 0.5, in which case the outlier value was excluded from the calculation of the mean. Expression of the following 10 inflammation-related genes and transcription factors was measured: chemokine (C-C motif) ligand 2 (*CCL2*), interleukin-18 (*IL18*), *IL6*, *IL1B*, nuclear factor kappa-B subunit 1 (*NFKB1*), natriuretic peptide receptor C (*NPR3*), peroxisome proliferator-activated receptor alpha (*PPARA*), sterol regulatory element-binding transcription factor 2 (*SREBF2*), tumor necrosis factor (*TNF*), and TNF receptor-associated factor 3 (*TRAF3*). *CCL2*, *IL6* and *NPR3* were not retained in the analyses since their level of expression was too low to be detected in approximately half of the samples and thus did not allow reliable data to be obtained. Normalization of the target genes to the expression of the internal control gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was also considered and led to similar results.

Supplemental File 3 Table. GenBank and Life Technologies numbers of the analyzed genes

	# GenBank	# Life Technologies
Target genes		
CCL2	NM_002982	Hs00234140_m1
IL18	NM_001562	Hs01038788_m1
IL6	NM_00600	Hs00985639_m1
IL1B	NM_000576	Hs01555410_m1
NFKB1	NM_003998	Hs00765730_m1
NPR3	-	Hs01099013_m1
PPARA	NM_005036	Hs00947536_m1
SREBF2	NM_004599	Hs01081784_m1
TNF	NM_000594	Hs01113624_g1
TRAF3	NM_145725	Hs00936781_m1
Control genes		
G6PD	-	Hs00166169_m1
GAPDH	NM_002046	Hs00266705_g1

References

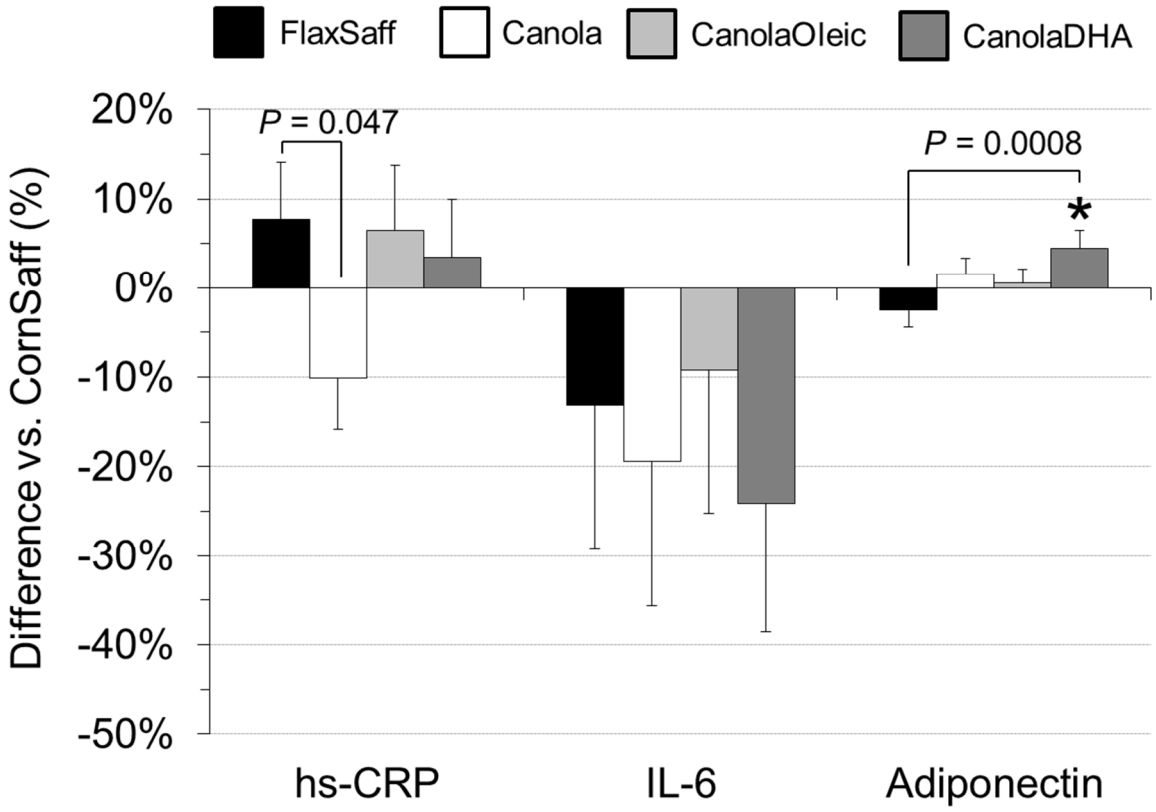
[1] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8. doi: 10.1006/meth.2001.1262.



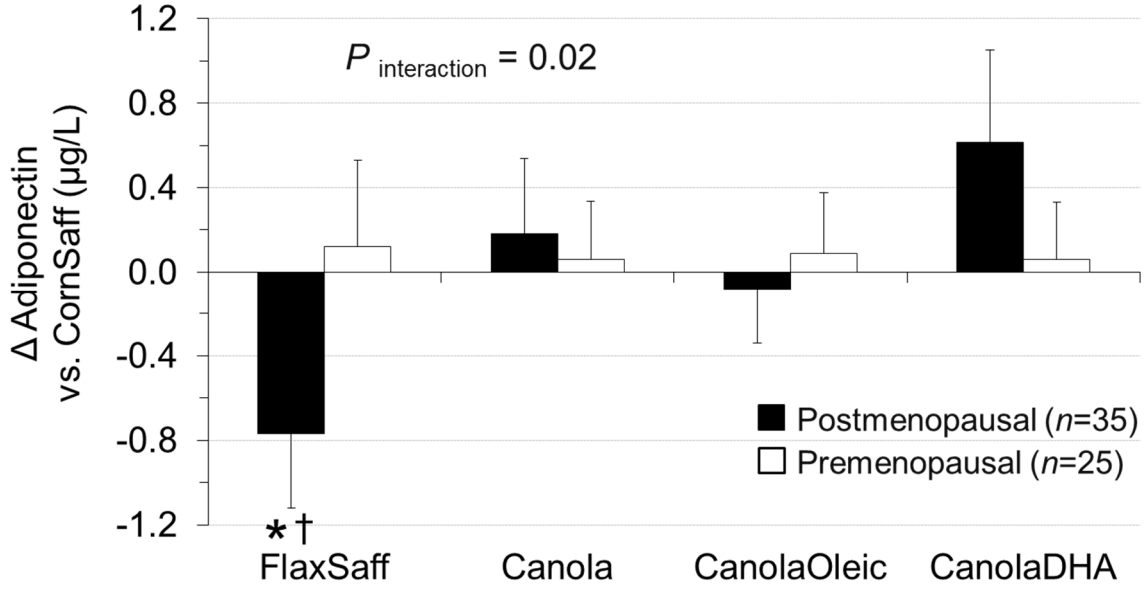
Supplemental File 4. Flow of participants through the study. This chart is adapted from Figure 1 in Senanayake VK, Pu S, Jenkins DA, Lamarche B, Kris-Etherton PM, West SG, et al. Plasma fatty acid changes following consumption of dietary oils containing n-3, n-6, and n-9 fatty acids at different proportions: preliminary findings of the Canola Oil Multicenter Intervention Trial (COMIT). Trials. 2014;15:136. doi: 10.1186/1745-6215-15-136. Copyright 2014 Senanayake *et al*, Publisher: BioMed Central.

Supplemental File 5. Dropout and exclusion rate in each participating center

- 11 dropouts and 1 exclusion at INAF (21.8%)
- 7 dropouts and 4 exclusions at RCFFN (20.8%)
- 11 dropouts and 2 exclusions at Penn State (30.2%).
- The majority of dropouts/exclusions (*n*=19, 53%) occurred during or after the completion of the first of the five feeding phases. Eight dropouts/exclusions occurred during or after the second feeding phase and 9 others during or after the third feeding phase.



Supplemental File 6. Data from Table 4 presented as mean percent differences from the control CornSaff oil treatment in plasma concentrations of inflammatory biomarkers (n=114). *Different from CornSaff (P = 0.04).



Supplemental File 7. Interaction between dietary treatments and menopausal status (pre- or postmenopausal) in modifying the response of adiponectin concentrations from the control CornSaff oil treatment among women (n=60). P interaction: P value of the treatment × menopausal status interaction, determined by the MIXED procedure. A significant treatment effect was observed among postmenopausal women (P = 0.001), but not among premenopausal women (P = 0.91), as determined using the SLICE statement in the MIXED procedure. * = different from CornSaff (P = 0.02) and † = different from the Δ CanolaDHA vs. CornSaff (P = 0.003) among postmenopausal women, as determined by the Tukey-Kramer adjustment for multiple comparisons. Adiponectin concentrations on the control CornSaff oil treatment were 11.4 ± 5.8 µg/L among postmenopausal women and 8.5 ± 3.1 µg/L among premenopausal women.

Supplemental File 8. Comparison of subjects' characteristics at screening between the whole study sample (*n*=114) and the subsample included in the inflammatory gene expression analysis (*n*=62)[a]

	Whole sample (*n*=114)	Subsample – gene expression analysis (*n*=62)	*P*[b]
Females (*n*, % of total *n*)	60 (53 %)	24 (39%)	0.08
Age (years)	47.5 ± 12.9	50.0 ± 12.5	0.21
Weight (kg)	85.4 ± 15.1	87.2 ± 14.5	0.44
BMI (kg/m^2)	29.9 ± 4.1	29.8 ± 3.7	0.85
Waist circumference (cm)	101.6 ± 10.0	103.5 ± 9.0	0.23
Systolic BP (mm Hg)[c]	125.2 ± 17.4	124.1 ± 13.2	0.64
Diastolic BP (mm Hg)[c]	80.4 ± 12.2	77.4 ± 10.7	0.10
Fasting glucose (mmol/L)	5.4 ± 1.2	5.3 ± 0.6	0.72
TG (mmol/L)	1.8 ± 1.0	1.8 ± 1.0	0.99
Cholesterol (mmol/L)	5.4 ± 1.1	5.4 ± 1.1	0.97
HDL-C (mmol/L)	1.3 ± 0.3	1.3 ± 0.4	0.79
LDL-C (mmol/L)[d]	3.3 ± 0.9	3.3 ± 0.9	0.97
n with MetSyn[c,e]	52 (46 %)	25 (41%)	0.49

[a] Data are presented as means ± SD unless stated otherwise. BMI, body mass index; BP, blood pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MetSyn, metabolic syndrome; TG, triglycerides.

[b] *P* values obtained using the Student's T-test (continuous variables) or the Chi-square test (categorical variables).

[c] *n*=112 in results for the whole study population as data were missing for 2 participants and *n*=61 in results for the subsample included in the gene expression analysis as data were missing for 1 participant.

[d] *n*=113 in results for the whole study population as data were missing for 1 participant.

[e] Percent of subjects with metabolic syndrome based on the International Diabetes Federation criteria [1].

References

[1] Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366:1059-62. doi: 10.1016/S0140-6736(05)67402-8.

CHAPITRE 13 :

SUPPLÉMENTATION EN ACIDES GRAS POLYINSATURÉS OMÉGA-3 À LONGUE CHAÎNE ET EXPRESSION DE GÈNES INFLAMMATOIRES DANS LE DUODÉNUM DE PATIENTS OBÈSES ATTEINTS DU DIABÈTE DE TYPE 2

Labonté ME, Couture P, Tremblay AJ, Hogue JC, Lemelin V, Lamarche B.

Eicosapentaenoic and docosahexaenoic acid supplementation and inflammatory gene expression in the duodenum of obese patients with type 2 diabetes

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http://www.nutritionj.com/content/12/1/98

RÉSUMÉ

Nous avons évalué l'impact d'une supplémentation en acides gras polyinsaturés oméga-3 à longue chaîne (acide eicosapentaénoïque [EPA] et acide docosahexaénoïque [DHA]) sur l'expression de gènes inflammatoires dans le duodénum de patients obèses atteints du diabète de type 2. Cette étude contrôlée, randomisée, en chassé-croisé, incluait 12 hommes. Suite à une période de rodage (« *run-in* ») de 4 semaines, les patients ont reçu dans un ordre aléatoire 5 g/jour d'huile de poisson (fournissant 3 g/jour d'EPA et de DHA) et un placebo (mélange d'huile de maïs et de soya). Chaque phase d'intervention durait 8 semaines et les deux phases étaient entrecoupées d'une période de repos (« *washout* ») de 12 semaines. L'expression de gènes a été mesurée à la fin de chaque phase par amplification en chaîne par polymérase à partir d'échantillons de tissu duodénal obtenus lors de biopsies chez les patients à jeun. Les niveaux d'ARN messager (ARNm) de l'IL-6 et de TNF-α dans le duodénum étaient difficilement détectables après chaque intervention (< 100 copies/10^5 copies du gène de référence ATP5o). Les niveaux d'ARNm de l'IL-18 et du facteur de transcription STAT3 (« *signal transducer and activator of transcription 3* ») dans le duodénum étaient plus élevés que ceux de l'IL-6 et de TNF-α (> 5000 copies/10^5 copies d'ATP5o), mais demeuraient tout de même relativement faibles. La supplémentation en EPA et DHA n'a eu aucun impact sur l'expression des gènes évalués (tous les $P \geq 0{,}73$). Ces résultats suggèrent que l'expression des gènes de cytokines pro-inflammatoires est faible dans le duodénum de patients atteints du diabète de type 2 et qu'elle n'est pas influencée par une supplémentation en EPA et DHA. Des études additionnelles sont requises afin de déterminer si l'expression de gènes inflammatoires dans d'autres tissus entourant l'intestin grêle est influencée par une supplémentation en EPA et DHA.

TITLE PAGE

TITLE

Eicosapentaenoic and docosahexaenoic acid supplementation and inflammatory gene expression in the duodenum of obese patients with type 2 diabetes

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KEYWORDS: Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), signal transducer and activator of transcription 3 (STAT3), inflammatory gene expression, n-3 supplementation, placebo-controlled, duodenum, type 2 diabetes

LIST OF ABBREVIATIONS: ATP5o, ATP synthase O subunit; BMI, body mass index; DHA, docosahexaenoic acid; DNA, deoxyribonucleic acid; EPA, eicosapentaenoic acid; HbA1c, hemoglobin A1c; IL, interleukin; LCn-3PUFA, long-chain omega-3 polyunsaturated fatty acids; mRNA; messenger RNA; RNA, ribonucleic acid; STAT3, signal transducer and activator of transcription 3; TG, triglycerides; TNF-α, tumor-necrosis factor α.

ABSTRACT

Background: The extent to which long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) from fish oil such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exert their anti-inflammatory effects by down-regulating intestinal inflammation in humans is unknown. We investigated the impact of LCn-3PUFA supplementation on inflammatory gene expression in the duodenum of obese patients with type 2 diabetes. **Findings:** This placebo-controlled randomized crossover study included 12 men with type 2 diabetes. After a 4-week run-in period, patients received in a random sequence 5 g/d of fish oil (providing 3 g of EPA+DHA) and a placebo (corn and soybean oil) for 8 weeks each. The two treatment phases were separated by a 12-week washout period. Gene expression was assessed by real-time polymerase chain reaction in duodenal biopsy samples obtained in the fasted state at the end of each treatment phase. Intestinal mRNA expression levels of interleukin(IL)-6 and tumor-necrosis factor(TNF)-α were hardly detectable after either treatment (<100 copies/10^5 copies of the reference gene ATP5o). Intestinal mRNA expression of IL-18 and of the transcription factor signal transducer and activator of transcription 3 (STAT3) was higher (>5000 copies/10^5 copies ATP5o) but still relatively low. EPA+DHA supplementation had no impact on any of these levels (all $P \geq 0.73$). **Conclusions:** These data suggest that duodenal cells gene expression of pro-inflammatory cytokines is low in patients with type 2 diabetes and not affected by EPA+DHA supplementation. Further studies are warranted to determine if inflammatory gene expression in other tissues surrounding the intestine is modulated by EPA+DHA supplementation. **ClinicalTrials.gov ID:** NCT01449773.

FINDINGS:

INTRODUCTION

It is widely recognized that obesity, metabolic syndrome, and type 2 diabetes are associated with low-grade chronic inflammation [1], attributed in part to an expanded adipose tissue mass infiltrated with macrophages that secrete pro-inflammatory cytokines [2, 3]. It has been suggested that diet-induced inflammation in the small intestine is also linked to obesity and insulin resistance [4]. Long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) such as eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) have been shown to have anti-inflammatory effects by down-regulating inflammatory gene expression in adipocytes and mononuclear cells [5-7]. Intervention studies evaluating the extent to which EPA and DHA may exert their anti-inflammatory effects by down-regulating intestinal inflammation are lacking. The aim of the present study was to investigate the impact of EPA and DHA supplementation on the expression of inflammatory genes in the duodenum of obese men with type 2 diabetes, who are likely to have intestinal inflammation [4] and therefore to respond favorably to LCn-3PUFA supplementation [8]. We hypothesized that EPA and DHA supplementation reduces pro-inflammatory gene expression in the duodenum of obese men with type 2 diabetes.

METHODS

Participants

Twelve adult men with type 2 diabetes were recruited via the Lipid Clinic of the Laval University Hospital Research Center, Québec, QC, Canada, between April 2008 and March 2009. Diagnosis of type 2 diabetes was based on criteria issued by the American Diabetes Association [9]. Inclusion criteria were: age between 18 and 55 y, plasma triglyceride (TG) levels above the 50^{th} percentile for age [10], non-smoker, body mass index (BMI) between 25.0 and 40.0 kg/m^2, stable body weight over the last 6 mo, hemoglobin A1c (HbA1c) between 6.5 and 8.5%, baseline fasting plasma glucose < 15.0 mmol/L and patients with *de novo* type 2 diabetes not taking oral hypoglycemic agents or patients having received stable doses of metformin for at least 3 mo before randomization. Exclusion criteria were: genetic

dyslipidemias, patients with secondary form of diabetes or acute metabolic diabetic complications, subjects having cardiovascular diseases or taking medication known to affect lipoprotein metabolism (e.g. lipid lowering agents). All participants signed an informed consent document approved by the Ethics Board of the Laval University Hospital Research Center.

Study Design

The study was undertaken according to a double-blind randomized crossover design with two balanced [1:1] treatments of 8 weeks each. All subjects received, in random order: 5 g/d (5 x 1 g capsules) of fish oil providing 3 g/d of EPA (64%) and DHA (36%) or a control supplementation (5 x 1 g capsules/d of a 50/50 blend of corn and soybean oil). Treatments were separated by a 12-week washout. During a 4-week run-in stabilization period that preceded the treatments, participants were advised to consume a low fat diet following the recommendations of the National Cholesterol Education Program - Adult Treatment Panel III [11]. Dietary intake of marine-derived LCn-3PUFA was limited by prohibiting the consumption of fish during the entire experimental period, including the washout period. Alcohol consumption, vitamin supplements and natural health products were also strictly forbidden during the entire experimental period.

Participants' baseline intake of marine-derived LCn-3PUFA was assessed using a validated interviewer-administered food frequency questionnaire [12]. Compliance to supplementation was assessed by counting the number of capsules returned to the research staff over the course of the experimentation.

Characterization of plasma lipids

Plasma TG concentrations were determined by enzymatic methods at the end of each supplementation period using the Technicon RA-1000 analyzer (Technicon Instruments Corporation, Tarrytown, NY). Plasma phospholipids fatty acid levels were determined as previously described [13].

Duodenal biopsies

Four biopsy samples (3 × 3 mm) were obtained at the end of each phase by gastro-duodenoscopy from the second portion of the duodenum using multiple sample single-use biopsy forceps and were immediately flash-frozen in liquid nitrogen and stored at -80°C, as previously described [14].

Total RNA extraction

Biopsy samples were homogenized in 1 ml of Qiazol. Ribonucleic acid (RNA) was then extracted using an RNeasy mini-kit (Qiagen). Tissue samples were also treated with an RNase-free DNase set to eliminate any contaminant deoxyribonucleic acid (DNA). Total RNA was then eluted into 100 μl RNase-free H_2O and stored at -80°C.

RNA quantification and quantitative real-time PCR

Details on RNA quantification and quantitative real-time PCR are provided in **Additional File 1**. Briefly, RNA quality was assessed with a 2100 Bioanalyzer (Agilent Technologies, Inc.) as previously described [15]. Messenger RNA (mRNA) expression data were normalized using the second derivative and double correction method [16] and are expressed as the number of copies/10^5 copies of the reference gene ATP synthase O subunit (ATP5o). The standard curve was established by using known amounts of purified polymerase chain reaction products and the LightCycler 480 version 1.5 software provided by the manufacturer (Roche Inc).

Statistical analyses

Nonparametric Wilcoxon matched-pairs signed-rank tests were used to compare the impact of LCn-3PUFA and placebo on inflammatory gene mRNA expression in duodenal tissues. Mixed models with proper interaction terms have shown no evidence of a carry-over effect of the LCn-3PUFA supplementation (not shown). Associations among key outcomes were assessed using Spearman correlation coefficients. Differences were considered significant at $P < 0.05$. Analyses were performed using SAS (version 10.1; SAS Institute, Cary, NC).

RESULTS

All 12 recruited participants completed the study and were included in the analyses. **Table 1** shows their characteristics. Body weight remained stable throughout the study. More than 80% of the capsules were taken by participants. No side effects were reported. Plasma phospholipids LCn-3PUFA levels were higher while plasma phospholipids levels of dihomo-γ-linoleic acid and arachidonic acid were lower after EPA+DHA supplementation than after placebo (all $P \leq 0.001$). This again reflected good compliance. There was no between-treatment difference in alpha-linolenic acid and linoleic acid levels (both $P \geq 0.09$, **Table 2**). Mean plasma TG concentrations tended to be lower after EPA+DHA supplementation than after placebo (2.30 ± 1.09 vs. 2.50 ± 1.22 mmol/L; $P = 0.08$).

Table 3 presents the duodenal mRNA expression of inflammatory genes measured after placebo and EPA+DHA treatments. mRNA expression levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α were hardly detectable after either treatment (< 100 copies/10^5 copies ATP5o). mRNA expression of IL-18 and of the transcription factor signal transducer and activator of transcription 3 (STAT3) was higher (≥ 5000 copies/10^5 copies ATP5o) but still relatively low. EPA+DHA supplementation had no impact on any of these levels (changes between -15 and +26% vs. placebo, all $P \geq 0.73$). Despite these low levels of expression and the lack of change with EPA+DHA supplementation, treatment-induced variations in mRNA STAT3 levels (EPA+DHA vs. placebo) were correlated with concurrent variations in mRNA expression of IL-6 ($r = 0.55$; $P = 0.06$), IL-18 ($r = 0.64$; $P = 0.03$), and TNF-α ($r = 0.67$; $P = 0.02$).

DISCUSSION

To the best of our knowledge, this study is the first to assess the impact of EPA+DHA supplementation on the expression of inflammatory genes in duodenal tissues of obese patients with type 2 diabetes.

Our results first showed that the expression of inflammatory genes in duodenal tissues of obese men with type 2 diabetes is relatively low, particularly in the case of IL-6 and TNF-α. This suggests that inflammation at the level of duodenal cells *per se* may not play a

significant role in modulating chronic low-grade inflammation in obese diabetic patients. As reviewed by Ding and Lund [4], very little is known on the crosstalk between the intestine and adipose tissue in fostering chronic inflammation. In the case of Crohn's disease, the extent of inflammation and cellular damage has been correlated with the accrual of mesenteric fat around the intestine (so-called "creeping" fat) [17]. As is the case with abdominal fat, mesenteric fat is characterized by infiltration with immune cells and increased levels of pro-inflammatory cytokines such as IL-6 and TNF-α [18]. The duodenal biopsy procedure in the present study collected basolateral cells and not cellular material from the outer surface of the intestine where mesenteric fat would be found. Thus, our study cannot resolve the possibility that mesenteric fat expresses pro-inflammatory genes in type 2 diabetes.

Our study also suggests that 3 g/d EPA+DHA supplementation has no impact on duodenal cells gene expression of pro-inflammatory cytokines and of transcription factor STAT3 compared with a placebo. Previous studies in animal models have shown that LCn-3PUFA from fish oil down-regulate the small intestine and gut-associated lymphoid tissue expression of inflammatory mediators (interferon-γ, chemokines) [19, 20]. Other recent studies in cell culture and animal models also suggest that LCn-3PUFA down-regulate inflammatory gene expression by modifying the activity of transcription factors such as nuclear factor-κB and peroxisome proliferator-activated receptor-γ, which are expressed in the gastrointestinal tract [21]. The lack of effect of LCn-3PUFA supplementation on mRNA expression of pro-inflammatory cytokines and of transcription factor STAT3 in duodenal tissues of obese patients with type 2 diabetes in the present study is most likely attributable to the fact that gene expression was extremely low and therefore unlikely to be further modified. However, consistent with our results, a study performed in 242 human subjects by Pot et al. [22] has shown that concentrations of local markers of inflammation measured in biopsy samples of the colon were unaffected by consumption of oily fish, which represents a naturally occurring source of EPA+DHA.

A limitation that needs to be pointed out is the limited number of subjects which yielded limited statistical power to detect treatment differences in gene expression. However, mRNA expression levels of inflammatory genes were very low in most cases, and it is

unlikely that such numbers would have changed with a larger sample size. The use of a randomized crossover design is a strength and the long intervention and washout periods have limited the possibility of a carry-over effect of the LCn-3PUFA supplementation. Analyses of plasma fatty acid profiles following placebo and EPA+DHA supplementation confirmed compliance to treatments.

In summary, we believe this is the first study suggesting that gene expression of pro-inflammatory cytokines in duodenal tissues from obese patients with type 2 diabetes is very low and not affected by EPA+DHA supplementation. Further studies will be needed to investigate if inflammatory gene expression in other tissues surrounding the intestine such as mesenteric fat is modulated by EPA+DHA supplementation.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

PC, VL and BL designed the research; AJT and JCH coordinated the study and participated in the data collection; VL performed duodenal biopsies; MEL and BL performed statistical analyses; MEL interpreted the data and wrote the manuscript; BL had primary responsibility for final content. All authors critically reviewed the manuscript and approved its final version.

REFERENCES

1. Pradhan A: **Obesity, metalic syndrome, and type 2 diabetes: Inflammatory basis of glucose metabolic disorders.** *Nutr Rev* 2007, **65:**S152-S156.

2. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: **Increased Adipose-Tissue Expression of Tumor-Necrosis-Factor-Alpha in Human Obesity and Insulin-Resistance.** *J Clin Invest* 1995, **95:**2409-2415.

3. Gonzalez-Periz A, Claria J: **Resolution of Adipose Tissue Inflammation.** *ScientificWorldJournal* 2010, **10:**832-856.

4. Ding S, Lund PK: **Role of intestinal inflammation as an early event in obesity and insulin resistance.** *Curr Opin Clin Nutr Metab Care* 2011, **14:**328-333.

5. Bouwens M, van de Rest O, Dellschaft N, Bromhaar MG, de Groot LCPG, Geleijnse JM, Muller M, Afman LA: **Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells.** *Am J Clin Nutr* 2009, **90:**415-424.

6. Hsueh HW, Zhou Z, Whelan J, Allen KGD, Moustaid-Moussa N, Kim H, Claycombe KJ: **Stearidonic and Eicosapentaenoic Acids Inhibit Interleukin-6 Expression in ob/ob Mouse Adipose Stem Cells via Toll-Like Receptor-2-Mediated Pathways.** *J Nutr* 2011, **141:**1260-1266.

7. Kopecky J, Rossmeisl M, Flachs P, Kuda O, Brauner P, Jilkova Z, Stankova B, Tvrzicka E, Bryhn M: **n-3 PUFA: bioavailability and modulation of adipose tissue function.** *Proc Nutr Soc* 2009, **68:**361-369.

8. Myhrstad MCW, Retterstol K, Telle-Hansen VH, Ottestad I, Halvorsen B, Holven KB, Ulven SM: **Effect of marine n-3 fatty acids on circulating inflammatory markers in healthy subjects and subjects with cardiovascular risk factors.** *Inflamm Res* 2011, **60:**309-319.

9. Genuth S, Alberti KGMM, Bennett P, Buse J, DeFronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P: **Follow-up report on the diagnosis of diabetes mellitus.** *Diabetes Care* 2003, **26:**3160-3167.

10. Heiss G, Tamir I, Davis CE, Tyroler HA, Rifkind BM, Schonfeld G, Jacobs D, Frantz ID: **Lipoprotein-Cholesterol Distributions in Selected North-American Populations - Lipid Research Clinics Program Prevalence Study.** *Circulation* 1980, **61:**302-315.

11. NCEP Expert Panel: **Executive Summary of the 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).** *JAMA* 2001, **285:**2486-2497.

12. 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.

13. Frasure-Smith N, Lesperance F, Julien P: **Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes.** *Biol Psychiatry* 2004, **55:**891-896.

14. Tremblay AJ, Lamarche B, Lemelin V, Hoos L, Benjannet S, Seidah NG, Davis HR, Couture P: **Atorvastatin increases intestinal expression of NPC1L1 in hyperlipidemic men.** *J Lipid Res* 2011, **52:**558-565.

15. Luu-The V, Paquet N, Calvo E, Cumps J: **Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction.** *Biotechniques* 2005, **38:**287-293.

16. Warrington JA, Nair A, Mahadevappa M, Tsyganskaya M: **Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes.** *Physiol Genomics* 2000, **2:**143-147.

17. Olivier I, Theodorou V, Valet P, Castan-Laurell I, Guillou H, Bertrand-Michel J, Cartier C, Bezirard V, Ducroc R, Segain JP, Portier G, Kirzin S, Moreau J, Duffas JP, Ferrier L, Eutamene H: **Is Crohn's Creeping Fat an Adipose Tissue?** *Inflamm Bowel Dis* 2011, **17:**747-757.

18. Desreumaux P, Ernst O, Geboes K, Gambiez L, Berrebi D, Muller-Alouf H, Hafraoui S, Emilie D, Ectors N, Peuchmaur M, Cortot A, Capron M, Auwerx J, Colombel JF: **Inflammatory alterations in mesenteric adipose tissue in Crohn's disease.** *Gastroenterology* 1999, **117:**73-81.

19. Kleemann R, Scott FW, Worz-Pagenstert U, Ratnayake WMN, Kolb H: **Impact of dietary fat on Th1/Tk2 cytokine gene expression in the pancreas and gut of diabetes-prone BB rats.** *J Autoimmun* 1998, **11:**97-103.

20. van Schothorst EM, Flachs P, Franssen-van Hal NLW, Kuda O, Bunschoten A, Molthoff J, Vink C, Hooiveld GJEJ, Kopecky J, Keijer J: **Induction of lipid oxidation by polyunsaturated fatty acids of marine origin in small intestine of mice fed a high-fat diet.** *BMC Genomics* 2009, **10:**110.

21. Calder PC: **Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases.** *Mol Nutr Food Res* 2008, **52:**885-897.

22. Pot GK, Geelen A, Majsak-Newman G, Harvey LJ, Nagengast FM, Witteman BJM, van de Meeberg PC, Hart AR, Schaafsma G, Lund EK, Rijkers GI, Kampman E: **Increased Consumption of Fatty and Lean Fish Reduces Serum C-Reactive Protein Concentrations but Not Inflammation Markers in Feces and in Colonic Biopsies.** *J Nutr* 2010, **140:**371-376.

TABLES

Table 1. Characteristics of the study participants at screening (*n* = 12 men with type 2 diabetes)

	Mean ± SD
Age (y)	54.1 ± 7.2
BMI (kg/m^2)	33.7 ± 6.0
LDL-C (mmol/L)	2.9 ± 0.5
HDL-C (mmol/L)	1.0 ± 0.2
TG (mmol/L)	3.1 ± 2.0
HbA1c (%)	7.0 ± 0.8
EPA+DHA intake (g/d)	0.23 ± 0.20

Abbreviations: BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HbA1c, hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

Table 2. Plasma phospholipids fatty acid levels after EPA+DHA and placebo supplementation in 12 men with type 2 diabetes

	Placebo	EPA+DHA	Difference	*P*
	Fatty acid levels (%)			
LA	19.9 (3.6)	19.3 (2.9)	-0.6	0.09
ALA	0.21 (0.09)	0.19 (0.11)	-0.02	0.85
DGLA	3.82 (1.57)	2.87 (0.65)	-0.95	0.001
AA	9.40 (2.05)	7.97 (1.37)	-1.43	0.0005
EPA	1.00 (0.93)	3.53 (1.65)	2.53	0.0005
DPA	1.03 (0.34)	1.25 (0.26)	0.22	0.0005
DHA	2.96 (1.15)	4.43 (1.26)	1.47	0.0005

Data are expressed as median and interquartile range in parentheses. *P* values were calculated using Wilcoxon matched-pairs signed-rank tests. Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; DGLA, dihomo-γ-linoleic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acids.

Table 3. Inflammatory gene expression in duodenal tissue after EPA+DHA and placebo supplementation in 12 men with type 2 diabetes

	Placebo	EPA+DHA	Difference	*P*
	mRNA copies/10^5 copies ATP5o			
IL-6	8 (9)	10 (3)	2	0.77
TNF-α	109 (51)	93 (50)	-16	0.75
IL-18	5226 (3552)	5398 (1486)	172	0.73
STAT3	4637 (1723)	4646 (1437)	9	0.91

Data are expressed as median and interquartile range in parentheses. *P* values were calculated using Wilcoxon matched-pairs signed-rank tests. Abbreviations: ATP5o, ATP synthase O subunit; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IL, interleukin; mRNA, messenger ribonucleic acid; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor α.

SUPPLEMENTARY FILES

Additional file 1. Details on RNA quantification and quantitative real-time PCR

RNA quality was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies Inc). First-strand complementary DNA (cDNA) synthesis was accomplished using 5 µg of isolated RNA in a reaction containing 200 U of Superscript III RNase H-RT (Invitrogen Life Technologies), 300 ng of oligo-dT_{18}, 50 ng of random hexamers, 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 500 µM deoxynucleotides triphosphate, 5 mM dithiothreitol, and 40 U of Protector RNase inhibitor (Roche Diagnostics); the volume of the final solution was 50 µl. The reaction was performed at 25°C for 10 minutes, followed by 50°C for 1 hour, and then the solution was treated with 1 µg of RNase A for 30 min at 37°C. The resulting products were purified with Qiaquick PCR purification kits (QIAGEN). cDNA corresponding to 20 ng of total RNA was used to perform fluorescent-based real-time PCR quantification using the LightCycler 480 (Roche). Reagent LightCycler 480 SYBRGreen I Master was obtained from the same company and used according to the manufacturer's instructions. Fifty cycles of PCR reactions were performed under the following conditions: denaturation at 95°C for 10 sec, annealing at 58-64°C for 10 sec and elongation at 72°C for 14 sec. The reaction was then heated for 5 sec at a temperature 2°C lower than the melting temperature of the DNA fragment. The fluorescence signal readings were recorded at the end of the heating period to avoid a non-specific signal. A melting curve was performed to assess for the presence of a non-specific signal. Oligoprimer pairs that amplify approximately 200 bp were designed using GeneTools software (Biotools Inc.), and their specificity was verified by blast in the GenBank database. Data analyses and normalization were performed using the second-derivative and double-correction methods as described by Luu-The *et al*. [1] (see references below); the following reference genes were used: hypoxanthine guanine phosphoribosyl transferase 1 (Hprt1), ATP synthase O subunit (ATP5o), glucose-6-phosphate dehydrogenase (G6PD) and 18S ribosomal RNA (18S). Previous studies have shown that Hprt1, ATP5o and G6PD have stable expression levels from the embryonic stage of life through adulthood in various tissues [1]. The mRNA expression levels are expressed as the number of copies/10^5 copies of the reference gene ATP5o, using a standard curve of

crossing points versus the logarithm of the quantity. The standard curve was established using known amounts of purified PCR products (10, 10^2, 10^3, 10^4, 10^5, and 10^6 copies) and the LightCycler 480 version 1.5 software provided by the manufacturer (Roche Inc.). The efficiency of the PCR amplification was verified.

Primer sets used in quantitative real-time PCR from duodenal biopsies

Gene Symbol	Description	Forward Primer (5'→3')	Reverse Primer (5'→3')
IL-6	Interleukin 6 (interferon, beta 2)	ACAGCCACTCACCTCTTCAGA	AGTGCCTCTTTGCTGCTTTCA
TNF-α	Homo sapiens tumor necrosis factor	CATCTATCTGGGAGGGGTCTT	GCAATGATCCCAAAGTAGACC
IL-18	Homo sapiens interleukin 18 (interferon-gamma-inducing factor)	GCTGAAGATGATGAAAACCTGGAAT	ATAAATATGGTCCGGGGTGCA
STAT3	Homo sapiens signal transducer and activator of transcription 3 (acute-phase response factor)	GGTTGGACATGATGCACACTAT	AGGGCAGACTCAAGTTTATCAG
ATP5o	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	ATTGAAGGTCGCTATGCCACAG	AACGACTCCTTGGGTATTGCTTAA

References

[1] Luu-The V, Paquet N, Calvo E, Cumps J: **Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction.** *Biotechniques* 2005, **38:**287-293

CHAPITRE 14 : ÉTUDE DE L'IMPACT DES ACIDES GRAS ALIMENTAIRES SUR L'INFLAMMATION – CONCLUSION

Ce troisième et dernier volet de mon projet de doctorat a porté sur l'étude de l'impact de différents acides gras sur l'inflammation et ses mécanismes sous-jacents (expression de gènes inflammatoires) dans le cadre de deux études d'intervention nutritionnelle randomisées contrôlées conçues selon un devis en chassé-croisé : 1) le projet COMIT, dans lequel divers acides gras aux effets potentiellement cardioprotecteurs ont été comparés simultanément et 2) le projet N-3 GUT, dans lequel une supplémentation en oméga-3 d'origine marine était comparée à un placebo (mélange d'huile de maïs et de soya). Mis à part leur utilisation d'un devis randomisé en chassé-croisé, les deux études différaient grandement en fonction de nombreuses caractéristiques (ex : nombre de sujets, caractéristiques des sujets, interventions réalisées, mesure ou non des biomarqueurs inflammatoires circulants, tissus dans lesquels l'expression de gènes a été mesurée). Je vais donc discuter de certains points importants séparément pour chacune des deux études, en débutant par le projet COMIT.

Conclusions sur le projet COMIT

Un des principaux constats dérivant du projet COMIT est que dans le cadre d'une alimentation caractérisée par un profil en AGS favorable sur le plan cardiovasculaire, les effets anti-inflammatoires d'une huile de canola enrichie en DHA comparativement aux AGPI de source végétale (n-6 et n-3) pourraient être attribuables, du moins en partie, à la réduction de l'expression de l'IL-1β.

D'abord, je désire mettre en lumière que l'huile de canola enrichie en DHA a exercé ses effets anti-inflammatoires comparativement à des huiles enrichies en AGPI n-6 ou n-3 de source végétale principalement en augmentant les concentrations d'un biomarqueur anti-inflammatoire, l'adiponectine, plutôt qu'en réduisant les concentrations de biomarqueurs pro-inflammatoires (hs-CRP, IL-6). Ce constat est tout à fait en accord avec la littérature actuelle (section 11.1.5 du chapitre 11) qui démontre que les oméga-3 d'origine marine engendrent assez clairement une augmentation des concentrations d'adiponectine, mais exercent des effets contradictoires sur les concentrations de biomarqueurs pro-

inflammatoires. Il pourrait donc s'avérer pertinent dans le futur d'évaluer l'adiponectine en combinaison avec d'autres molécules anti-inflammatoires (ex : IL-10) afin de confirmer éventuellement si les effets anti-inflammatoires des oméga-3 d'origine marine au plan « clinique/physiologique » passent d'abord et avant tout par l'augmentation des concentrations de molécules anti-inflammatoires plutôt que par la réduction des biomarqueurs pro-inflammatoires.

Tel que mentionné plus haut, les données du projet COMIT nous ont amené à émettre l'hypothèse que l'augmentation des concentrations d'adiponectine suite à la consommation de l'huile de canola enrichie en DHA puisse être attribuable, du moins en partie, à la réduction de l'expression de l'IL-1β. Cette hypothèse reste toutefois à confirmer dans le futur. De plus, une évaluation davantage complète des mécanismes sous-jacents à l'augmentation des concentrations d'adiponectine suite à la consommation de DHA devrait prendre en compte la mesure de PPAR-γ, dont il fut question dans la discussion du chapitre 12. Parmi les PPAR, seule l'expression de PPAR-α a été mesurée dans le cadre du présent projet.

Il m'apparaît également important de souligner, dans le contexte d'une perspective plus globale entourant la santé cardiovasculaire, que les effets bénéfiques de la consommation de l'huile de canola enrichie en DHA ne se limitaient pas seulement à des effets anti-inflammatoires. En effet, nos collaborateurs de l'Université du Manitoba ont démontré que la consommation de l'huile de canola enrichie en DHA, comparativement aux autres huiles, avait exercé des effets bénéfiques significatifs sur le HDL-cholestérol, les triglycérides, le ratio cholestérol-total : HDL-cholestérol, la tension artérielle diastolique et le score du risque de Framingham (314).

Un autre constat du projet COMIT, auquel on s'attendait plus ou moins, est que l'augmentation des AGMI (acide oléique ; avec l'huile de canola régulière) aux dépens des AGPI n-6 et n-3 de source végétale (huile *FlaxSaff*) a réduit les concentrations de la hs-CRP. J'aimerais ajouter, par rapport à ce qui a déjà été discuté au chapitre 12, que de nombreux facteurs incluant le nombre élevé de sujets dans le projet (n = 114), le contexte d'alimentation contrôlée, le devis en chassé-croisé et le fait que la hs-CRP ait été mesurée à deux reprises à la fin de chaque diète expérimentale peuvent possiblement expliquer

l'observation d'effets bénéfiques significatifs sur la hs-CRP suite au remplacement d'AGPI d'origine végétale par de l'acide oléique. Nous émettons également l'hypothèse que cet effet serait particulièrement notable lorsque le ratio n-6 : n-3 est faible. En effet, la principale différence entre l'huile de canola régulière et l'huile de canola enrichie en OA (*CanolaOleic*) était leur ratio n-6 : n-3 (3 fois plus faible dans la première que dans la seconde). Contrairement aux résultats observés avec l'huile de canola régulière, l'huile de canola enrichie en OA n'a eu aucun impact sur la hs-CRP comparativement à la consommation de l'huile riche en AGPI n-6 et n-3 de source végétale.

Contrairement à notre hypothèse, l'ALA n'a entraîné aucun effet anti-inflammatoire comparativement aux AGPI oméga-6 (acide linoléique). Tel qu'indiqué au chapitre 12, ce résultat supporte la notion que les oméga-6 n'exerceraient pas d'effets pro-inflammatoires (3, 247), du moins dans le contexte d'une alimentation faible en AGS. En ce qui concerne plus spécifiquement l'ALA, nos résultats semblent supporter l'hypothèse mentionnée lors du survol de la littérature (section 11.1.4 du chapitre 11) comme quoi la consommation de cet acide gras, peu importe sa dose (ex : 19 g pour un apport énergétique de 3000 kcal/jour dans la présente étude), n'a pas d'effet sur les biomarqueurs pro-inflammatoires lorsque le statut inflammatoire initial des participants est « relativement faible ». En effet, même si tous nos sujets étaient caractérisés par de l'obésité abdominale, leur concentration moyenne de la hs-CRP « en tant que groupe » n'était pas sévèrement détériorée, se situant dans les environs de 2 mg/L.

Un dernier constat en lien avec le projet COMIT, mais non le moindre, est que la consommation des différentes huiles à l'étude a globalement eu peu d'impact sur l'expression de gènes inflammatoires dans le sang. Cette absence d'effet peut nous amener à se questionner concernant le type de cellules utilisées pour mesurer l'expression de gènes. En effet, nous avons mesuré l'expression de gènes dans les cellules sanguines complètes (méthode *PaxGene*) alors que plusieurs études ont plus spécifiquement évalué l'expression de gènes inflammatoires dans les PBMC. Les PBMC sont des cellules immunitaires faisant partie du « sang complet », mais ce dernier comprend également les érythrocytes et les cellules polynucléaires (315, 316). Il a été démontré que le nombre de gènes exprimés et que les valeurs d'expression de gènes seraient plus faibles alors que la variabilité des

mesures serait plus grande en isolant l'ARN à partir des cellules sanguines complètes comparativement aux PBMC (316-318). Cela pourrait peut-être expliquer pourquoi, globalement, nous n'avons observé aucun effet sur l'expression de gènes inflammatoires en réponse aux différentes huiles. Cela pourrait également expliquer pourquoi nous n'avons pas pu analyser les données provenant de 3 des 10 gènes inflammatoires que nous avions ciblés (« *Supplemental File 3* » du chapitre 12), deux de ces gènes étant une cytokine et un facteur chimiotactique bien connus, soit l'IL-6 et MCP-1 (qui porte aussi le nom CCL2, « *chemokine (C-C motif) ligand 2* »). Il est suggéré que les profils d'expression de gènes différents obtenus à partir des cellules sanguines complètes comparativement aux PBMC pourraient être attribuables à l'abondance d'ARN messager de globine dans les cellules sanguines complètes (316, 319, 320). Apparemment, des étapes additionnelles dans l'isolation de l'ARN provenant du « sang complet » impliquant la réduction de la globine pourraient améliorer la sensibilité et réduire la variabilité de la méthode (318-321). Malgré tout, il est possible que la consommation des différentes huiles n'ait *tout simplement eu aucun effet*, de façon globale, sur les gènes évalués ici-même. Tel que souligné un peu plus haut, il s'avèrerait pertinent dans le futur de considérer davantage la mesure de l'expression de gènes anti-inflammatoires, particulièrement en réponse aux oméga-3 d'origine marine (DHA). Enfin, comme le souligne la discussion du chapitre 12, les interventions avaient une durée relativement courte (4 semaines). Les effets à plus long terme de différents acides gras sur l'expression de gènes inflammatoires méritent donc également d'être investigués.

Conclusions sur le projet N-3 GUT

Je rappelle que le principal constat du projet N-3 GUT est que l'expression de gènes pro-inflammatoires dans le duodénum d'hommes obèses atteints du diabète de type 2 est très faible et n'est pas influencée par une supplémentation de 3 g/jour en EPA+DHA durant 8 semaines.

En plus des éléments soulevés dans la discussion du chapitre 13, il faut reconnaître que nous avons mesuré l'expression des gènes de seulement trois cytokines pro-inflammatoires (IL-6, TNF-α, IL-18) et d'un facteur de transcription (STAT3). Ce petit nombre de gènes évalués limite la portée de nos résultats. Il aurait donc pu être pertinent d'évaluer

l'expression d'un plus grand nombre de gènes associés à des effets pro-inflammatoires (ex : IL-1β, MCP-1, NF-κB) de même que des gènes associés à des effets anti-inflammatoires (ex : PPAR-α, PPAR-γ). Toutefois, nous émettons l'hypothèse que les niveaux d'expression de ces autres gènes dans le duodénum auraient probablement été aussi faibles que ceux des gènes que nous avons évalués. Il est intéressant de noter qu'une étude publiée récemment par Veilleux *et al.* (322), qui semble actuellement la seule autre étude que la nôtre à avoir mesuré l'expression de gènes inflammatoires dans le duodénum chez l'humain, a démontré une expression augmentée du gène TNF-α, mais pas des gènes TLR4 ni IL-1β, chez des individus obèses résistants à l'insuline comparativement à des individus obèses non résistants à l'insuline de même âge et de même sexe (n = 10 par groupe). Cette étude a aussi démontré une plus grande production d'IL-6 par des cellules intestinales en culture et une plus grande activation de NF-κB dans le duodénum (reflétée par un ratio plus grand de NF-κB/I-κB, calculé en fonction de l'expression des protéines) chez les résistants à l'insuline comparativement aux individus non résistants à l'insuline. Il faut savoir que le degré de sévérité de l'obésité des sujets était plus grand dans l'étude de Veilleux *et al.* (322) (IMC moyen = 53,8 kg/m^2) que dans la présente étude (IMC moyen = 33,7 kg/m^2). Il faut aussi dire que Veilleux *et al.* (322) ont évalué l'expression relative de certains gènes inflammatoires entre deux groupes de sujets, alors que nous avons plutôt mesuré l'expression absolue (nombre de copies) en réponse à une intervention nutritionnelle chez un seul et même type de sujets. Les buts poursuivis et les mesures réalisées n'étaient pas les mêmes dans notre étude et dans celle de Veilleux *et al.* (322). Il est donc difficile de comparer les résultats obtenus. Malgré tout, au contraire de nos résultats, Veilleux *et al.* (322) suggèrent la présence d'inflammation dans le duodénum de sujets sévèrement obèses et résistants à l'insuline.

Ensuite, nous avons déjà été critiqués par rapport au fait que nous ayons seulement réalisé des biopsies du duodénum pour mesurer l'expression de gènes inflammatoires, alors que l'iléon serait le principal site de l'inflammation intestinale d'après les études chez l'animal (302, 306, 307). Il faut toutefois savoir que de prélever des échantillons de l'iléon chez l'humain dans un contexte de recherche représente un défi de taille, difficilement réalisable à moins de procéder par iléo-coloscopie ou par chirurgie. Entre autres, l'iléo-coloscopie demande un certain niveau de préparation au patient quelques jours avant l'intervention

(ex : régime sans résidus) et elle est généralement effectuée sous anesthésie générale. Les procédures pour l'obtention de biopsies de l'iléon sont donc encore plus invasives que la gastro-duodénoscopie réalisée dans la présente étude. Autant d'un point de vue éthique que pratique, réaliser des biopsies du duodénum demeure davantage réaliste dans un contexte de recherche.

Enfin, un aspect relativement important que je n'ai pas encore abordé dans la thématique de l'inflammation intestinale est celui du microbiote. Il est de plus en plus suggéré que l'interaction d'une alimentation riche en lipides avec le microbiote intestinal est nécessaire dans l'activation de l'inflammation intestinale associée au développement de l'obésité et de la résistance à l'insuline (306, 323). En effet, des souris élevées dans des conditions exemptes de germes (« *germ-free mice* ») consommant une diète riche en lipides ne montreraient pas une expression augmentée du gène TNF-α dans l'iléon et seraient résistantes à l'obésité comparativement à des souris consommant la même diète, mais étant élevées dans des conditions normales (302). D'après une récente revue de la littérature (323), de nombreuses études chez l'animal suggèrent que la consommation d'une diète riche en lipides, particulièrement en AGS, induirait des signaux inflammatoires dans le tractus gastro-intestinal en passant par l'augmentation de certaines classes de *Firmicutes* aux dépens des *Bacteriodetes*. Cette revue de la littérature indique également que l'impact des oméga-3 sur le microbiote intestinal n'a pas encore été étudié (323). Combiner la mesure de l'expression de gènes inflammatoires dans l'intestin à la mesure des populations de bactéries présentes dans le microbiote intestinal pourrait donc s'avérer une avenue intéressante afin de déterminer le rôle précis des oméga-3 sur l'inflammation intestinale. Toutefois, il faut admettre que l'étude du microbiote va au-delà des travaux qui ont été réalisés ici-même.

Mis ensemble, les résultats des projets COMIT et N-3 GUT indiquent, sur le plan « métabolique », que la consommation de divers acides gras, dont des acides gras oméga-3 d'origine marine, influence peu ou pas l'expression de gènes inflammatoires dans le sang de sujets avec obésité abdominale ou dans l'intestin grêle de sujets obèses et diabétiques de type 2. Le fait que les analyses d'expression de gènes réalisées ici étaient principalement de nature exploratoire illustre l'importance de réaliser des études futures spécifiquement

conçues en fonction de la mesure de l'expression de gènes inflammatoires. En effet, encore trop peu d'études ont vérifié l'impact de la consommation de divers acides gras sur l'expression de gènes inflammatoires *in vivo* chez l'humain en tant que mesure principale (« *primary outcome* »).

CHAPITRE 15 : CONCLUSION GÉNÉRALE

Je rappelle que le présent projet de doctorat visait, de façon globale, à étudier les liens entre l'alimentation et l'inflammation en passant de la population, à l'individu, à la cellule (**Figure 15.1**). L'alimentation a elle-même été étudiée sous différents angles, incluant de simples nutriments (acides gras, dont les oméga-3), des aliments (produits laitiers) ainsi que des profils alimentaires reflétant l'alimentation d'une population dans sa globalité.

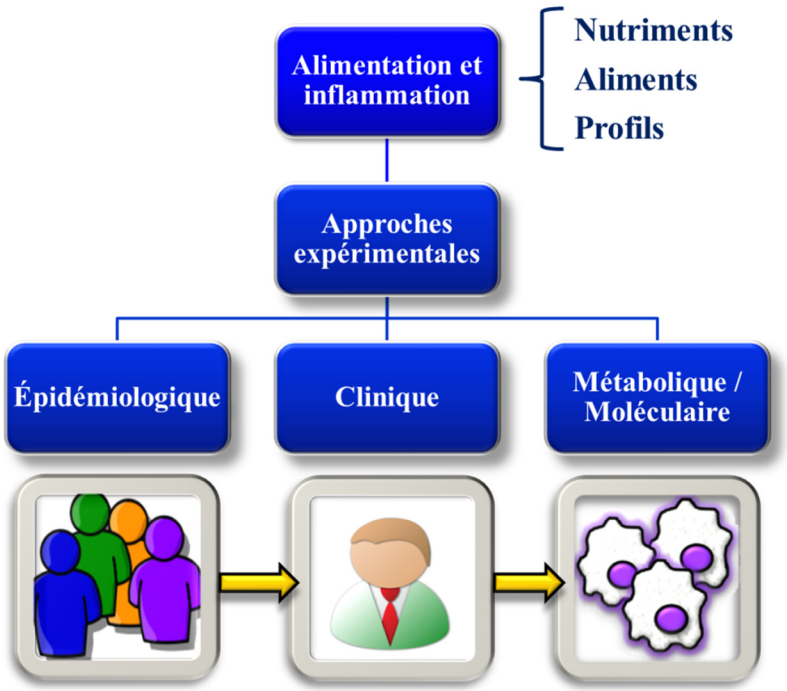

Figure 15.1 : Intégration des différents éléments du présent projet de doctorat qui visait, de façon globale, à étudier les liens entre l'alimentation et l'inflammation en passant de la population, à l'individu, à la cellule.

Sur la base des travaux présentés dans cette thèse, il n'est pas possible de confirmer l'hypothèse générale émise au chapitre 2 comme quoi « *les facteurs nutritionnels globalement considérés comme bénéfiques pour la santé sont associés à des effets anti-inflammatoires, alors que les facteurs nutritionnels globalement considérés comme néfastes pour la santé sont associés à des effets pro-inflammatoires* ». Peu importe l'angle sous lequel l'alimentation a été étudiée, le constat global ressortant des résultats autant de nature épidémiologique, clinique, que métabolique est qu'il est difficile de détecter des

associations claires et nettes entre l'alimentation et les paramètres inflammatoires. Dans la plupart des cas, on note que l'alimentation exercerait une influence mineure sur l'inflammation.

Ce projet de doctorat m'a entre autres permis de réaliser que l'étude des liens entre l'alimentation et l'inflammation est plutôt complexe. Tel que mis en lumière lors de l'étude des associations entre l'alimentation et le profil inflammatoire des nations autochtones du Nord-du-Québec, certaines caractéristiques des participants peuvent représenter d'importants facteurs confondants. Ces facteurs confondants peuvent possiblement masquer et même dénaturer les associations attendues entre l'alimentation et l'inflammation, comme on le suspecte pour l'association inverse entre l'adhésion au profil alimentaire « À faible valeur nutritive » et la hs-CRP chez les Inuits du Nunavik.

Un autre facteur qui complexifie l'étude des liens entre l'alimentation et l'inflammation est l'alimentation elle-même. Non seulement l'alimentation peut être étudiée sous plusieurs angles (nutriments, aliments, alimentation globale), mais chaque méthode d'évaluation alimentaire est sujette à un certain nombre de biais systématiques et erreurs de mesure aléatoires. Des biais et erreurs de mesure trop grands peuvent eux aussi masquer la présence d'associations entre l'alimentation et l'inflammation. Cela met donc en lumière l'importance, dans le domaine de la recherche en nutrition, de connaître les forces et limites des différentes méthodes d'évaluation alimentaire et de choisir celle la plus appropriée à nos besoins.

Il faut également retenir, peu importe l'étude réalisée, que les biomarqueurs inflammatoires sont eux-mêmes caractérisés par beaucoup de variabilité intra- et interindividuelle. Par curiosité, dans le projet COMIT, nous avons calculé la variabilité intra-individuelle des trois biomarqueurs inflammatoires évalués (hs-CRP, IL-6, adiponectine) en fonction des valeurs à la fin de chacune des 5 diètes. Nous avons observé que la variabilité intra-individuelle de la hs-CRP et de l'IL-6 était en moyenne de 3 à 4 fois plus élevée que celle de l'adiponectine (11%), qui elle était relativement semblable à la variabilité calculée pour le HDL-cholestérol (8%) et même plus faible que la variabilité calculée pour les triglycérides (18%) (données exploratoires non publiées). Une variabilité plus grande pour la hs-CRP et l'IL-6 limite donc la possibilité d'observer des différences significatives entre

divers traitements ou groupes de sujets. Cela peut peut-être expliquer, du moins en partie, pourquoi la littérature scientifique actuelle (incluant le projet COMIT) démontre que les oméga-3 d'origine marine augmentent les concentrations d'adiponectine, mais n'exercent pas d'effets clairement définis sur les biomarqueurs pro-inflammatoires.

Par ailleurs, il n'y a pas de consensus à l'heure actuelle concernant quel biomarqueur inflammatoire devrait préférentiellement être mesuré (en d'autres mots, quel biomarqueur inflammatoire est le « meilleur »). Tel que souligné à quelques endroits dans cette thèse, je crois qu'il demeure important de considérer la balance entre les biomarqueurs pro- et anti-inflammatoires plutôt que de s'attarder à un seul type de biomarqueurs. Certains membres de la communauté scientifique suggèrent par ailleurs que l'avenir dans le domaine de l'inflammation repose probablement sur l'évaluation de groupes/combinaisons de biomarqueurs (« *clusters* ») et/ou sur la mesure de paramètres reliés à l'immunité (marqueurs à la surface des cellules immunitaires) (8). Par exemple, une étude chez des sujets âgés aurait démontré qu'un regroupement de biomarqueurs incluant l'IL-6, la hs-CRP, l'hormone thyroïdienne et la transthyrétine serait positivement et indépendamment associé à la mortalité, alors que chaque biomarqueur pris séparément serait peu informatif (324).

De plus, bien que les différents facteurs nutritionnels évalués dans ce projet de doctorat aient globalement eu peu d'influence sur le profil inflammatoire des participants, nous ne pouvons pas écarter la possibilité, sur le plan génétique, que ces facteurs nutritionnels puissent exercer un impact différent sur le profil inflammatoire en fonction de polymorphismes présents dans les gènes des participants. Toutefois, comme cette avenue n'a pas été explorée, je ne suis pas vraiment en mesure de me prononcer davantage sur le sujet.

Pour terminer, je crois que la nature diversifiée de ce projet de doctorat sur l'alimentation et l'inflammation et le constat global qui en ressort nous permettent de prendre conscience de l'importance de planifier judicieusement les études que l'on désire réaliser concernant, entre autres, le choix des participants (caractéristiques, taille de l'échantillon), le choix des interventions (si applicable ; incluant le choix de l'intervention « témoin »), le choix de la méthode d'évaluation alimentaire, le choix des paramètres inflammatoires à mesurer et le

choix des analyses à effectuer. Cela dit, je ne remets aucunement en question les projets de recherche/travaux qui ont été réalisés. Je veux simplement dire que bien que ce projet de doctorat réponde à quelques interrogations entourant les liens entre l'alimentation et l'inflammation, je crois qu'il soulève autant sinon plus de questions qui demeurent sans réponse. La bonne nouvelle est que les quelques réponses obtenues et les nombreuses questions qu'elles soulèvent peuvent constituer la base même de travaux futurs dans le domaine. Ce projet m'a également permis de prendre conscience que chaque type d'approche expérimentale est nécessaire en recherche afin de se forger une idée globale et réaliste sur un sujet.

BIBLIOGRAPHIE

1. The Conference Board of Canada. *Le Québec peut réduire la prévalence et le fardeau économique des maladies chroniques en favorisant l'adoption de saines habitudes de vie,* [En ligne]. http://www.conferenceboard.ca/press/newsrelease/14-11-24/le_qu%C3%A9bec_peut_r%C3%A9duire_la_pr%C3%A9valence_et_le_fardeau_%C3%A9conomique_des_maladies_chroniques_en_favorisant_l_adoption_de_saines_habitudes_de_vie.aspx (page consultée le 25 novembre 2014).

2. Scrivo R, Vasile M, Bartosiewicz I, Valesini G. Inflammation as "common soil" of the multifactorial diseases. Autoimmun Rev 2011;10:369-74.

3. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, Esposito K, Jonsson LS, Kolb H, Lansink M et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr 2011;106 Suppl 3:S5-78.

4. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F et al. Inflammatory disease processes and interactions with nutrition. Br J Nutr 2009;101 Suppl 1:S1-45.

5. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007;449:819-26.

6. Barton GM. A calculated response: control of inflammation by the innate immune system. J Clin Invest 2008;118:413-20.

7. Medzhitov R. Origin and physiological roles of inflammation. Nature 2008;454:428-35.

8. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, Holgate ST, Jonsson LS, Latulippe ME, Marcos A et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br J Nutr 2013;109 Suppl 1:S1-34.

9. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 2008;8:349-61.

10. Rader DJ. Inflammatory markers of coronary risk. N Engl J Med 2000;343:1179-82.

11. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746-9.

12. Swarbrick MM, Havel PJ. Physiological, pharmacological, and nutritional regulation of circulating adiponectin concentrations in humans. Metab Syndr Relat Disord 2008;6:87-102.

13. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131-5.

14. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, D'Andrea F, Molinari AM, Giugliano D. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation 2002;105:804-9.

15. Kim CS, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, Kwon BS, Erickson KL, Yu R. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes (Lond) 2006;30:1347-55.

16. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. Am J Physiol Endocrinol Metab 2003;285:E527-33.

17. Gray B, Steyn F, Davies PS, Vitetta L. Omega-3 fatty acids: a review of the effects on adiponectin and leptin and potential implications for obesity management. Eur J Clin Nutr 2013;67:1234-42.

18. Dietrich M, Jialal I. The effect of weight loss on a stable biomarker of inflammation, C-reactive protein. Nutr Rev 2005;63:22-8.

19. Selvin E, Paynter NP, Erlinger TP. The effect of weight loss on C-reactive protein: a systematic review. Arch Intern Med 2007;167:31-9.

20. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. JAMA 2003;289:1799-804.

21. Lapice E, Maione S, Patti L, Cipriano P, Rivellese AA, Riccardi G, Vaccaro O. Abdominal adiposity is associated with elevated C-reactive protein independent of BMI in healthy nonobese people. Diabetes Care 2009;32:1734-6.

22. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004;145:2273-82.

23. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998;83:847-50.

24. Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Coussieu C, Basdevant A, Bar Hen A, Bedossa P et al. Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes 2006;55:1554-61.

25. Flower L, Gray R, Pinkney J, Mohamed-Ali V. Stimulation of interleukin-6 release by interleukin-1beta from isolated human adipocytes. Cytokine 2003;21:32-7.

26. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112:1821-30.

27. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-808.

28. Trayhurn P, Wang B, Wood IS. Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? Br J Nutr 2008;100:227-35.

29. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 2005;46:2347-55.

30. Vigushin DM, Pepys MB, Hawkins PN. Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. J Clin Invest 1993;91:1351-7.

31. Ablij H, Meinders A. C-reactive protein: history and revival. Eur J Intern Med 2002;13:412.

32. Nanri A, Moore MA, Kono S. Impact of C-reactive protein on disease risk and its relation to dietary factors: Literature review. Asian Pac J Cancer Prev 2007;8:167-77.

33. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999;45:2136-41.

34. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-12.

35. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.

36. Ridker PM, Danielson E, Fonseca FAH, Genest J, Gotto AM, Kastelein JJP, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG et al. Rosuvastatin to Prevent Vascular

Events in Men and Women with Elevated C-Reactive Protein. N Engl J Med 2008;359:2195-207.

37. Galli C, Calder PC. Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. Ann Nutr Metab 2009;55:123-39.

38. Eder K, Baffy N, Falus A, Fulop AK. The major inflammatory mediator interleukin-6 and obesity. Inflamm Res 2009;58:727-36.

39. Fernandez-Real JM, Broch M, Vendrell J, Gutierrez C, Casamitjana R, Pugeat M, Richart C, Ricart W. Interleukin-6 gene polymorphism and insulin sensitivity. Diabetes 2000;49:517-20.

40. Klein CL, Kohler H, Bittinger F, Wagner M, Hermanns I, Grant K, Lewis JC, Kirkpatrick CJ. Comparative studies on vascular endothelium in vitro. I. Cytokine effects on the expression of adhesion molecules by human umbilical vein, saphenous vein and femoral artery endothelial cells. Pathobiology 1994;62:199-208.

41. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 1994;94:1543-9.

42. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996;271:665-8.

43. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.

44. Nakamura K, Fuster JJ, Walsh K. Adipokines: a link between obesity and cardiovascular disease. J Cardiol 2014;63:250-9.

45. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, Pedersen AA, Kalthoff C, Tullin S, Sams A et al. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. J Biol Chem 2010;285:6153-60.

46. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N et al. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation 2004;109:2046-9.

47. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296-301.

48. Yamaguchi N, Argueta JG, Masuhiro Y, Kagishita M, Nonaka K, Saito T, Hanazawa S, Yamashita Y. Adiponectin inhibits Toll-like receptor family-induced signaling. FEBS Lett 2005;579:6821-6.

49. Kumar A, Takada Y, Boriek AM, Aggarwal BB. Nuclear factor-kappaB: its role in health and disease. J Mol Med (Berl) 2004;82:434-48.

50. Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol 2007;8:49-62.

51. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation 2001;103:1057-63.

52. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327-34.

53. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, Illig T, Martin S, Herder C. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984-2002. Diabetes 2005;54:2932-8.

54. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P et al. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 2013;36:166-75.

55. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009;302:179-88.

56. Kim DH, Kim C, Ding EL, Townsend MK, Lipsitz LA. Adiponectin levels and the risk of hypertension: a systematic review and meta-analysis. Hypertension 2013;62:27-32.

57. Groblewska M, Mroczko B, Sosnowska D, Szmitkowski M. Interleukin 6 and C-reactive protein in esophageal cancer. Clin Chim Acta 2012;413:1583-90.

58. Candido J, Hagemann T. Cancer-related inflammation. J Clin Immunol 2013;33 Suppl 1:S79-84.

59. Liao Q, Long C, Deng Z, Bi X, Hu J. The role of circulating adiponectin in prostate cancer: a meta-analysis. Int J Biol Markers 2014:0.

60. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.

61. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767-72.

62. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men - Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999;99:237-42.

63. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A(2), high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Circulation 2004;109:837-42.

64. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291:1730-7.

65. Luo S, Lei H, Liu Q. Correlation between serum adiponectin and risk factors in patients with coronary artery disease. Clin Lab 2013;59:121-6.

66. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation 2000;101:2149-53.

67. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-74.

68. Libby P, Okamoto Y, Rocha VZ, Folco E. Inflammation in atherosclerosis: transition from theory to practice. Circ J 2010;74:213-20.

69. Ridker PM. C-Reactive Protein: Eighty Years from Discovery to Emergence as a Major Risk Marker for Cardiovascular Disease. Clin Chem 2009;55:209-15.

70. Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132-40.

71. Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K, Blumenthal RS, Budoff MJ. High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? J Am Coll Cardiol 2013;62:397-408.

72. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Erdmann J, Braund P, Engert JC, Bennett D et al. Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA 2009;302:37-48.

73. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. N Engl J Med 2008;359:1897-908.

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

75. Interleukin-6 Receptor Mendelian Randomisation Analysis C, Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet 2012;379:1214-24.

76. Collaboration IRGCERF, Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, Gorman DN, Gao P, Saleheen D, Rendon A et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. Lancet 2012;379:1205-13.

77. Ridker PM. Moving beyond JUPITER: will inhibiting inflammation reduce vascular event rates? Curr Atheroscler Rep 2013;15:295.

78. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J 2011;162:597-605.

79. Everett BM, Pradhan AD, Solomon DH, Paynter N, Macfadyen J, Zaharris E, Gupta M, Clearfield M, Libby P, Hasan AA et al. Rationale and design of the Cardiovascular Inflammation Reduction Trial: a test of the inflammatory hypothesis of atherothrombosis. Am Heart J 2013;166:199-207 e15.

80. Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464-9.

81. Albert MA, Glynn RJ, Buring J, Ridker PM. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). Am J Cardiol 2004;93:1238-42.

82. Morimoto Y, Conroy SM, Ollberding NJ, Kim Y, Lim U, Cooney RV, Franke AA, Wilkens LR, Hernandez BY, Goodman MT et al. Ethnic differences in serum adipokine and C-reactive protein levels: the multiethnic cohort. Int J Obes (Lond) 2014;38:1416-22.

83. Khan UI, Wang D, Sowers MR, Mancuso P, Everson-Rose SA, Scherer PE, Wildman RP. Race-ethnic differences in adipokine levels: the Study of Women's Health Across the Nation (SWAN). Metabolism 2012;61:1261-9.

84. Anand SS, Razak F, Yi QL, Davis B, Jacobs R, Vuksan V, Lonn E, Teo K, McQueen M, Yusuf S. C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. Arterioscler Thromb Vasc Biol 2004;24:1509-15.

85. Wener MH, Daum PR, McQuillan GM. The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. J Rheumatol 2000;27:2351-9.

86. Wang TJ, Nam BH, Wilson PW, Wolf PA, Levy D, Polak JF, D'Agostino RB, O'Donnell CJ. Association of C-reactive protein with carotid atherosclerosis in men and women: the Framingham Heart Study. Arterioscler Thromb Vasc Biol 2002;22:1662-7.

87. Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB, Herrington DM. Gender and C-reactive protein: Data from the Multiethnic Study of Atherosclerosis (MESA) cohort. Am Heart J 2006;152:593-8.

88. Cartier A, Cote M, Lemieux I, Perusse L, Tremblay A, Bouchard C, Despres JP. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? Am J Clin Nutr 2009;89:1307-14.

89. Ford ES, Giles WH, Mokdad AH, Myers GL. Distribution and correlates of C-reactive protein concentrations among adult US women. Clin Chem 2004;50:574-81.

90. Rexrode KM, Pradhan A, Manson JE, Buring JE, Ridker PM. Relationship of total and abdominal adiposity with CRP and IL-6 in women. Ann Epidemiol 2003;13:674-82.

91. Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE. Hormone replacement therapy and increased plasma concentration of C-reactive protein. Circulation 1999;100:713-6.

92. Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, Wallace RB, Jackson RD, Pettinger MB, Ridker PM. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. JAMA 2002;288:980-7.

93. Marques-Vidal P, Bochud M, Bastardot F, Luscher T, Ferrero F, Gaspoz JM, Paccaud F, Urwyler A, von Kanel R, Hock C et al. Levels and determinants of inflammatory biomarkers in a Swiss population-based sample (CoLaus study). PLoS One 2011;6:e21002.

94. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000;51:245-70.

95. An J, Ribeiro RC, Webb P, Gustafsson JA, Kushner PJ, Baxter JD, Leitman DC. Estradiol repression of tumor necrosis factor-alpha transcription requires estrogen

receptor activation function-2 and is enhanced by coactivators. Proc Natl Acad Sci U S A 1999;96:15161-6.

96. Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol 2003;149:331-5.

97. Bottner A, Kratzsch J, Muller G, Kapellen TM, Bluher S, Keller E, Bluher M, Kiess W. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J Clin Endocrinol Metab 2004;89:4053-61.

98. Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD, Guralnik JM, Longo DL. The origins of age-related proinflammatory state. Blood 2005;105:2294-9.

99. Bruunsgaard H, Skinhoj P, Pedersen AN, Schroll M, Pedersen BK. Ageing, tumour necrosis factor-alpha (TNF-alpha) and atherosclerosis. Clin Exp Immunol 2000;121:255-60.

100. Ballou SP, Lozanski FB, Hodder S, Rzewnicki DL, Mion LC, Sipe JD, Ford AB, Kushner I. Quantitative and qualitative alterations of acute-phase proteins in healthy elderly persons. Age Ageing 1996;25:224-30.

101. Fagiolo U, Cossarizza A, Scala E, Fanales-Belasio E, Ortolani C, Cozzi E, Monti D, Franceschi C, Paganelli R. Increased cytokine production in mononuclear cells of healthy elderly people. Eur J Immunol 1993;23:2375-8.

102. Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhoj P, Pedersen BK. A high plasma concentration of TNF-alpha is associated with dementia in centenarians. J Gerontol A Biol Sci Med Sci 1999;54:M357-64.

103. Wei J, Xu H, Davies JL, Hemmings GP. Increase of plasma IL-6 concentration with age in healthy subjects. Life Sci 1992;51:1953-6.

104. Paolisso G, Rizzo MR, Mazziotti G, Tagliamonte MR, Gambardella A, Rotondi M, Carella C, Giugliano D, Varricchio M, D'Onofrio F. Advancing age and insulin resistance: role of plasma tumor necrosis factor-alpha. Am J Physiol 1998;275:E294-9.

105. Franceschi C. Inflammaging as a major characteristic of old people: can it be prevented or cured? Nutr Rev 2007;65:S173-6.

106. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med 2003;138:891-7.

107. Lao XQ, Jiang CQ, Zhang WS, Adab P, Lam TH, Cheng KK, Thomas GN. Smoking, smoking cessation and inflammatory markers in older Chinese men: The Guangzhou Biobank Cohort Study. Atherosclerosis 2009;203:304-10.

108. Ryu SY, Lee YS, Park J, Kang MG, Kim KS. Relations of plasma high-sensitivity C-reactive protein to various cardiovascular risk factors. J Korean Med Sci 2005;20:379-83.

109. Lowe GD, Yarnell JW, Rumley A, Bainton D, Sweetnam PM. C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in the Speedwell study: are inflammation and fibrin turnover linked in pathogenesis? Arterioscler Thromb Vasc Biol 2001;21:603-10.

110. Helmersson J, Larsson A, Vessby B, Basu S. Active smoking and a history of smoking are associated with enhanced prostaglandin F(2alpha), interleukin-6 and F2-isoprostane formation in elderly men. Atherosclerosis 2005;181:201-7.

111. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. Chest 2007;131:1557-66.

112. Iwashima Y, Katsuya T, Ishikawa K, Kida I, Ohishi M, Horio T, Ouchi N, Ohashi K, Kihara S, Funahashi T et al. Association of hypoadiponectinemia with smoking habit in men. Hypertension 2005;45:1094-100.

113. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 2002;13:561-8.

114. Abramson JL, Vaccarino V. Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. Arch Intern Med 2002;162:1286-92.

115. Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between physical activity and markers of inflammation in a healthy elderly population. Am J Epidemiol 2001;153:242-50.

116. Wannamethee SG, Lowe GD, Whincup PH, Rumley A, Walker M, Lennon L. Physical activity and hemostatic and inflammatory variables in elderly men. Circulation 2002;105:1785-90.

117. Mora S, Lee IM, Buring JE, Ridker PM. Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. JAMA 2006;295:1412-9.

118. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Rimm EB. Leisure-time physical activity and reduced plasma levels of obesity-related inflammatory markers. Obes Res 2003;11:1055-64.

119. Stauffer BL, Hoetzer GL, Smith DT, DeSouza CA. Plasma C-reactive protein is not elevated in physically active postmenopausal women taking hormone replacement therapy. J Appl Physiol (1985) 2004;96:143-8.

120. Kuo HK, Yen CJ, Chen JH, Yu YH, Bean JF. Association of cardiorespiratory fitness and levels of C-reactive protein: data from the National Health and Nutrition Examination Survey 1999-2002. Int J Cardiol 2007;114:28-33.

121. Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. Arterioscler Thromb Vasc Biol 2002;22:1869-76.

122. Giallauria F, Palomba S, De Sio I, Maresca L, Vuolo L, Savastano S, Lombardi G, Colao A, Vigorito C, Orio F. Inflammatory markers and visceral fat are inversely associated with maximal oxygen consumption in women with polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf) 2009;70:394-400.

123. McGavock JM, Mandic S, Vonder Muhll I, Lewanczuk RZ, Quinney HA, Taylor DA, Welsh RC, Haykowsky M. Low cardiorespiratory fitness is associated with elevated C-reactive protein levels in women with type 2 diabetes. Diabetes Care 2004;27:320-5.

124. Beavers KM, Brinkley TE, Nicklas BJ. Effect of exercise training on chronic inflammation. Clin Chim Acta 2010;411:785-93.

125. Song Y, Ridker PM, Manson JE, Cook NR, Buring JE, Liu S. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care 2005;28:1438-44.

126. King DE, Mainous AG, 3rd, Geesey ME, Woolson RF. Dietary magnesium and C-reactive protein levels. J Am Coll Nutr 2005;24:166-71.

127. Bo S, Durazzo M, Guidi S, Carello M, Sacerdote C, Silli B, Rosato R, Cassader M, Gentile L, Pagano G. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. Am J Clin Nutr 2006;84:1062-9.

128. Chacko SA, Song Y, Nathan L, Tinker L, de Boer IH, Tylavsky F, Wallace R, Liu S. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. Diabetes Care 2010;33:304-10.

129. Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RB, Sr., Dawson-Hughes B, Ordovas JM, O'Donnell CJ, Kathiresan S, Keaney JF, Jr. et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. Am J Epidemiol 2008;167:313-20.

130. Reddi K, Henderson B, Meghji S, Wilson M, Poole S, Hopper C, Harris M, Hodges SJ. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. Cytokine 1995;7:287-90.

131. Ohsaki Y, Shirakawa H, Hiwatashi K, Furukawa Y, Mizutani T, Komai M. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. Biosci Biotechnol Biochem 2006;70:926-32.

132. Levitan EB, Cook NR, Stampfer MJ, Ridker PM, Rexrode KM, Buring JE, Manson JE, Liu S. Dietary glycemic index, dietary glycemic load, blood lipids, and C-reactive protein. Metabolism 2008;57:437-43.

133. Du H, van der AD, van Bakel MM, van der Kallen CJ, Blaak EE, van Greevenbroek MM, Jansen EH, Nijpels G, Stehouwer CD, Dekker JM et al. Glycemic index and glycemic load in relation to food and nutrient intake and metabolic risk factors in a Dutch population. Am J Clin Nutr 2008;87:655-61.

134. Qi L, Rimm E, Liu S, Rifai N, Hu FB. Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. Diabetes Care 2005;28:1022-8.

135. Schwingshackl L, Hoffmann G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. Nutr Metab Cardiovasc Dis 2013;23:699-706.

136. King DE, Egan BM, Geesey ME. Relation of dietary fat and fiber to elevation of C-reactive protein. Am J Cardiol 2003;92:1335-9.

137. Qi L, van Dam RM, Liu S, Franz M, Mantzoros C, Hu FB. Whole-grain, bran, and cereal fiber intakes and markers of systemic inflammation in diabetic women. Diabetes Care 2006;29:207-11.

138. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from national health and nutrition examination survey data. J Nutr 2004;134:1181-5.

139. North CJ, Venter CS, Jerling JC. The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. Eur J Clin Nutr 2009;63:921-33.

140. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006;83:1505S-19S.

141. Baker RG, Hayden MS, Ghosh S. NF-kappaB, inflammation, and metabolic disease. Cell Metab 2011;13:11-22.

142. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol 2008;8:923-34.

143. Davis JE, Gabler NK, Walker-Daniels J, Spurlock ME. Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. Obesity (Silver Spring) 2008;16:1248-55.

144. Lefevre M, Jonnalagadda S. Effect of whole grains on markers of subclinical inflammation. Nutr Rev 2012;70:387-96.

145. Watzl B, Kulling SE, Moseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. Am J Clin Nutr 2005;82:1052-8.

146. Azadbakht L, Esmaillzadeh A. Red meat intake is associated with metabolic syndrome and the plasma C-reactive protein concentration in women. J Nutr 2009;139:335-9.

147. Hodgson JM, Ward NC, Burke V, Beilin LJ, Puddey IB. Increased lean red meat intake does not elevate markers of oxidative stress and inflammation in humans. J Nutr 2007;137:363-7.

148. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. Circulation 2012;125:1735-41, S1.

149. Aeberli I, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, Berthold HK, Spinas GA, Berneis K. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. Am J Clin Nutr 2011;94:479-85.

150. Kant AK. Dietary patterns and health outcomes. J Am Diet Assoc 2004;104:615-35.

151. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002;13:3-9.

152. Hoffmann K, Schulze MB, Schienkiewitz A, Nothlings U, Boeing H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. Am J Epidemiol 2004;159:935-44.

153. Barbaresko J, Koch M, Schulze MB, Nothlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutr Rev 2013;71:511-27.

154. Krajcovicova-Kudlackova M, Blazicek P. C-reactive protein and nutrition. Bratisl Lek Listy 2005;106:345-7.

155. Szeto YT, Kwok TC, Benzie IF. Effects of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. Nutrition 2004;20:863-6.

156. Pilis W, Stec K, Zych M, Pilis A. Health benefits and risk associated with adopting a vegetarian diet. Rocz Panstw Zakl Hig 2014;65:9-14.

157. Ruiz-Nunez B, Pruimboom L, Dijck-Brouwer DA, Muskiet FA. Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context. J Nutr Biochem 2013;24:1183-201.

158. Galland L. Diet and inflammation. Nutr Clin Pract 2010;25:634-40.

159. Secrétariat aux affaires autochtones. Gouvernement du Québec. *Relations avec les Autochtones. Profil des nations,* [En ligne]. http://www.autochtones.gouv.qc.ca/relations_autochtones/profils_nations/profil.htm (Page consultée le 13 novembre 2014).

160. Secrétariat aux affaires autochtones. Gouvernement du Québec. *Amérindiens et Inuits - Portrait des nations autochtones du Québec - 2e édition,* [En ligne]. http://www.autochtones.gouv.qc.ca/publications_documentation/publications/document-11-nations-2e-edition.pdf (Page consultée le 13 novembre 2014).

161. Nieboer E, Dewailly E, Johnson-Down L, Sampasa-Kanyinga H, Château-Degat M-L, Egeland GM, Atikessé L, Robinson E, Torrie J. *Nituuchischaayihtitaau Aschii Multi-community Environment-and-Health Study in Eeyou Istchee 2005-2009: Final Technical Report.* Nieboer E, Robinson E, Petrov K, editors. Public Health Report Series 4 on the Health of the Population. Chisasibi QC: Cree Board of Health and Social Services of James Bay; 2013.

162. Rochette L, Blanchet C. *Methodological Report. Nunavik Inuit Health Survey 2004, Qanuippitaa? How are we?* Québec, Canada : Gouvernement du Québec - Institut national de santé publique du Québec (INSPQ) & Nunavik Regional Board of Health and Social Services (NRBHSS); 2007.

163. Sharma S. Assessing diet and lifestyle in the Canadian Arctic Inuit and Inuvialuit to inform a nutrition and physical activity intervention programme. J Hum Nutr Diet 2010;23 Suppl 1:5-17.

164. Bjerregaard P, Curtis T, Greenland Population S. Cultural change and mental health in Greenland: the association of childhood conditions, language, and urbanization with mental health and suicidal thoughts among the Inuit of Greenland. Soc Sci Med 2002;54:33-48.

165. Berkes F, Farkas CS. Eastern James Bay Cree Indians: changing patterns of wild food use and nutrition. Ecol Food Nutr 1978;7:155-72.

166. Receveur O, Boulay M, Kuhnlein HV. Decreasing traditional food use affects diet quality for adult Dene/Metis in 16 communities of the Canadian Northwest Territories. J Nutr 1997;127:2179-86.

167. Kuhnlein HV, Receveur O, Soueida R, Egeland GM. Arctic Indigenous Peoples experience the nutrition transition with changing dietary patterns and obesity. J Nutr 2004;134:1447-53.

168. Sheikh N, Egeland GM, Johnson-Down L, Kuhnlein HV. Changing dietary patterns and body mass index over time in Canadian Inuit communities. Int J Circumpolar Health 2011;70:511-9.

169. Johnson-Down LM, Egeland GM. How is nutrition transition affecting dietary adequacy in Eeyouch (Cree) adults of Northern Quebec, Canada? Appl Physiol Nutr Metab 2013;38:300-5.

170. Hopping BN, Mead E, Erber E, Sheehy C, Roache C, Sharma S. Dietary adequacy of Inuit in the Canadian Arctic. J Hum Nutr Diet 2010;23 Suppl 1:27-34.

171. Popkin BM. Nutritional patterns and transitions. Popul Dev Rev 1993;19:138-57.

172. Sagild U, Littauer J, Jespersen CS, Andersen S. Epidemiological studies in Greenland 1962-1964. I. Diabetes mellitus in Eskimos. Acta Med Scand 1966;179:29-39.

173. Davies LE, Hanson S. The Eskimos of the Northwest Passage: A Survey of Dietary Composition and Various Blood and Metabolic Measurements. Can Med Assoc J 1965;92:205-16.

174. Mouratoff GJ, Carroll NV, Scott EM. Diabetes mellitus in Eskimos. JAMA 1967;199:107-12.

175. Robinson E. The health of the james bay cree. Can Fam Physician 1988;34:1606-13.

176. Chateau-Degat ML, Dewailly E, Louchini R, Counil E, Noël M, Ferland A, Lucas M, Valera B, Ekoé JM, Ladouceur R et al. Cardiovascular burden and related risk factors among Nunavik (Quebec) Inuit: insights from baseline findings in the circumpolar Inuit health in transition cohort study. Can J Cardiol 2010;26:190-6.

177. Chateau-Degat ML, Dewailly E, Charbonneau G, Laouan-Sidi EA, Tremblay A, Egeland GM. Obesity risks: towards an emerging Inuit pattern. Int J Circumpolar Health 2011;70:166-77.

178. Dewailly E, Chateau-Degat ML, Ekoé JM, Ladouceur R, Rochette L. *Status of Cardiovascular Disease and Diabetes in Nunavik. Nunavik Inuit Health Survey 2004, Qanuippitaa? How are we?* Québec, Canada : Gouvernement du Québec -

Institut national de santé publique du Québec (INSPQ) & Nunavik Regional Board of Health and Social Services (NRBHSS); 2007.

179. Haman F, Fontaine-Bisson B, Batal M, Imbeault P, Blais JM, Robidoux MA. Obesity and type 2 diabetes in Northern Canada's remote First Nations communities: the dietary dilemma. Int J Obes (Lond) 2010;34 Suppl 2:S24-31.

180. Garriguet D. Obesity and the eating habits of the Aboriginal population. Health Rep 2008;19:21-35.

181. Dannenbaum D, Kuzmina E, Lejeune P, Torrie J, Gangbe M. Prevalence of diabetes and diabetes-related complications in First Nations communities in northern Quebec (Eeyou Istchee), Canada. Can J Diabetes 2008;32:46-52.

182. Ayach BB, Korda H. Commentary: Type 2 Diabetes Epidemic in First Nations People of Canada. Ethn Dis 2010;20:300-3.

183. Young TK, Reading J, Elias B, O'Neil JD. Type 2 diabetes mellitus in Canada's first nations: status of an epidemic in progress. CMAJ 2000;163:561-6.

184. Shah BR, Hux JE, Zinman B. Increasing rates of ischemic heart disease in the native population of Ontario, Canada. Arch Intern Med 2000;160:1862-6.

185. Lee DS, Chiu M, Manuel DG, Tu K, Wang X, Austin PC, Mattern MY, Mitiku TF, Svenson LW, Putnam W et al. Trends in risk factors for cardiovascular disease in Canada: temporal, socio-demographic and geographic factors. CMAJ 2009;181:E55-66.

186. Labonte ME, Dewailly E, Lucas M, Couture P, Lamarche B. Association of red blood cell n-3 polyunsaturated fatty acids with plasma inflammatory biomarkers among the Quebec Cree population. Eur J Clin Nutr 2014;68:1042-7.

187. Labonte ME, Dewailly E, Chateau-Degat ML, Couture P, Lamarche B. Population-based study of high plasma C-reactive protein concentrations among the Inuit of Nunavik. Int J Circumpolar Health 2012;71 doi: 10.3402/ijch.v71i0.19066.

188. Eilat-Adar S, Mete M, Nobmann ED, Xu JQ, Fabsitz RR, Ebbesson SOE, Howard BV. Dietary Patterns are Linked to Cardiovascular Risk Factors but Not to Inflammatory Markers in Alaska Eskimos. J Nutr 2009;139:2322-8.

189. Makhoul Z, Kristal AR, Gulati R, Luick B, Bersamin A, Boyer B, Mohatt GV. Associations of very high intakes of eicosapentaenoic and docosahexaenoic acids with biomarkers of chronic disease risk among Yup'ik Eskimos. Am J Clin Nutr 2010;91:777-85.

190. Makhoul Z, Kristal AR, Gulati R, Luick B, Bersamin A, O'Brien D, Hopkins SE, Stephensen CB, Stanhope KL, Havel PJ et al. Associations of obesity with

triglycerides and C-reactive protein are attenuated in adults with high red blood cell eicosapentaenoic and docosahexaenoic acids. Eur J Clin Nutr 2011;65:808-17.

191. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr 2007;86:74-81.

192. Vidgren HM, Agren JJ, Schwab U, Rissanen T, Hanninen O, Uusitupa MI. Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men. Lipids 1997;32:697-705.

193. 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;38:2012-22.

194. Hedrick VE, Dietrich AM, Estabrooks PA, Savla J, Serrano E, Davy BM. Dietary biomarkers: advances, limitations and future directions. Nutr J 2012;11:109.

195. Dewailly E, Blanchet C, Gingras S, Lemieux S, Holub BJ. Fish consumption and blood lipids in three ethnic groups of Quebec (Canada). Lipids 2003;38:359-65.

196. Solis-Pereyra B, Aattouri N, Lemonnier D. Role of food in the stimulation of cytokine production. Am J Clin Nutr 1997;66:521S-5S.

197. Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect Immun 1996;64:5403-5.

198. Miettinen M, Matikainen S, Vuopio-Varkila J, Pirhonen J, Varkila K, Kurimoto M, Julkunen I. Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. Infect Immun 1998;66:6058-62.

199. Salas-Salvado J, Garcia-Arellano A, Estruch R, Marquez-Sandoval F, Corella D, Fiol M, Gomez-Gracia E, Vinoles E, Aros F, Herrera C et al. Components of the mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. Eur J Clin Nutr 2008;62:651-9.

200. Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy Products Consumption Is Associated with Decreased Levels of Inflammatory Markers Related to Cardiovascular Disease in Apparently Healthy Adults: The ATTICA Study. J Am Coll Nutr 2010;29:357-64.

201. Esmaillzadeh A, Azadbakht L. Dairy consumption and circulating levels of inflammatory markers among Iranian women. Public Health Nutr 2010;13:1395-402.

202. Yannakoulia M, Yiannakouris N, Melistas L, Fappa E, Vidra N, Kontogianni MD, Mantzoros CS. Dietary factors associated with plasma high molecular weight and total adiponectin levels in apparently healthy women. Eur J Endocrinol 2008;159:R5-10.

203. Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. Metabolism 2008;57:824-30.

204. Sadeghi M, Khosravi-Boroujeni H, Sarrafzadegan N, Asgary S, Roohafza H, Gharipour M, Sajjadi F, Khalesi S, Rafieian-Kopaei M. Cheese consumption in relation to cardiovascular risk factors among Iranian adults- IHHP Study. Nutr Res Pract 2014;8:336-41.

205. Seo D, Ginsburg GS, Goldschmidt-Clermont PJ. Gene expression analysis of cardiovascular diseases: novel insights into biology and clinical applications. J Am Coll Cardiol 2006;48:227-35.

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

207. Van Loan MD, Keim NL, Adams SH, Souza E, Woodhouse LR, Thomas A, Witbracht M, Gertz ER, Piccolo B, Bremer AA et al. Dairy Foods in a Moderate Energy Restricted Diet Do Not Enhance Central Fat, Weight, and Intra-Abdominal Adipose Tissue Losses nor Reduce Adipocyte Size or Inflammatory Markers in Overweight and Obese Adults: A Controlled Feeding Study. J Obes 2011;2011:989657.

208. Jones KW, Eller LK, Parnell JA, Doyle-Baker PK, Edwards AL, Reimer RA. Effect of a dairy- and calcium-rich diet on weight loss and appetite during energy restriction in overweight and obese adults: a randomized trial. Eur J Clin Nutr 2013;67:371-6.

209. Serra MC, Beavers KM, Beavers DP, Willoughby DS. Effects of 28 days of dairy or soy ingestion on skeletal markers of inflammation and proteolysis in post-menopausal women. Nutr Health 2012;21:117-30.

210. Nestel PJ, Mellett N, Pally S, Wong G, Barlow CK, Croft K, Mori TA, Meikle PJ. Effects of low-fat or full-fat fermented and non-fermented dairy foods on selected cardiovascular biomarkers in overweight adults. Br J Nutr 2013;110:2242-9.

211. Sun X, Zemel MB. Calcium and 1,25-dihydroxyvitamin D-3 regulation of adipokine expression. Obesity 2007;15:340-8.

212. Sun XC, Zemel MB. 1 alpha,25-dihydroxyvitamin D-3 modulation of adipocyte reactive oxygen species production. Obesity 2007;15:1944-53.

213. VanAmerongen BM, Dijkstra CD, Lips P, Polman CH. Multiple sclerosis and vitamin D: an update. Eur J Clin Nutr 2004;58:1095-109.

214. Rosa FT, Zulet MA, Marchini JS, Martinez JA. Bioactive compounds with effects on inflammation markers in humans. Int J Food Sci Nutr 2012;63:749-65.

215. Producteurs laitiers du Canada. *Données sur la consommation. Données quantitatives. Les produits laitiers : un groupe négligé,* [En ligne]. http://www.savoirlaitier.ca/donnees-sur-la-consommation/donnees-quantitatives/les-produits-laitiers-un-groupe-neglige (Page consultée le 10 décembre 2014).

216. Santos S, Oliveira A, Lopes C. Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines. Nutr Res 2013;33:687-95.

217. Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res 2002;43:445-52.

218. Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. Am J Clin Nutr 2004;79:969-73.

219. Teng KT, Voon PT, Cheng HM, Nesaretnam K. Effects of partially hydrogenated, semi-saturated, and high oleate vegetable oils on inflammatory markers and lipids. Lipids 2010;45:385-92.

220. Bendsen NT, Stender S, Szecsi PB, Pedersen SB, Basu S, Hellgren LI, Newman JW, Larsen TM, Haugaard SB, Astrup A. Effect of industrially produced trans fat on markers of systemic inflammation: evidence from a randomized trial in women. J Lipid Res 2011;52:1821-8.

221. Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, Couture P, Lamarche B. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. Am J Clin Nutr 2008;87:593-9.

222. Kuhnt K, Kraft J, Vogelsang H, Eder K, Kratzsch J, Jahreis G. Dietary supplementation with trans-11- and trans-12-18 : 1 increases cis-9, trans-11-conjugated linoleic acid in human immune cells, but without effects on biomarkers of immune function and inflammation. Br J Nutr 2007;97:1196-205.

223. Lichtenstein AH, Erkkila AT, Lamarche B, Schwab US, Jalbert SM, Ausman LM. Influence of hydrogenated fat and butter on CVD risk factors: remnant-like

particles, glucose and insulin, blood pressure and C-reactive protein. Atherosclerosis 2003;171:97-107.

224. Vega-Lopez S, Matthan NR, Ausman LM, Ai M, Otokozawa S, Schaefer EJ, Lichtenstein AH. Substitution of vegetable oil for a partially-hydrogenated fat favorably alters cardiovascular disease risk factors in moderately hypercholesterolemic postmenopausal women. Atherosclerosis 2009;207:208-12.

225. Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup EM. Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. Am J Clin Nutr 2006;83:237-43.

226. Yoneyama S, Miura K, Sasaki S, Yoshita K, Morikawa Y, Ishizaki M, Kido T, Naruse Y, Nakagawa H. Dietary intake of fatty acids and serum C-reactive protein in Japanese. J Epidemiol 2007;17:86-92.

227. Rajaram S, Connell KM, Sabate J. Effect of almond-enriched high-monounsaturated fat diet on selected markers of inflammation: a randomised, controlled, crossover study. Br J Nutr 2010;103:907-12.

228. Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI, Fiol M, Gomez-Gracia E, Lopez-Sabater MC, Vinyoles E et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 2006;145:1-11.

229. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004;292:1440-6.

230. Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis 2007;190:181-6.

231. Schwingshackl L, Strasser B, Hoffmann G. Effects of monounsaturated fatty acids on cardiovascular risk factors: a systematic review and meta-analysis. Ann Nutr Metab 2011;59:176-86.

232. Gillingham LG, Gustafson JA, Han SY, Jassal DS, Jones PJ. High-oleic rapeseed (canola) and flaxseed oils modulate serum lipids and inflammatory biomarkers in hypercholesterolaemic subjects. Br J Nutr 2011;105:417-27.

233. Keogh JB, Grieger JA, Noakes M, Clifton PM. Flow-mediated dilatation is impaired by a high-saturated fat diet but not by a high-carbohydrate diet. Arterioscler Thromb Vasc Biol 2005;25:1274-9.

234. Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care 2007;30:1717-23.

235. Garcia-Escobar E, Rodriguez-Pacheco F, Garcia-Serrano S, Gomez-Zumaquero JM, Haro-Mora JJ, Soriguer F, Rojo-Martinez G. Nutritional regulation of interleukin-6 release from adipocytes. Int J Obes (Lond) 2010;34:1328-32.

236. Granados N, Amengual J, Ribot J, Palou A, Bonet ML. Distinct effects of oleic acid and its trans-isomer elaidic acid on the expression of myokines and adipokines in cell models. Br J Nutr 2011;105:1226-34.

237. van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG, de Groot LC, de Vries JH, Muller M, Afman LA. 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;90:1656-64.

238. Adkins Y, Kelley DS. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J Nutr Biochem 2010;21:781-92.

239. Choque B, Catheline D, Rioux V, Legrand P. Linoleic acid: between doubts and certainties. Biochimie 2014;96:14-21.

240. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. Annu Rev Pathol 2008;3:279-312.

241. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie 2009;91:791-5.

242. Miles EA, Allen E, Calder PC. In vitro effects of eicosanoids derived from different 20-carbon Fatty acids on production of monocyte-derived cytokines in human whole blood cultures. Cytokine 2002;20:215-23.

243. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik JM. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 2006;91:439-46.

244. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. Circulation 2003;108:155-60.

245. Poudel-Tandukar K, Nanri A, Matsushita Y, Sasaki S, Ohta M, Sato M, Mizoue T. Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. Nutrition Research 2009;29:363-70.

246. Fernandez-Real JM, Broch M, Vendrell J, Ricart W. Insulin resistance, inflammation, and serum fatty acid composition. Diabetes Care 2003;26:1362-8.

247. Johnson GH, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. J Acad Nutr Diet 2012;112:1029-41, 41 e1-15.

248. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, Berglund J, Pulkki K, Basu S, Uusitupa M 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;95:1003-12.

249. Lopez-Garcia E, Schulze MB, Manson JAE, Meigs JB, Albert CM, Rifai N, Willett WC, Hu FB. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. J Nutr 2004;134:1806-11.

250. Ohsawa M, Itai K, Onoda T, Tanno K, Sasaki S, Nakamura M, Ogawa A, Sakata K, Kawamura K, Kuribayashi T et al. Dietary intake of n-3 polyunsaturated fatty acids is inversely associated with CRP levels, especially among male smokers. Atherosclerosis 2008;201:184-91.

251. Kontogianni MD, Vlassopoulos A, Gatzieva A, Farmaki AE, Katsiougiannis S, Panagiotakos DB, Kalogeropoulos N, Skopouli FN. Flaxseed oil does not affect inflammatory markers and lipid profile compared to olive oil, in young, healthy, normal weight adults. Metabolism 2013;62:686-93.

252. Paschos GK, Zampelas A, Panagiotakos DB, Katsiougiannis S, Griffin BA, Votteas V, Skopouli FN. Effects of flaxseed oil supplementation on plasma adiponectin levels in dyslipidemic men. Eur J Nutr 2007;46:315-20.

253. Nelson TL, Stevens JR, Hickey MS. Adiponectin levels are reduced, independent of polymorphisms in the adiponectin gene, after supplementation with alpha-linolenic acid among healthy adults. Metabolism 2007;56:1209-15.

254. Geleijnse JM, de Goede J, Brouwer IA. Alpha-linolenic acid: is it essential to cardiovascular health? Curr Atheroscler Rep 2010;12:359-67.

255. Robinson LE, Mazurak VC. N-3 polyunsaturated fatty acids: relationship to inflammation in healthy adults and adults exhibiting features of metabolic syndrome. Lipids 2013;48:319-32.

256. Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 1996;63:116-22.

257. Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM. Dietary alpha-linolenic acid inhibits proinflammatory cytokine production by peripheral

blood mononuclear cells in hypercholesterolemic subjects. Am J Clin Nutr 2007;85:385-91.

258. Kew S, Banerjee T, Minihane AM, Finnegan YE, Muggli R, Albers R, Williams CM, Calder PC. Lack of effect of foods enriched with plant- or marine-derived n-3 fatty acids on human immune function. Am J Clin Nutr 2003;77:1287-95.

259. Thies F, Miles EA, Nebe-von-Caron G, Powell JR, Hurst TL, Newsholme EA, Calder PC. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. Lipids 2001;36:1183-93.

260. Wallace FA, Miles EA, Calder PC. Comparison of the effects. of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. Br J Nutr 2003;89:679-89.

261. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr 2004;134:2991-7.

262. Bemelmans WJE, Lefrandt JD, Feskens EJM, van Haelst PL, Broer J, Meyboom-de Jong B, May JF, Tervaert JWC, Smit AJ. Increased alpha-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. Eur J Clin Nutr 2004;58:1083-9.

263. Rallidis LS, Paschos G, Liakos GK, Velissaridou AH, Anastasiadis G, Zampelas A. Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. Atherosclerosis 2003;167:237-42.

264. Paschos G, Yiannakouris N, Rallidis LS, Davies I, Griffin BA, Panagiotakos DB, Skopouli FN, Votteas V, Zampelas A. Apolipoprotein E genotype in dyslipidemic patients and response of blood lipids and inflammatory markers to alpha-linolenic Acid. Angiology 2005;56:49-60.

265. Paschos G, Rallidis LS, Liakos GK, Panagiotakos DB, Anastasiadis G, Votteas V, Zampelas A. Background diet influences the anti-inflammatory effect of alpha-linolenic acid in dyslipidaemic subjects. Br J Nutr 2004;92:649-55.

266. Bloedon LT, Balikai S, Chittams J, Cunnane SC, Berlin JA, Rader DJ, Szapary PO. Flaxseed and cardiovascular risk factors: Results from a double blind, randomized, controlled clinical trial. J Am Coll Nutr 2008;27:65-74.

267. Nelson TL, Stevens JR, Hickey MS. Inflammatory markers are not altered by an eight week dietary alpha-linolenic acid intervention in healthy abdominally obese adult males and females. Cytokine 2007;38:101-6.

268. Kaul N, Kreml R, Austria JA, Richard MN, Edel AL, Dibrov E, Hirono S, Zettler ME, Pierce GN. A comparison of fish oil, flaxseed oil and hempseed oil supplementation on selected parameters of cardiovascular health in healthy volunteers. J Am Coll Nutr 2008;27:51-8.

269. Dewell A, Marvasti FF, Harris WS, Tsao P, Gardner CD. Low- and high-dose plant and marine (n-3) fatty acids do not affect plasma inflammatory markers in adults with metabolic syndrome. J Nutr 2011;141:2166-71.

270. Zhao G, Etherton TD, Martin KR, Vanden Heuvel JP, Gillies PJ, West SG, Kris-Etherton PM. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. Biochem Biophys Res Commun 2005;336:909-17.

271. Jangale NM, Devarshi PP, Dubal AA, Ghule AE, Koppikar SJ, Bodhankar SL, Chougale AD, Kulkarni MJ, Harsulkar AM. Dietary flaxseed oil and fish oil modulates expression of antioxidant and inflammatory genes with alleviation of protein glycation status and inflammation in liver of streptozotocin-nicotinamide induced diabetic rats. Food Chem 2013;141:187-95.

272. Enos RT, Velazquez KT, McClellan JL, Cranford TL, Walla MD, Murphy EA. Reducing the dietary omega-6:omega-3 utilizing alpha-linolenic acid; not a sufficient therapy for attenuating high-fat-diet-induced obesity development nor related detrimental metabolic and adipose tissue inflammatory outcomes. PLoS One 2014;9:e94897.

273. Purushothaman D, Brown WY, Vanselow BA, Quinn K, Wu SB. Flaxseed oil supplementation alters the expression of inflammatory-related genes in dogs. Genet Mol Res 2014;13:5322-32.

274. Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysohoou C, Skoumas Y, Stefanadis C. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease - The ATTICA study. J Am Coll Cardiol 2005;46:120-4.

275. He K, Liu K, Daviglus ML, Jenny NS, Mayer-Davis E, Jiang R, Steffen L, Siscovick D, Tsai M, Herrington D. Associations of Dietary Long-Chain n-3 Polyunsaturated Fatty Acids and Fish With Biomarkers of Inflammation and Endothelial Activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). Am J Cardiol 2009;103:1238-43.

276. Micallef MA, Munro IA, Garg ML. An inverse relationship between plasma n-3 fatty acids and C-reactive protein in healthy individuals. Eur J Clin Nutr 2009;63:1154-6.

277. Farzaneh-Far R, Harris WS, Garg S, Na B, Whooley MA. Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study. Atherosclerosis 2009;205:538-43.

278. Reinders I, Virtanen JK, Brouwer IA, Tuomainen TP. Association of serum n-3 polyunsaturated fatty acids with C-reactive protein in men. Eur J Clin Nutr 2012;66:736-41.

279. Kalogeropoulos N, Panagiotakos DB, Pitsavos C, Chrysohoou C, Rousinou G, Toutouza M, Stefanadis C. Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. Clin Chim Acta 2010;411:584-91.

280. Mozaffarian D, Lemaitre RN, King IB, Song X, Spiegelman D, Sacks FM, Rimm EB, Siscovick DS. Circulating long-chain omega-3 fatty acids and incidence of congestive heart failure in older adults: the cardiovascular health study: a cohort study. Ann Intern Med 2011;155:160-70.

281. Wu JH, Cahill LE, Mozaffarian D. Effect of fish oil on circulating adiponectin: a systematic review and meta-analysis of randomized controlled trials. J Clin Endocrinol Metab 2013;98:2451-9.

282. Robinson LE, Buchholz AC, Mazurak VC. Inflammation, obesity, and fatty acid metabolism: influence of n-3 polyunsaturated fatty acids on factors contributing to metabolic syndrome. Appl Physiol Nutr Metab 2007;32:1008-24.

283. Rangel-Huerta OD, Aguilera CM, Mesa MD, Gil A. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomakers: a systematic review of randomised clinical trials. Br J Nutr 2012;107 Suppl 2:S159-70.

284. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, Vandermeer JWM, Cannon JG, Rogers TS, Klempner MS, Weber PC et al. The Effect of Dietary Supplementation with N-3 Poly-Unsaturated Fatty-Acids on the Synthesis of Interleukin-1 and Tumor Necrosis Factor by Mononuclear-Cells. N Engl J Med 1989;320:265-71.

285. Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrilllabrode A, Dinarello CA, Gorbach SL. Oral (N-3) Fatty-Acid Supplementation Suppresses Cytokine Production and Lymphocyte-Proliferation - Comparison Between Young and Older Women. J Nutr 1991;121:547-55.

286. Abbate R, Gori AM, Martini F, Brunelli T, Filippini M, Francalanci I, Paniccia R, Prisco D, Gensini GF, Serneri GGN. n-3 PUFA supplementation, monocyte PCA expression and interleukin-6 production. Prostaglandins Leukot Essent Fatty Acids 1996;54:439-44.

287. Gallai V, Sarchielli P, Trequattrini A, Franceschini M, Floridi A, Firenze C, Alberti A, Di Benedetto D, Stragliotto E. Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. J Neuroimmunol 1995;56:143-53.

288. Trebble T, Arden NK, Stroud MA, Wootton SA, Burdge GC, Miles EA, Ballinger AB, Thompson RL, Calder PC. Inhibition of tumour necrosis factor-alpha and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. Br J Nutr 2003;90:405-12.

289. Kelley DS, Taylor PC, Nelson GJ, Schmidt PC, Ferretti A, Erickson KL, Yu R, Chandra RK. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. Lipids 1999;34:317-24.

290. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510:92-101.

291. Calder PC. N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. Lipids 2003;38:343-52.

292. Nakamura MT, Cheon Y, Li Y, Nara TY. Mechanisms of regulation of gene expression by fatty acids. Lipids 2004;39:1077-83.

293. Bouwens M, van de Rest O, Dellschaft N, Bromhaar MG, de Groot LCPG, Geleijnse JM, Muller M, Afman LA. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr 2009;90:415-24.

294. Weaver KL, Ivester P, Seeds M, Case LD, Arm JP, Chilton FH. Effect of Dietary Fatty Acids on Inflammatory Gene Expression in Healthy Humans. J Biol Chem 2009;284:15400-7.

295. 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.

296. Skulas-Ray AC, Kris-Etherton PM, Harris WS, Heuvel JPV, Wagner PR, West SG. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. Am J Clin Nutr 2011;93:243-52.

297. de Mello VDF, Erkkila AT, Schwab US, Pulkkinen L, Kolehmainen M, Atalay M, Mussalo H, Lankinen M, Oresic M, Lehto S et al. The effect of fatty or lean fish intake on inflammatory gene expression in peripheral blood mononuclear cells of patients with coronary heart disease. Eur J Nutr 2009;48:447-55.

298. Waehre T, Yndestad A, Smith C, Haug T, Tunheim SH, Gullestad L, Froland SS, Semb AG, Aukrust P, Damas JK. Increased expression of interleukin-1 in coronary

artery disease with downregulatory effects of HMG-CoA reductase inhibitors. Circulation 2004;109:1966-72.

299. Itariu BK, Zeyda M, Hochbrugger EE, Neuhofer A, Prager G, Schindler K, Bohdjalian A, Mascher D, Vangala S, Schranz M et al. Long-chain n-3 PUFAs reduce adipose tissue and systemic inflammation in severely obese nondiabetic patients: a randomized controlled trial. Am J Clin Nutr 2012;96:1137-49.

300. Kratz M, Kuzma JN, Hagman DK, van Yserloo B, Matthys CC, Callahan HS, Weigle DS. n3 PUFAs do not affect adipose tissue inflammation in overweight to moderately obese men and women. J Nutr 2013;143:1340-7.

301. Kabir M, Skurnik G, Naour N, Pechtner V, Meugnier E, Rome S, Quignard-Boulange A, Vidal H, Slama G, Clement K et al. Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. Am J Clin Nutr 2007;86:1670-9.

302. Ding S, Chi MM, Scull BP, Rigby R, Schwerbrock NM, Magness S, Jobin C, Lund PK. High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. PLoS One 2010;5:e12191.

303. Ji Y, Sakata Y, Tso P. Nutrient-induced inflammation in the intestine. Curr Opin Clin Nutr Metab Care 2011;14:315-21.

304. Fujiyama Y, Hokari R, Miura S, Watanabe C, Komoto S, Oyama T, Kurihara C, Nagata H, Hibi T. Butter feeding enhances TNF-alpha production from macrophages and lymphocyte adherence in murine small intestinal microvessels. J Gastroenterol Hepatol 2007;22:1838-45.

305. Yoshida H, Miura S, Kishikawa H, Hirokawa M, Nakamizo H, Nakatsumi RC, Suzuki H, Saito H, Ishii H. Fatty acids enhance GRO/CINC-1 and interleukin-6 production in rat intestinal epithelial cells. J Nutr 2001;131:2943-50.

306. Ding S, Lund PK. Role of intestinal inflammation as an early event in obesity and insulin resistance. Curr Opin Clin Nutr Metab Care 2011;14:328-33.

307. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am J Physiol Gastrointest Liver Physiol 2010;299:G440-8.

308. de Wit NJ, Bosch-Vermeulen H, de Groot PJ, Hooiveld GJ, Bromhaar MM, Jansen J, Muller M, van der Meer R. The role of the small intestine in the development of dietary fat-induced obesity and insulin resistance in C57BL/6J mice. BMC Med Genomics 2008;1:14.

309. Pendyala S, Neff LM, Suarez-Farinas M, Holt PR. Diet-induced weight loss reduces colorectal inflammation: implications for colorectal carcinogenesis. Am J Clin Nutr 2011;93:234-42.

310. van Schothorst EM, Flachs P, Franssen-van Hal NL, Kuda O, Bunschoten A, Molthoff J, Vink C, Hooiveld GJ, Kopecky J, Keijer J. Induction of lipid oxidation by polyunsaturated fatty acids of marine origin in small intestine of mice fed a high-fat diet. BMC Genomics 2009;10:110.

311. Ohtsuka Y, Okada K, Yamakawa Y, Ikuse T, Baba Y, Inage E, Fujii T, Izumi H, Oshida K, Nagata S et al. omega-3 fatty acids attenuate mucosal inflammation in premature rat pups. J Pediatr Surg 2011;46:489-95.

312. Liu Y, Chen F, Odle J, Lin X, Jacobi SK, Zhu H, Wu Z, Hou Y. Fish oil enhances intestinal integrity and inhibits TLR4 and NOD2 signaling pathways in weaned pigs after LPS challenge. J Nutr 2012;142:2017-24.

313. Lu J, Borthwick F, Hassanali Z, Wang Y, Mangat R, Ruth M, Shi D, Jaeschke A, Russell JC, Field CJ et al. Chronic dietary n-3 PUFA intervention improves dyslipidaemia and subsequent cardiovascular complications in the JCR:LA- cp rat model of the metabolic syndrome. Br J Nutr 2011;105:1572-82.

314. Jones PJ, Senanayake VK, Pu S, Jenkins DJ, Connelly PW, Lamarche B, Couture P, Charest A, Baril-Gravel L, West SG et al. DHA-enriched high-oleic acid canola oil improves lipid profile and lowers predicted cardiovascular disease risk in the canola oil multicenter randomized controlled trial. Am J Clin Nutr 2014;100:88-97.

315. Mesko B, Poliska S, Nagy L. Gene expression profiles in peripheral blood for the diagnosis of autoimmune diseases. Trends Mol Med 2011;17:223-33.

316. Min JL, Barrett A, Watts T, Pettersson FH, Lockstone HE, Lindgren CM, Taylor JM, Allen M, Zondervan KT, McCarthy MI. Variability of gene expression profiles in human blood and lymphoblastoid cell lines. BMC Genomics 2010;11:96.

317. Debey S, Schoenbeck U, Hellmich M, Gathof BS, Pillai R, Zander T, Schultze JL. Comparison of different isolation techniques prior gene expression profiling of blood derived cells: impact on physiological responses, on overall expression and the role of different cell types. Pharmacogenomics J 2004;4:193-207.

318. Feezor RJ, Baker HV, Mindrinos M, Hayden D, Tannahill CL, Brownstein BH, Fay A, MacMillan S, Laramie J, Xiao W et al. Whole blood and leukocyte RNA isolation for gene expression analyses. Physiol Genomics 2004;19:247-54.

319. Wright C, Bergstrom D, Dai H, Marton M, Morris M, Tokiwa G, Wang Y, Fare T. Characterization of globin RNA interference in gene expression profiling of whole-blood samples. Clin Chem 2008;54:396-405.

320. Liu J, Walter E, Stenger D, Thach D. Effects of globin mRNA reduction methods on gene expression profiles from whole blood. J Mol Diagn 2006;8:551-8.

321. Vartanian K, Slottke R, Johnstone T, Casale A, Planck SR, Choi D, Smith JR, Rosenbaum JT, Harrington CA. Gene expression profiling of whole blood: comparison of target preparation methods for accurate and reproducible microarray analysis. BMC Genomics 2009;10:2.

322. Veilleux A, Grenier E, Marceau P, Carpentier AC, Richard D, Levy E. Intestinal lipid handling: evidence and implication of insulin signaling abnormalities in human obese subjects. Arterioscler Thromb Vasc Biol 2014;34:644-53.

323. Shen W, Gaskins HR, McIntosh MK. Influence of dietary fat on intestinal microbes, inflammation, barrier function and metabolic outcomes. J Nutr Biochem 2014;25:270-80.

324. Wikby A, Nilsson BO, Forsey R, Thompson J, Strindhall J, Lofgren S, Ernerudh J, Pawelec G, Ferguson F, Johansson B. The immune risk phenotype is associated with IL-6 in the terminal decline stage: findings from the Swedish NONA immune longitudinal study of very late life functioning. Mech Ageing Dev 2006;127:695-704.

ANNEXE A :

La répartition des Autochtones sur le territoire

Les 17 régions administratives du Québec

01 Bas-Saint-Laurent	07 Outaouais	12 Chaudière-Appalaches
02 Saguenay–Lac-Saint-Jean	08 Abitibi-Témiscamingue	13 Laval
03 Capitale-Nationale	09 Côte-Nord	14 Lanaudière
04 Mauricie	10 Nord-du-Québec	15 Laurentides
05 Estrie	11 Gaspésie–Îles-de-la-Madeleine	16 Montérégie
06 Montréal		17 Centre-du-Québec

Carte du Québec illustrant la situation géographique des différents villages autochtones de la province, dont les villages cris (points oranges) et les villages inuits (igloos blancs). ***Source :*** Secrétariat aux affaires autochtones, Ministère du Conseil exécutif, Gouvernement du Québec, 2011. Document reproduit avec la permission du Centre de services partagés du Gouvernement du Québec.

ANNEXE B :

ÉTUDE DE POPULATION DES CONCENTRATIONS ÉLEVÉES DE LA PROTÉINE C-RÉACTIVE CHEZ LES INUITS DU NUNAVIK

Labonté ME, Dewailly E, Chateau-Degat ML, Couture P, Lamarche B.

Population-based study of high plasma C-reactive protein concentrations among the Inuit of Nunavik

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RÉSUMÉ

L'impact de la transition d'un mode de vie traditionnel vers un mode de vie « américanisé » sur la hs-CRP, un biomarqueur pro-inflammatoire, n'a pas été documenté à ce jour dans la population inuite du Nunavik. Dans cette étude transversale, nous avons évalué la prévalence de concentrations élevées de la hs-CRP chez les Inuits du Nunavik (province de Québec) et identifié les facteurs de risque anthropométriques, biochimiques et reliés au mode de vie associés à des concentrations élevées de la hs-CRP. Un échantillon représentatif de la population inuite constitué de 801 résidents des 14 villages côtiers du Nunavik, âgés entre 18 et 74 ans, a été inclus dans les analyses. Les individus ont participé à une session clinique et complété des questionnaires sur leur mode de vie. Des analyses de régression logistique multivariées ont été utilisées pour déterminer les facteurs de risque de présenter des concentrations élevées de la hs-CRP. Des concentrations élevées de la hs-CRP (≥ 2 mg/L) étaient présentes chez 32,7% (IC 95% 29,5-35,8) de la population inuite et étaient davantage prévalentes chez les femmes que chez les hommes (36,7% vs 29,0%, P = 0,007). Les analyses de régression logistique multivariées ont indiqué que chaque augmentation de 1 mmHg de la tension artérielle systolique était associée à une augmentation de 3% de la probabilité de présenter des concentrations de la hs-CRP plus grandes ou égales à 2 mg/L (IC 95% 1,01-1,04). La combinaison d'un âge avancé (≥ 50 vs < 30 ans) et d'un tour de taille élevé (valeurs seuil spécifiques à chaque sexe) dans une analyse de régression logistique multivariée était également associée à une augmentation de 13,3 fois des probabilités de présenter des concentrations de la hs-CRP plus grandes ou égales à 2 mg/L (IC 95% 5,8-30,9). Ces résultats suggèrent que des concentrations élevées de la hs-CRP sont relativement prévalentes chez les Inuits avec des valeurs similaires à celles observées dans la population canadienne caucasienne. Le sexe, l'âge, le tour de taille et la tension artérielle systolique sont les principaux facteurs qui augmentent le risque de présenter ce phénotype inflammatoire chez les Inuits du Nunavik malgré leur mode de vie initialement différent de celui des Caucasiens.

TITLE PAGE

TITLE

Population-based study of high plasma C-reactive protein concentrations among the Inuit of Nunavik

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SHORT TITLE: Prevalence of high CRP among Inuit

KEYWORDS: Nunavik, risk factors, waist circumference, aging, sex, systolic blood pressure, Inuit, C-reactive protein, prevalence

ABSTRACT

Background: The shift away from traditional lifestyle in the Inuit population over the last few decades has been associated with an increased prevalence of coronary heart disease (CHD) risk factors such as obesity, high blood pressure, and diabetes. However, the impact of this transition on the pro-inflammatory marker high-sensitivity C-reactive protein (hs-CRP) has not been documented. **Objectives:** To examine the prevalence of elevated plasma hs-CRP concentrations in Inuit from Nunavik in the province of Quebec (Canada) and identify anthropometric, biochemical and lifestyle risk factors associated with elevated hs-CRP. **Design:** A population-representative sample of 801 Inuit residents from fourteen villages of Nunavik, aged between 18-74 years, were included in the analyses. Subjects participated in a clinical session and completed questionnaires on lifestyle. Multivariate logistic regression was used to determine risk factors for elevated hs-CRP. **Results:** Elevated plasma hs-CRP concentrations (≥2 mg/L) were present in 32.7% (95% confidence interval (CI) 29.5-35.8) of the Inuit adult population and were more prevalent among women than among men (36.7% vs. 29.0%, *P*=0.007). Multivariate logistic regression analysis indicated that every 1 mmHg increase in systolic blood pressure was associated with a 3% increase in the odds of having hs-CRP concentrations ≥2 mg/L in the Inuit population (95% CI 1.01-1.04). The combination of older age (≥50 vs. <30 years) and elevated waist circumference (gender-specific cut-off values) in a multivariate logistic model was also associated with a 13.3-fold increase in the odds of having plasma hs-CRP concentrations ≥2 mg/L (95% CI 5.8-30.9). **Conclusions:** These data indicate that elevated hs-CRP is relatively prevalent among Inuit with values that are similar to those seen in Canadian Caucasian populations. Sex, age, waist circumference, and systolic blood pressure are major factors that increase the risk of this inflammatory phenotype among Inuit from Nunavik, despite its different lifestyle background compared with Caucasians.

INTRODUCTION

Inuit populations have experienced an important transition from a traditional to a modernized lifestyle over the last decades (1). This shift away from the traditional Inuit lifestyle has been associated with an increased prevalence of coronary heart disease (CHD) risk factors such as obesity (2,3), high blood pressure (2), and diabetes (4). High-sensitivity C-reactive protein (hs-CRP), a pro-inflammatory marker that has previously been shown to independently predict CHD outcomes in Caucasian populations (5,6), has recently been positively associated with cardiovascular disease (CVD) prevalence and carotid intima media thickness in the Inuit population of Alaska (7,8). Such data in Canadian Inuit populations have not been described yet.

This study investigated for the first time the prevalence of elevated plasma hs-CRP concentrations in Inuit from Nunavik in Northern Quebec and identified its risk factors, including anthropometric, biochemical and lifestyle characteristics, using unique data from the Nunavik Inuit Health Survey (NIHS) 2004 entitled "Qanuippitaa? -- How are we?" (9).

METHODS

The present cross-sectional study included Inuit residents aged 18 years or more who participated in the extensive NIHS between August 27th and October 1st 2004. Residents of collective dwellings (i.e. hotels, hospitals, jails) and households in which there were no Inuit aged 18 years or more were excluded. The survey used a stratified random sampling of private Inuit households across the fourteen coastal villages of Nunavik, Quebec. The assumption was that recruiting members of households rather than specific individuals would increase coverage of the target population. To obtain a good representation of each community, a proportional allocation of sample units based on households was chosen. Details of the sampling process and other methodological aspects are reported elsewhere (9).

Ethics committees of Laval University and *Institut national de santé publique du Québec* approved the survey. All participants provided written informed consent after watching a video describing the study.

Clinical measures

The NIHS data collection included a 3-h clinical session conducted by research team nurses aboard the Canadian Coast Guard ship (CCGS) Amundsen, which visited each of the fourteen coastal villages of Nunavik. During the session, participants had to answer a clinical questionnaire. They also had a blood test and physical measurements were taken.

Height, body weight, body fat composition and waist circumference measurements were described previously (10). Elevated waist circumference was defined as ≥ 90 cm in men and ≥ 80 cm in women according to the International Diabetes Federation (IDF) classification of central obesity for First Nations (11). Blood pressure (BP) was measured according to the Canadian Coalition for High Blood Pressure technique (12). The presence of the metabolic syndrome (MetS) was determined using the IDF classification in First Nations (11). Diagnosis of type 2 diabetes status was based on self report.

Participants were advised to fast for at least 8 h prior to blood sample collection. Collected blood samples were centrifuged and stored at -80°C onboard the CCGS Amundsen and then sent at the Centre Hospitalier Universitaire de Québec analyses of the cardiometabolic risk factors (2). Plasma hs-CRP concentrations were measured using a commercially available, highly sensitive CRP assay (Behring Latex-Enhanced on the Behring Nephelometer BN-100; Behring Diagnostic, Westwood, Mass) and the calibrators provided by the manufacturer (N Rheumatology Standards SL; Behring Diagnostic). The mean interassay coefficient of variation for plasma hs-CRP concentrations was less than 1% at low and high plasma hs-CRP concentrations, as previously described (13).

Lifestyle and socioeconomic data

Participants completed an individual questionnaire administered in face-to-face interviews. It collected, among other information, data on living habits (e.g. smoking, physical activity) and socioeconomic characteristics (e.g. education level). A confidential questionnaire was self-administered to participants to document more delicate subjects such as alcohol use. Examples of the questionnaires can be viewed in the methodological report of the survey

(9). Classification of participants according to smoking status, drinking habits, physical activity levels and education level is described in Supplemental File 1.

Statistical analyses

Elevated plasma hs-CRP concentrations were defined as ≥ 2.0 mg/L, as suggested by the 2009 Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of CVD in the adult (14). As body mass index (BMI) may overestimate the prevalence of overweight and obesity among the Inuit (15), we used waist circumference and body fat composition (%) to evaluate the impact of adiposity on hs-CRP concentrations.

Statistical analyses were performed using the SAS software (version 9.2; SAS Institute, Cary, NC). Differences in characteristics of men vs. women were assessed using Student's t-tests for continuous variables and the Chi-square test for categorical data. The Chi-square test was also performed to compare the prevalence of elevated plasma hs-CRP concentrations between subgroups of individuals stratified on the basis of sex (men/women), age (< 30, 30-49, ≥ 50 years), waist circumference (low/high), and MetS (yes/no).

Multivariate logistic regression analysis was used to characterize the risk of elevated plasma hs-CRP concentrations according to lifestyle, anthropometric and biochemical variables. Odds ratio (OR) with 95% confidence intervals (CI) were calculated for each variable individually, using a multivariate model that included sex, age, waist circumference, and smoking as covariates. The model also took into account missing values for each of the predictor variables. In order to estimate missing data for continuous variables, multiple imputation (MI procedure in SAS) was performed prior to logistic regression analysis using the Markov Chain Monte Carlo method with a single chain to create five imputations (16). Missing data for categorical variables were adjusted for in the logistic model by including, for each variable, a separate group representing subjects with missing data. Because results from "missing data" subgroups are not interpretable and data assumed to be missing at random, their respective OR are not presented. Percent missing data for each independent variable in the models was 0% (no missing data) for sex and age; 0.4% for low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol

(HDL-C), triacylglycerol (TG), and glucose; 0.9% for insulin; 3.1% for systolic and diastolic BP; 4.0% for waist circumference; 6.5% for smoking; 8.0% for physical activity; 8.2% for education level; and 23.5% for drinking habits. Finally, all variables were analyzed simultaneously in a single multivariate logistic model to determine overall which risk factors remained significant and independent from one another. Two-way interactions among variables were also investigated using specific interaction terms in the multivariate logistic model. Significant interactions are presented graphically using different strata of relevant variables.

All data were analysed by the bootstrap technique in order to account for the complex sampling strategy employed and to correct for related sampling errors (SURVEY procedure in SAS or SUDAAN software (RTI International, NC) when the procedure was unavailable in SAS). All analyses were weighted to achieve population representativeness. Weights were adapted to the non-response rate of each measurement instrument. Since all results were weighted, the number of participants included in analyses is indicated for informational purposes only. Non-normally distributed variables were log-transformed prior to analysis. Statistical significance was set at a *P* value of <0.05.

RESULTS

Figure 1 illustrates the flow of participants throughout the study. Analyses were based on a sample of 801 Inuit adults among 919 recruited participants from whom a blood sample was taken. Twenty participants were excluded from analyses because they were non-Inuit, twenty-six women were excluded because they were pregnant and fourteen subjects were excluded because of missing data on hs-CRP concentrations. Fifty-eight subjects (7.0% of the population, 95% CI 5.3-8.7) were excluded because their hs-CRP concentrations were $\geq$ 10 mg/L, which is indicative of an active acute inflammatory response (17).

Characteristics of the participants, stratified by sex, are shown in Table 1. Compared with women, men were generally characterized by a deteriorated metabolic profile including lower plasma HDL-C and apolipoprotein (apo) AI concentrations as well as higher total-C/HDL-C ratio and blood pressure (all $P<0.0001$). Women had higher plasma interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) concentrations than men (both $P=0.006$).

Estimated proportion of individuals with plasma hs-CRP concentrations ≥ 2.0 mg/L among the Nunavik Inuit population was 32.7% (95% CI 29.5-35.8). The estimated proportion of the population with plasma hs-CRP concentrations > 3.0 mg/L was 21.4% (95% CI 18.7-24.0). As shown in Figure 2, elevated plasma hs-CRP concentrations (≥ 2.0 mg/L) were more prevalent among women than among men (P=0.007). The prevalence of elevated hs-CRP concentrations also increased with age, waist circumference, and presence of MetS (Figure 2).

As shown in Table 2, age and waist circumference were significantly associated with the odds of having plasma hs-CRP concentrations ≥ 2.0 mg/L (all P≤0.003) in a multivariate logistic model that also included sex and smoking as covariates. HDL-C, TG (log transformed), insulin (log transformed), systolic BP, and diastolic BP were also significantly associated with the odds of having plasma hs-CRP concentrations ≥ 2.0 mg/L (all P≤0.008; Table 2) when considered individually with adjustment for sex, age, waist circumference and smoking. When considering all risk factors simultaneously in a single multivariate model, only age, waist circumference and systolic BP remained significantly and independently associated with the odds of having plasma hs-CRP concentrations ≥ 2.0 mg/L (all P≤0.02; not shown). Female sex was also significantly and independently associated with the odds of having plasma hs-CRP concentrations ≥ 2.0 mg/L in this single multivariate model (OR=1.54; 95% CI 1.04-2.28; P=0.03; not shown). Replacing waist circumference with percent body fat in the various multivariate logistic models had no impact on results (not shown). Further adjustment for the use of antihypertensive, diabetic, and lipid lowering medications or excluding subjects on these medications (7.5%, 3.2%, and 5.1% of participants, respectively) also yielded similar results (not shown).

A two-way multiplicative interaction was observed between age and waist circumference on the odds of having elevated plasma hs-CRP concentrations (P interaction=0.04) in a multivariate logistic model that adjusted for HDL-C, TG (log transformed), insulin (log transformed), systolic BP, diastolic BP, sex and smoking. This interaction is presented graphically in Figure 3 using combinations of different strata of age (< 30, 30-49, ≥ 50 years) and waist circumference (low/high based on sex-specific cut-points). The combination of older age (≥ 50 vs. < 30 years) and abdominal obesity (≥ 90 cm in men and

≥ 80 cm in women vs. normal waist) was associated with a 13.3-fold increase in the odds of having high hs-CRP concentrations (95% CI 5.8-30.9, $P<0.0001$) independent of other risk factors associated with high hs-CRP concentrations.

DISCUSSION

To the best of our knowledge, this population-based study was the first to determine the prevalence of elevated plasma hs-CRP concentrations, defined as ≥ 2.0 mg/L, in the Inuit population from Nunavik. Our results showed that elevated hs-CRP is present in nearly one third of the Inuit adult population. We identified sex, age, waist circumference, and systolic BP as the primary risk factors of having plasma hs-CRP concentrations ≥ 2.0 mg/L in this population. In addition to their independent association with elevated hs-CRP concentrations, age and waist circumference appear to exert a synergistic influence on hs-CRP concentrations.

Previous studies have shown that the prevalence of high hs-CRP concentrations in Canadian aboriginal populations is higher than among individuals of European ancestry (18,19). Anand et al. (19) have indeed shown that 54.8% of Aboriginals from the Six Nations Reservation in Ontario, Canada, had elevated hs-CRP concentrations based on a cut-off of 3.0 mg/L compared with 25.0% of Canadians of European origin. Only 21.4% of the Nunavik Inuit population in the present study had hs-CRP concentrations > 3.0 mg/L. The prevalence of elevated hs-CRP concentrations among Inuit adults thus appears to be less than half than in other Canadian aboriginal populations. The prevalence in the Inuit population is actually comparable to the prevalence observed in Caucasians, despite the fact that other CHD risk factors such as obesity and smoking are more prevalent in Inuit vs. Caucasians (10). Our observation is population-based and therefore most likely to represent the true prevalence of elevated hs-CRP in the Nunavik Inuit population. Further investigations are warranted to determine if this relatively low prevalence of high hs-CRP is also seen in other circumpolar populations.

Our data derived from multivariate logistic regression analysis are consistent with previous studies that have identified female sex (18-20), aging (21,22), and obesity indices (23,24) as the most significant and independent predictors of high hs-CRP concentrations. Despite

a traditional lifestyle background, the Inuit population appears to share similar risk factors for elevated hs-CRP concentrations as Caucasian populations. This observation is further supported by similar results gathered in the Canadian Oji-Cree population (18) and in urban Chinese men facing a rapid lifestyle transition (25).

Very few observational studies have reported an association between systolic BP and elevated plasma hs-CRP concentrations. Consistent with our data in Inuit, a study in Australian men and women showed that hypertension (vs. no hypertension) predicted an increased risk of having elevated hs-CRP concentrations (OR=1.6 in men and 1.4 in women for hs-CRP concentrations > 3.0 mg/L) even after adjustment for BMI, diabetes status, total-C, HDL-C, TG, smoking, exercise and, in women, menopausal status and hormone replacement therapy (26). The positive association between systolic BP and hs-CRP may reflect the activation of the renin-angiotensin system (RAS) (27,28). Indeed, one of the main products of the RAS is Angiotensin II (Ang II), a powerful vasoconstrictor that has been shown to induce inflammation in the vascular wall (28) as well as in the adipose tissue (29). Increasing evidence suggests that Ang II stimulates the secretion of pro-inflammatory cytokines such as TNF-α, IL-6, or IL-1β from vascular smooth muscle cells (30,31), immune cells (32), and adipocytes (33) through activation of the transcription factor nuclear factor kappa B (NF-κB) (29,34). IL-6 has been shown to upregulate the messenger RNA (mRNA) transcription of CRP in hepatocytes while IL-1β stimulates CRP mRNA translation (35).

Finally, the combination of different strata of age and waist circumference in a multivariate model showed that Inuit of older age with an elevated waist circumference had a 13-fold increase in their odds of having elevated plasma hs-CRP. Although highly significant, this estimate has a relatively high degree of imprecision and additional studies are therefore needed to confirm that Inuit people portrayed by these characteristics should be targeted in public health interventions aimed at reducing the risk for elevated hs-CRP in the Inuit population. Most importantly, further research is warranted to investigate how elevated hs-CRP concentrations relate to future CHD events especially in Inuit showing these attributes.

Limitations

First, the cross-sectional design of the study precludes identification of causal associations between elevated hs-CRP concentrations and identified risk factors. However, the main correlates of hs-CRP have been determined using a large and representative sample of the Nunavik Inuit population as well as standardized methods for collection of risk factor data. Although our analyses were adjusted for several confounders, other factors such as infectious agents (e.g. helicobacter pylori, zoonoses) may have affected hs-CRP concentrations upward in the Inuit population. However, the association observed between inflammation and the MetS in the Persian Gulf Healthy Heart Study was independent of pathogen burden (36). There is also recent evidence indicating that obesity status remains a strong predictor of elevated hs-CRP concentrations even above 10 mg/L (37).

CONCLUSION

Elevated hs-CRP concentrations are relatively prevalent among the Nunavik Inuit adult population with values that resemble those seen in Canadian Caucasian populations, but that are significantly lower than in other native populations. Well-known risk factors for elevated hs-CRP including female sex, aging, and waist circumference were identified as major correlates of this inflammatory phenotype among Inuit, despite their different lifestyle background compared with Caucasians. The extent to which elevated hs-CRP concentrations predict future CHD events in the Nunavik Inuit population requires further assessment using longitudinal, prospective data.

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CONFLICT OF INTEREST AND FUNDING

The authors have no conflict of interest to declare. This study was funded by the Ministère de la santé et des services sociaux du Québec, the Nunavik Regional Board of Health and Social Services (NRBHSS), the CIHR, the Northern Contaminant Program (Indian and Northern Development), the FRSQ and the ArcticNet network. The director of the NRBHSS participated in the design of the study and the collection of data. The other funding agencies played no role in the design and conduct of the study; the collection, analysis and interpretation of the data; or in the preparation, review, or approval of the manuscript.

REFERENCES

1. Sharma S. Assessing diet and lifestyle in the Canadian Arctic Inuit and Inuvialuit to inform a nutrition and physical activity intervention programme. J Hum Nutr Diet. 2010;23 Suppl 1:5-17.

2. Dewailly E, Chateau-Degat ML, Ekoé JM, Ladouceur R, Rochette L. Status of cardiovascular disease and diabetes in Nunavik. Nunavik Inuit Health Survey 2004, Qanuippitaa? How are we?. Québec, Canada: Gouvernement du Québec – Institut national de santé publique du Québec (INSPQ) & Nunavik Regional Board of Health and Social Services (NRBHSS); 2007.

3. Chateau-Degat ML, Dewailly E, Charbonneau G, Laouan-Sidi EA, Tremblay A, Egeland GM. Obesity risks: towards an emerging Inuit pattern. Int J Circumpolar Health. 2011;70:166-77.

4. Ayach BB, Korda H. Commentary: Type 2 Diabetes Epidemic in First Nations People of Canada. Ethn Dis. 2010;20(3):300-3.

5. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men - Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation. 1999;99(2):237-42.

6. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342(12):836-43.

7. Howard BV, Comuzzie A, Devereux RB, Ebbesson SOE, Fabsitz RR, Howard WJ et al. Cardiovascular disease prevalence and its relation to risk factors in Alaska Eskimos. Nutr Metab Cardiovasc Dis. 2010;20(5):350-8.

8. Cutchins A, Roman MJ, Devereux RB, Ebbesson SOE, Umans JG, Zhu J et al. Prevalence and Correlates of Subclinical Atherosclerosis in Alaska Eskimos The GOCADAN Study. Stroke. 2008;39(11):3079-82.

9. Rochette L, Blanchet C. Methodological Report. Nunavik Inuit Health Survey 2004, Qanuippitaa? How are we?. Québec, Gouvernement du Québec - Institut national de santé publique du Québec (INSPQ) & Nunavik Regional Board of Health and Social Services (NRBHSS); 2007.

10. Chateau-Degat ML, Dewailly E, Louchini R, Counil E, Noël M, Ferland A, et al. Cardiovascular burden and related risk factors among Nunavik (Quebec) Inuit: insights from baseline findings in the circumpolar Inuit health in transition cohort study. Can J Cardiol. 2010;26:190-6.

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

12. Chockalingham A. Canadian coalition for high blood pressure prevention and control: referral and treatment guidelines. St. John, Newfoundland: Faculty of Medecine, Memorial University of Newfoundland; 1988.

13. 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(20):2474-80.

14. Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier A et al. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult-2009 recommendations. Can J Cardiol. 2009;25(10):567-79.

15. Charbonneau-Roberts G, Saudny-Unterberger H, Kuhnlein HV, Egeland GM. Body mass index may overestimate the prevalence of overweight and obesity among the Inuit. Int J Circumpolar Health. 2005;64(2):163-9.

16. SAS Institute Inc. SAS/STAT® Software: Changes and Enhancements, Release 8.2. Chapter 9: The MI Procedure. Cary, NC: SAS Institute Inc; 2001. pp.131-6.

17. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003;107(3):363-9.

18. Connelly PW, Hanley AJ, Harris SB, Hegele RA, Zinman B. Relation of waist circumference and glycemic status to C-reactive protein in the Sandy Lake Oji-Cree. Int J Obes. 2003;27(3):347-54.

19. Anand SS, Razak F, Yi QL, Davis B, Jacobs R, Vuksan V et al. C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. Arterioscler Thromb Vasc Biol. 2004;24(8):1509-15.

20. Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W et al. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol. 2005;46(3):464-9.

21. Bruunsgaard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokines. Curr Opin Hematol. 2001;8(3):131-6.

22. Hegele RA, Ban MR, Young TK. Serum C-reactive protein in Canadian Inuit and its association with genetic variation on chromosome 1q21. Clin Chem. 2001;47(9):1707-9.

23. Neyestani TR, Salekzamani S, Kalayi A, Alavi-Majd H, Houshiarrad A, Nikooyeh B et al. Predictors of Serum Levels of High Sensitivity C-Reactive Protein and Systolic Blood Pressure in Overweight and Obese Nondiabetic Women in Tehran: A Cross-Sectional Study. Metab Syndr Relat Disord. 2011;9(1):41-7.

24. Gentile M, Panico S, Rubba F, Mattiello A, Chiodini P, Jossa F et al. Obesity, overweight, and weight gain over adult life are main determinants of elevated hs-CRP in a cohort of Mediterranean women. Eur J Clin Nutr. 2010;64(8):873-8.

25. Villegas R, Xiang YB, Cai H, Elasy T, Cai Q, Zhang X et al. Lifestyle determinants of c-reactive protein in middle-aged, urban Chinese men. Nutr Metab Cardiovasc Dis. 2012;22(3):223-30.

26. Hung J, Knuiman MW, Divitini ML, Davis T, Beilby JP. Prevalence and risk factor correlates of elevated C-reactive protein in an adult Australian population. Am J Cardiol. 2008;101(2):193-8.

27. Simões E Silva AC, Flynn JT. The renin-angiotensin-aldosterone system in 2011: role in hypertension and chronic kidney disease. Pediatr Nephrol. 2012;27(10):1835-45.

28. Savoia C, Burger D, Nishigaki N, Montezano A, Touyz RM. Angiotensin II and the vascular phenotype in hypertension. Expert Rev Mol Med. 2011;13:e11.

29. Kalupahana NS, Moustaid-Moussa N. The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. Obes Rev. 2012;13(2):136-49.

30. Han Y, Runge MS, Brasier AR. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. Circ Res. 1999;84(6):695-703.

31. Kranzhofer R, Schmidt J, Pfeiffer CAH, Hagl S, Libby P, Kubler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 1999;19(7):1623-9.

32. Hoch NE, Guzik TJ, Chen W, Deans T, Maalouf SA, Gratze P et al. Regulation of T-cell function by endogenously produced angiotensin II. Am J Physiol Regul Integr Comp Physiol. 2009;296(2):R208-16.

33. Yvan-Charvet L, Massiera F, Lamande N, Ailhaud G, Teboul M, Moustaid-Moussa N et al. Deficiency of Angiotensin Type 2 Receptor Rescues Obesity But Not Hypertension Induced by Overexpression of Angiotensinogen in Adipose Tissue. Endocrinology. 2009;150(3):1421-8.

34. Ruiz-Ortega M, Esteban V, Ruperez M, Sanchez-Lopez E, Rodriguez-Vita J, Carvajal G et al. Renal and vascular hypertension-induced inflammation: role of angiotensin II. Curr Opin Nephrol Hypertens. 2006;15(2):159-66.

35. Ganter U, Arcone R, Toniatti C, Morrone G, Ciliberto G. Dual Control of C-Reactive Protein Gene-Expression by Interleukin-1 and Interleukin-6. EMBO J. 1989;8(12):3773-9.

36. Ebrahimi A, Nabipour I, Vahdat K, Jafari SM, Fouladvand M, Assadi M et al. High sensitivity C-reactive protein is associated with the metabolic syndrome independent to viral and bacterial pathogen burden. Diabetes Res Clin Pract. 2009;84(3):296-302.

37. Ishii S, Karlamangla AS, Bote M, Irwin MR, Jacobs DRJ, Cho HJ et al. Gender, obesity and repeated elevation of C-reactive protein: Data from the CARDIA cohort. PLoS One. 2012;7(4):e36062. Epub 2012 Apr 30.

TABLES

Table 1. Characteristics of a random sample of the Nunavik Inuit adult population.

Characteristics	Men[a]	Women[a]	*P*[b]
Age (y)[c]	35.9±0.37	36.7±0.33	0.09
Weight (kg)[c]	73.8±0.83	65.4±0.68	<0.0001
BMI (kg/m^2)[c]	26.8±0.26	27.6±0.27	0.03
Body fat (%)[c]	20.9±0.38	31.6±0.41	<0.0001
Waist circumference (cm)[c]	90.8±0.66	91.3±0.64	0.62
Cholesterol (mmol/L)			
Total-C[c]	4.9±0.05	5.1±0.04	0.0007
LDL-C[c]	2.8±0.04	2.7±0.04	0.17
HDL-C[c]	1.5±0.02	1.8±0.02	<0.0001
Total-C/HDL-C ratio[c,d]	3.5±0.06	3.0±0.04	<0.0001
Triacylglycerol (mmol/L)[c,d]	1.2±0.04	1.2±0.03	0.12
Apolipoproteins			
ApoB100 (g/L)	0.9±0.01	0.9±0.01	0.35
ApoAI (g/L)	1.6±0.01	1.8±0.02	<0.0001
Blood pressure (mmHg)			
Systolic[c]	122.1±0.64	114.9±0.58	<0.0001
Diastolic[c]	75.5±0.49	72.5±0.39	<0.0001
Inflammatory markers			
hs-CRP (mg/L)[d]	1.8±0.09	2.0±0.10	0.38
IL-6 (pg/mL)[d]	2.1±0.09	2.3±0.09	0.006
TNF-α (pg/mL)[d]	2.1±0.10	2.5±0.13	0.006
Insulin (pmol/L)[c,d]	62.7±3.15	67.1±2.61	0.004
Fasting glucose (mmol/L)[c,d]	4.6±0.05	4.6±0.04	0.38
Physical activity (≥ 3.5 h/wk, %)	52.7	39.3	0.0002
Smoking (current, %)	75.9	81.4	0.03
Drinking (≥ 1 drink/day, %)	30.1	23.2	0.03
Education level (≥ high school, %)	23.3	22.6	0.80

Note: Apo = apolipoprotein; BMI = body mass index; C = cholesterol; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; LDL = low density lipoprotein; TNF-α = tumor necrosis factor-α.

[a]n=280-367 for men and n=333-434 for women, depending on the variable. Values are means±SEM unless stated otherwise. All means were weighted to achieve population representativeness. Hence, the number of participants is indicated for informational purposes only.

[b]*P* values based on a Student's t-test except for physical activity, smoking, drinking and education level which were determined using the Chi-square test in SAS.

[c]These data have already been reported in a previous publication (3).

[d]Variables were log transformed prior to analysis, but non transformed data are presented for better interpretability.

Table 2. Adjusted OR for elevated hs-CRP concentrations according to demographic, anthropometric, biochemical, and lifestyle risk factors.

Variables	Adjusted OR hs-CRP ≥ 2.0 mg/L[a]	95% CI	*P*
Sex			
Men	1.00		
Women	1.06	0.78-1.44	0.71
Age (years)			
< 30	1.00		
30-49	1.63	1.18-2.27	0.003
≥ 50	4.93	3.27-7.42	<0.0001
Waist circumference (cm)[b]			
Low	1.00		
High	3.48	2.19-5.53	<0.0001
LDL-C (mmol/L)	0.99	0.82-1.20	0.92
HDL-C (mmol/L)	0.51	0.32-0.81	0.005
Log TG (mmol/L)	3.38	1.59-7.19	0.002
Log fasting glucose (mmol/L)	3.15	0.42-23.61	0.26
Log insulin (pmol/L)	2.20	1.24-3.90	0.007
Systolic BP (mmHg)	1.03	1.01-1.04	0.0001
Diastolic BP (mmHg)	1.02	1.01-1.04	0.008
Physical activity[c]			
< 3.5h/wk	1.00		
≥ 3.5h/wk	0.83	0.61-1.13	0.23
Smoking status[d]			
Non smokers	1.00		
Ex smokers	0.51	0.25-1.03	0.06
Current smokers	0.61	0.34-1.10	0.10
Drinking habits[e]			
Never	1.00		
Light	0.66	0.39-1.11	0.12
Moderate	0.73	0.40-1.32	0.30

Heavy	0.80	0.41-1.59	0.53
Education level[f]			
< High school	1.00		
= High school	1.05	0.68-1.64	0.82
> High school	1.25	0.69-2.28	0.46

Note: BP = blood pressure; C = cholesterol; CI = confidence intervals; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; LDL = low density lipoprotein; OR = odds ratio; TG = triacylglycerol.

[a] OR and 95% CI were determined for each variable individually, using a multivariate logistic regression model in SAS that included sex, age, waist circumference and smoking status as covariates. The model also took into account missing values for each of the predictor variables.

[b]High waist circumference cut-offs were ≥ 90 cm in men and ≥ 80 cm in women.

[c]Physical activity < or ≥ 3.5 h/wk for at least one of the four seasons of the previous year.

[d]"Non smokers" = Inuit not smoking at the time of interview, who also never smoked up to 100 cigarettes in their lifetime; "Ex smokers" = Inuit not smoking at the time of interview, who smoked a total of 100 cigarettes or more in their lifetime; "Current smokers" = occasional and daily smokers.

[e]"Never drinkers" = no alcohol consumed during the previous year; "Light drinkers" = <1 drink/d; "Moderate drinkers" = 1-2 drinks/d; "Heavy drinkers" = >2 drinks/d.

[f]The "= high school" category represents Inuit who completed high school as well as those who undertook a *partial* training in a community college, a trade school, a private commercial college, a technical institute, a CEGEP or a nursing school.

FIGURES

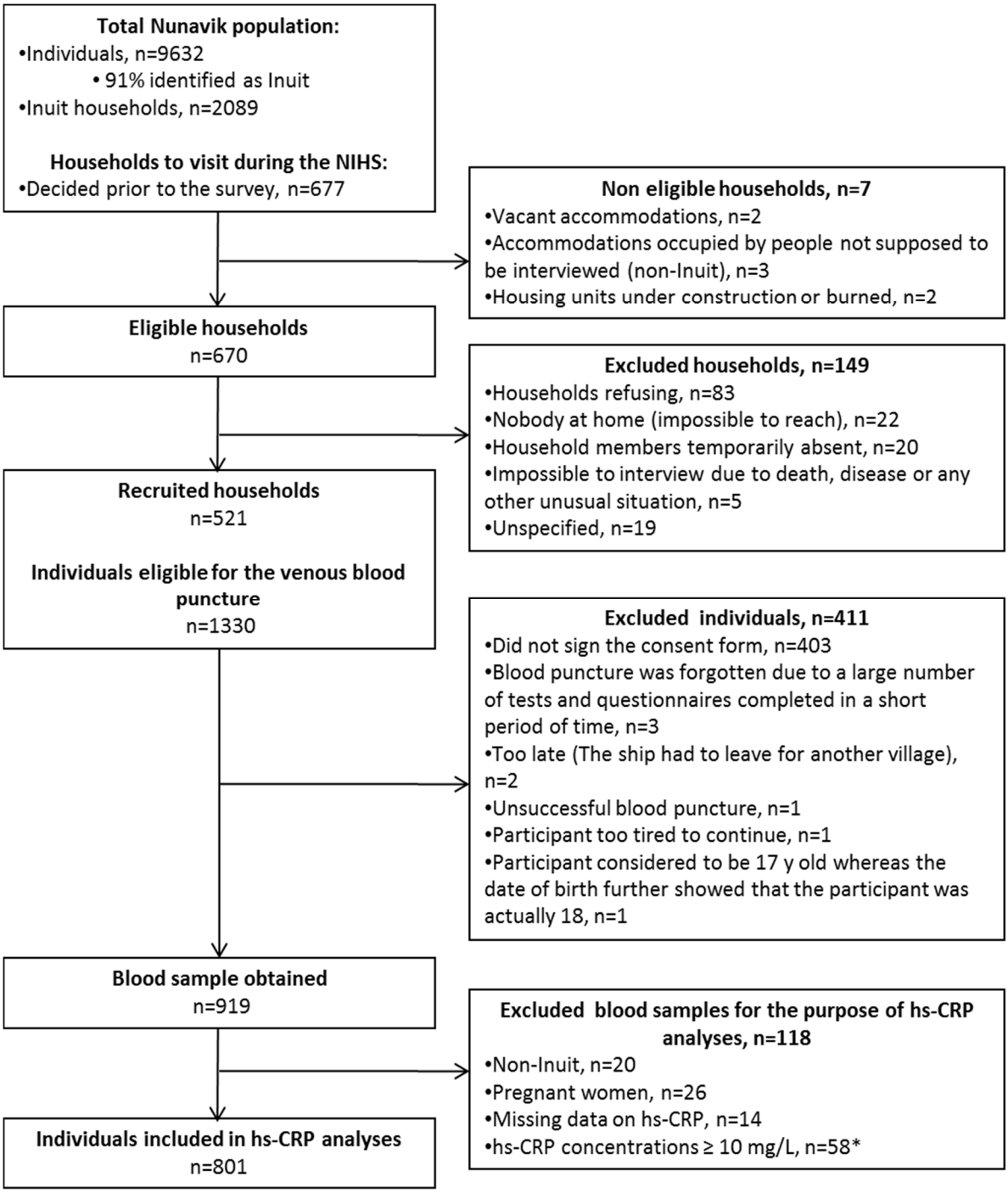

Figure 1. Flow of participants through the Nunavik Inuit Health Survey 2004. *58 subjects were excluded because they had hs-CRP concentrations ≥ 10 mg/L, which is indicative of an acute inflammatory response (17).

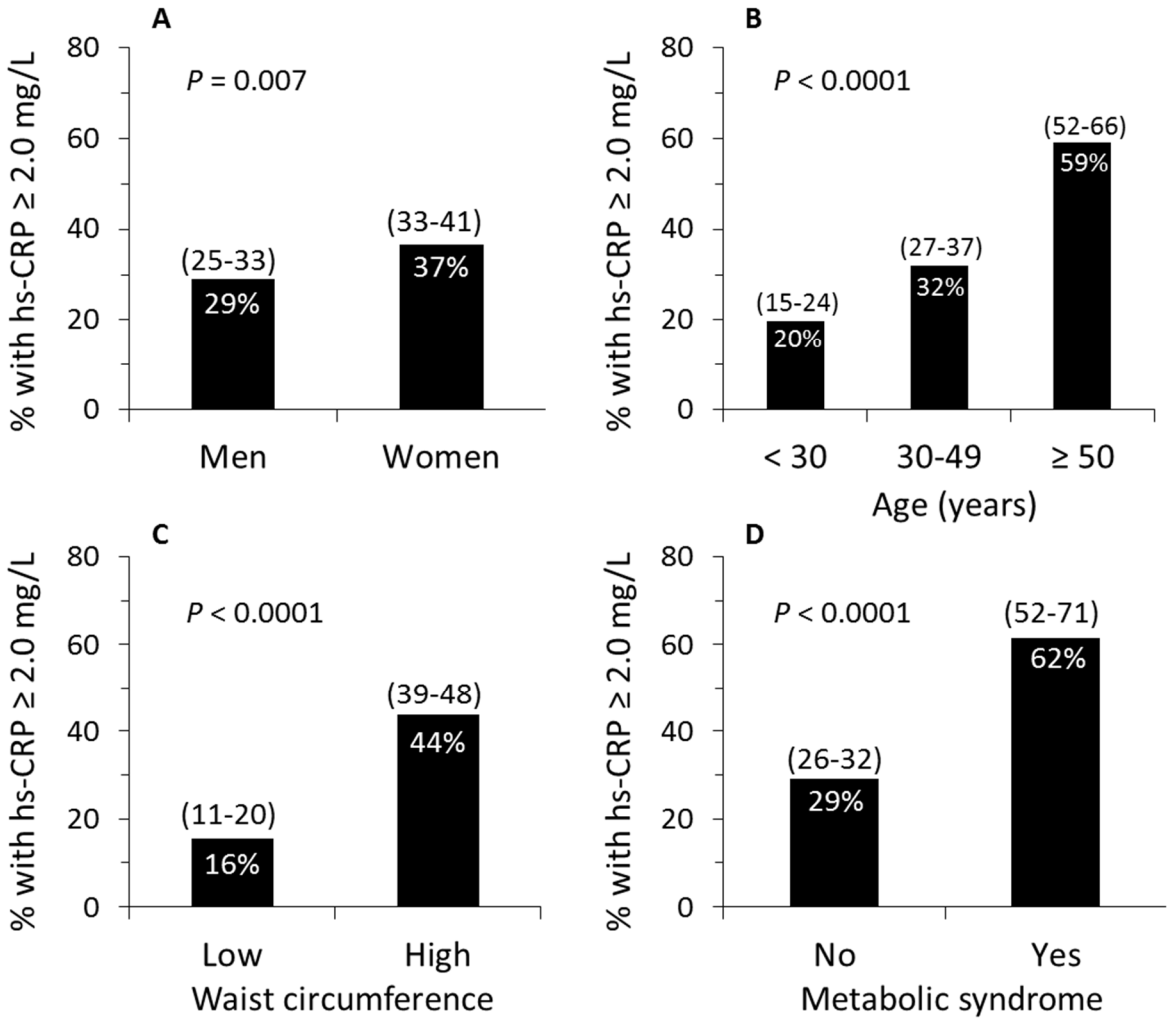

Figure 2. Population prevalence of elevated hs-CRP concentrations among Inuit from Nunavik. Note: hs-CRP = high-sensitivity C-reactive protein; MetS = metabolic syndrome. Population prevalence of elevated hs-CRP concentrations (≥ 2.0 mg/L) is presented according to sex (A), age (B), waist circumference (C) and the presence of MetS (D). *P* values for between-groups differences in frequencies were obtained using the Chi-square test. Values within parentheses are 95% confidence intervals. Prevalence values are unadjusted for other variables. In panels C and D, high waist circumference cut-offs (≥ 90 cm in men and ≥ 80 cm in women) and MetS criteria are those suggested by the International Diabetes Federation (11).

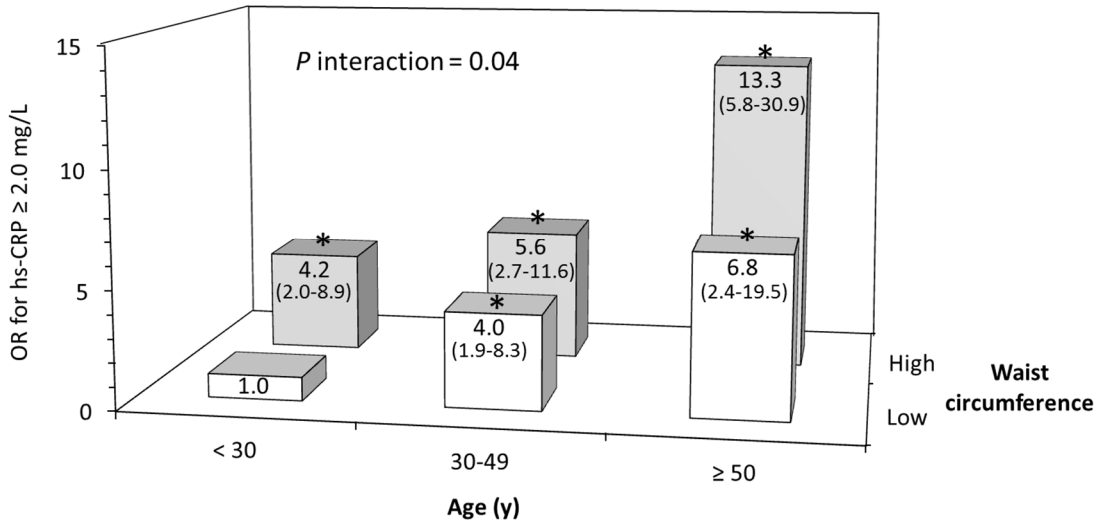

Figure 3. OR for elevated hs-CRP concentrations according to the combined impact of age and waist circumference. Note: HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; OR = odds ratio. A significant age*waist circumference multiplicative interaction (*P* interaction=0.04) was found on the odds of having elevated hs-CRP concentrations (≥ 2.0 mg/L) among the Nunavik Inuit population, using a multivariate logistic regression model. To illustrate the interaction, six groups were created based on the combination of different strata of age (< 30, 30-49, ≥ 50 years) and waist circumference (low/high) and were simultaneously entered into a logistic model, with the combination of age < 30 years and low waist circumference as the reference group (OR=1). OR and 95% confidence intervals (in parentheses) were obtained for each group and are adjusted for HDL-C, triacylglycerol (log transformed), insulin (log transformed), systolic blood pressure, diastolic blood pressure, sex and smoking. Positive associations of female sex and systolic blood pressure with elevated hs-CRP concentrations remained significant in this model ($P \leq 0.01$). High waist circumference cut-offs were ≥ 90 cm in men and ≥ 80 cm in women, as suggested by the International Diabetes Federation (11). *OR significantly higher than the reference group, $P \leq 0.0003$.

SUPPLEMENTARY FILES

Supplemental File 1. Description of the classification of Nunavik Inuit adults according to their smoking status, drinking habits, physical activity levels, and education level.

Smoking status: Participants were asked how often they smoked cigarettes at the time of the interview. Those who were not smoking were also asked if they had smoked (yes or no) a total of 100 cigarettes or more (about 4 packs) in their lifetime. Hence, participants were classified according to their smoking status into one of three categories, namely "non smokers" (not smoking at the time of the interview, including Inuit who never smoked up to 100 cigarettes in their lifetime), "ex smokers" (not smoking at the time of the interview, including Inuit who smoked a total of 100 cigarettes or more in their lifetime) and "current smokers" (occasional and daily smokers).

Drinking habits: Participants were asked if they ever had a drink of alcohol (yes or no), how often they drank alcoholic beverages in the past 12 mo as well as how many drinks they usually had on any given occasion. Participants were then classified according to their daily consumption of alcoholic beverages: "never drinkers" (i.e. Inuit who did not drink alcohol during the previous year), "light drinkers" (< 1 drink/d), "moderate drinkers" (1-2 drinks/d) and "heavy drinkers" (>2 drinks/d). A drink corresponded to one beer, one glass of wine or of liquor.

Physical activity levels: Subjects were asked how often they participated into physical activities such as sport, an outdoor pastime, fitness training, dancing or walking for each of the four seasons of the previous year. If they participated in physical activities once a week or more for at least one season, they were asked how many days a week and how much time on a typical day they engaged in physical activity. Inuit were then classified into two categories according to the number of hours per week spent on physical activity: <3.5 h/wk or ≥3.5 h/wk of physical activity for at least one season during the previous year. Inuit who participated in physical activities less than once a week for *each* season of the previous year were classified in the "<3.5 h/wk" category.

Education level: Participants were asked what the highest level of schooling they had undertaken was. Subjects were consequently classified into three categories using the high school level as a cut-off: “Less than completed high school”, “Completed high school” or “More than completed high school”. The “Completed high school” category also included Inuit with a *partial* training in a community college, a trade school, a private commercial college, a technical institute, a CEGEP or a nursing school.