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Présentée et soutenue publiquement par

Chantal JULIA

Le 15 Octobre 2013

Titre : Aspects épidémiologiques des relations entre Nutrition et Inflammation

Directeur de thèse : Pr. Serge Hercberg

Co-encadrant : Emmanuelle Kesse-Guyot

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Rapporteurs :

Pr. Jean-Pascal de Bandt

Pr. Jacques Delarue

Membres du jury :

Pr. Nathalie Charnaux

Pr. Sébastien Czernichow

Pr. Jean-Claude Melchior

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Résumé

L'inflammation apparaît comme un mécanisme ubiquitaire sous-tendant de nombreuses pathologies chroniques. Les nutriments participent à de multiples niveaux, de façon directe ou indirecte à la régulation de la réaction inflammatoire. Les acides gras polyinsaturés (PUFAs) sont les précurseurs de nombreux médiateurs de l'inflammation. Les nutriments antioxydants (vitamine C, E et caroténoïdes) interviennent dans la prévention et la régulation des réactions oxydantes participant à la réaction inflammatoire et aux dommages cellulaires l'accompagnant. L'approche épidémiologique, notamment au travers des études de cohortes, contribue à une meilleure compréhension des relations entre nutrition et santé dans la population.

L'étude des relations entre statut en antioxydants (concentrations sanguines en vitamine C, E et β-carotène) et CRP augmentée (C-reactive protein) à long terme nous a permis de montrer que le statut en β-carotène était négativement associé à une CRP augmentée.

L'étude des relations entre apports en PUFAs n-3 et n-6 et CRP augmentée nous ont permis de montrer que les apports en PUFAs n-3 et n-6 étaient négativement associés à une CRP augmentée. Les apports en vitamine E étaient modulateurs de cette relation, qui n'apparaissait significative que pour les sujets avec des apports faibles en vitamine E.

Enfin, la construction de profils alimentaires spécifiquement associés aux nutriments connus pour avoir des propriétés anti ou pro-inflammatoires (PUFAs, nutriments antioxydants) nous a permis de montrer qu'un profil alimentaire riche en acides gras essentiels et en nutriments antioxydants était négativement associé à une CRP augmentée à long terme.

Ces résultat concordants montrent bien l'intérêt d'apports adéquats en PUFAs et en nutriments antioxydants dans les mécanismes inflammatoires. Néanmoins, la balance entre ces différents nutriments doit être prise en compte, en particulier du fait d'interactions multiples entre leurs effets.

Mots-clés

Inflammation, Acides gras polyinsaturés, Nutriments antioxydants, Profils alimentaires, Epidémiologie nutritionnelle

Abstract

Inflammation appears as an ubiquitous mechanism involved in numerous chronic diseases, including metabolic diseases, cardiovascular diseases and cancer. Nutrients are involved at multiple levels and in multiple pathways of inflammation regulation. Polyunsaturated fatty acids are precursors for a range of inflammatory mediators: eicosanoids, resolvins and protectins. Antioxidant nutrients (vitamin C, E and β-carotene) prevent and regulate oxidant reactions, preventing in particular lipid peroxidation in cell membranes, which participate in inflammatory reactions and cellular damages. Epidemiological approaches, particularly through cohort studies allow for a better understanding of the relationships between nutrition and health in the population.

The study of the long-term relationships between antioxidant status (circulating concentrations of vitamin C, E and β-carotene) and C reactive protein (CRP) allowed us to show that β-carotene circulating concentrations were negatively associated to elevated CRP.

The study of relationships between dietary intakes of n-3 and n-6 PUFA and elevated CRP allowed us to show a negative association between n-3 PUFA intakes (particularly docosapentaenoic acid DPA) and elevated CRP and between n-6 PUFA intakes (particularly linoleic acid LA) and elevated CRP

The construction of dietary patterns specifically associated to nutrients having pro- or anti-inflammatory properties (PUFA and antioxidant nutrients' intakes) allowed us to show that a dietary pattern rich in essential fatty acids and antioxidant nutrients was negatively associated to elevated CRP in the long-term.

These consistent results corroborate the importance of adequate intakes of PUFA and antioxidant nutrients in inflammatory mechanisms. However, dietary balance between these nutrients needs to be carefully considered, given the multiple interactions existing in the mechanisms they are involved in.

Keywords

Inflammation, Polyunsaturated fatty acids, Antioxidant nutrients, Dietary patterns, Nutritional epidemiology

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INTRODUCTION

Les maladies chroniques telles que le cancer et les maladies cardiovasculaires sont responsables de la majorité des décès dans les pays occidentaux.(World Health Organization, 2013) Les tumeurs représentent la première cause de mortalité en France (29,6 % de l'ensemble des décès de l'année 2008), suivie des maladies cardiovasculaires (27,5 % des décès en 2008).(Aouba *et al.*, 2011) La prévention de ces maladies est donc un enjeu majeur de santé publique, étant donné l'impact considérable de ces maladies au niveau humain, social et économique.

Ces maladies sont reconnues comme étant multifactorielles, impliquant des facteurs génétiques, biologiques et comportementaux. Depuis plusieurs années, la nutrition (recouvrant l'alimentation et l'activité physique) a été reconnue comme pouvant constituer un facteur de risque ou de protection vis-à-vis de nombreuses maladies chroniques. Si dans certains cas les niveaux de preuves sont encore insuffisants pour démontrer l'existence d'un lien entre nutrition et maladie, certaines relations font en revanche l'objet d'un consensus international, en particulier dans le domaine du cancer.(Institut National du Cancer et Réseau National Alimentation Cancer Recherche, 2009, Lichtenstein *et al.*, 2006, World Cancer Research Fund / American Institute for Cancer Research, 2007, World Cancer Research Fund / American Institute for Cancer Research, 2009)

L'approche épidémiologique permet de mettre en évidence dans les populations humaines les relations entre nutrition et maladies chroniques. S'appuyant sur les résultats d'études menées in vitro ou chez l'animal, elle permet d'appuyer ou d'inférer les hypothèses soulevées par ces dernières chez l'homme. Les études épidémiologiques ayant des méthodes ne permettant pas de conclure avec le même degré de preuve, et étant réalisées sur des échantillons de population variés, c'est la convergence des arguments provenant de différentes études qui permet d'affirmer le lien entre nutrition et maladies chroniques.

Il est donc nécessaire de multiplier les études, en utilisant des approches épidémiologiques complémentaires. En particulier, en épidémiologie nutritionnelle, combiner des approches évaluant l'impact des aliments et groupes alimentaires, nutriments, profils alimentaires et

biomarqueurs du statut nutritionnel sont à même de permettre une meilleure compréhension des relations entre nutrition et maladies.

Parmi les phénomènes intervenant dans la genèse des maladies chroniques, l'inflammation est apparue récemment comme étant un mécanisme sous-tendant de nombreuses maladies, dont les maladies cardiovasculaires, les cancers et les maladies métaboliques.(Coussens et Werb, 2002, Hotamisligil, 2006, Libby, 2002)

Les nutriments interviennent à de multiples niveaux dans les processus inflammatoires. Des études in vitro en sur des modèles animaux ont permis d'expliciter certaines voies de signalisation de l'inflammation impliquant les nutriments.

Ce travail de thèse se propose d'étudier, à travers des approches complémentaires de l'épidémiologie nutritionnelle, les relations entre nutrition et inflammation de bas grade.

Une première partie reprenant les éléments biologiques connus de l'inflammation et des relations entre nutrition et inflammation est proposée, afin de présenter le contexte et les hypothèses à l'origine des questions soulevées dans les relations entre nutrition et inflammation.

Puis, les résultats de recherche de ce travail de thèse, sous la forme d'articles scientifiques publiés ou soumis sont présentés.

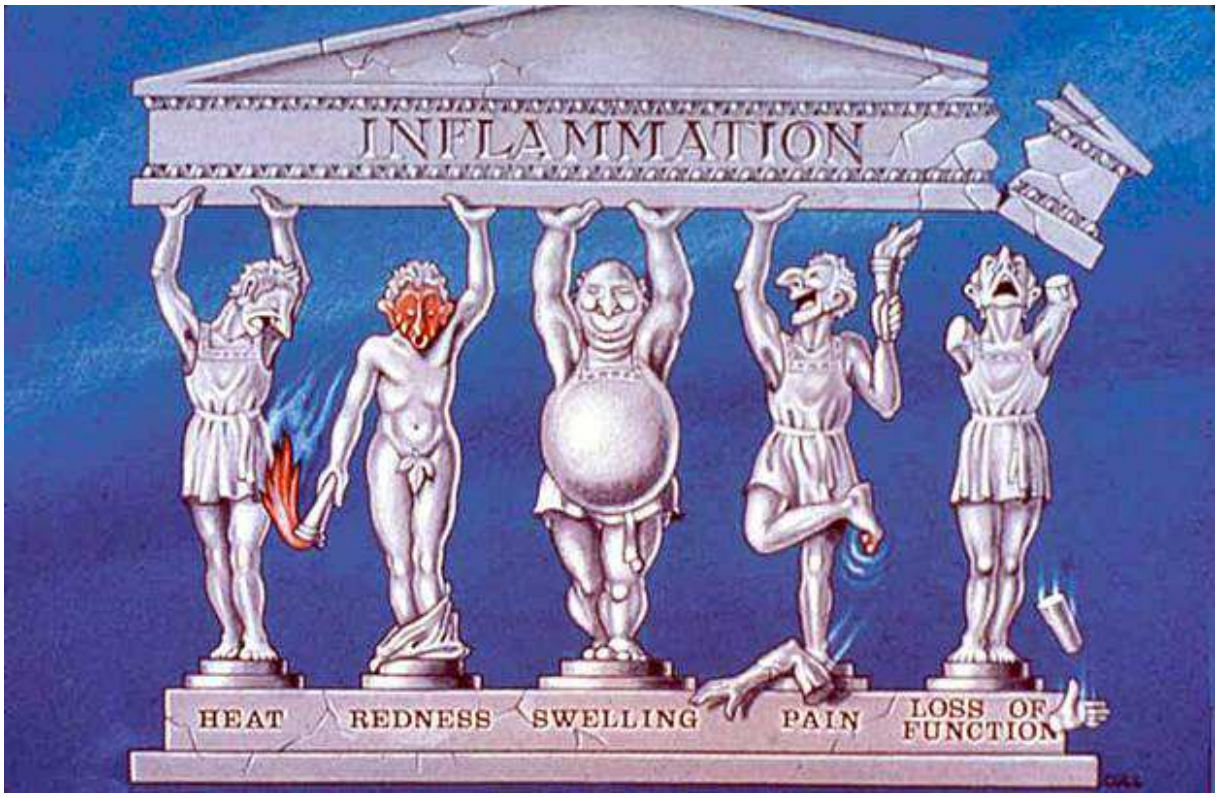
Enfin les éléments de résultats sont discutés et les perspectives de travail ouvertes à partir de ces travaux sont présentées.

ETAT DES CONNAISSANCES

1. Inflammation – Un concept ancien loin d'être élucidé

La première description des signes cardinaux de l'inflammation est attribuée à Celsus, médecin romain du début du premier siècle (env. 25 apr. JC – 50 apr. JC) : *rubor* (rougeur), *calor* (chaleur), *dolor* (douleur), *tumor* (gonflement) (**Figure 1**).(Celsus (appr.25 AC - 50 AC), 1971) La définition de l'inflammation comme une réaction de l'organisme à une agression est, elle, attribuée à Galien, au 3ème siècle apr. JC, sous le terme de *Phlogosis*.(Silva, 1994) La tétrade des signes de l'inflammation a été complétée plus tardivement par un cinquième signe : la perte de fonction (functio laesa). Ce dernier signe a été longtemps attribué à Galien lui-même, mais sa paternité reste controversée.(Rather, 1971)

Figure 1 Illustration des cinq signes cardinaux de l'inflammation (Lawrence *et al.*, 2002)



Une description plus systématique des processus impliqués dans l'inflammation a été initiée au milieu du XIXème siècle, dans la ligne des découvertes anatomo-pathologiques de Claude Bernard.

C'est à cette époque qu'ont été décrits les phénomènes macro- et microscopiques associés à l'inflammation, et que les premières théories sur la physiopathologie de celle-ci ont été formulées. Parmi les scientifiques pathologistes, Virchow (1821-1902) est l'un de ceux qui documente le mieux les phénomènes inflammatoires. Il considère ceux-ci comme une réaction active à une 'irritation externe moléculaire', et les décrit comme la succession d'étapes conduisant progressivement à sa résolution. L'irritation inflammatoire est décrite comme 'la réception d'une plus grande quantité de substances contenues dans le parenchyme particulier

de l'organe, […], dans l'intérieur des cellules et de leurs dérivés ; de cette façon les éléments du tissu s'accroissaient, végétaient pour ainsi dire, et qu'alors il se produisait des altérations plus avancées des éléments, et en même temps la terminaison de cet état.'(Virchow, 1859) Plus généralement, Virchow est l'un des premiers à considérer l'inflammation comme un phénomène ubiquitaire, et à la rattacher à de nombreux états pathologiques, en particulier le cancer ou l'athérosclérose.(Heidland *et al.*, 2006) Par ailleurs, Virchow est le premier à considérer la cellule comme le principal élément des processus physiologiques et pathologiques. Son traité 'Pathologie Cellulaire' de 1859 est reconnu à ce titre comme fondateur de la science moderne.(Silva, 1994)

Les découvertes du XIX^{ème} siècle ont permis d'établir les altérations morphologiques et lésions histologiques relevant de l'inflammation. L'élucidation des processus physiopathologiques complexes impliqués dans l'inflammation est quant à elle loin d'être acquise, et les connaissances relatives à ces processus sont en constante mutation.

2. Inflammation – réponse stéréotypée de l'organisme à une agression

Le processus inflammatoire est une réaction stéréotypée et contrôlée de l'organisme à une agression, que celle-ci soit d'origine infectieuse, traumatique ou auto-immune. La réaction inflammatoire se caractérise par une succession d'étapes, initialement locales, puis mettant en jeu des mécanismes systémiques d'activation, d'entretien et de résolution.

Nous décrirons tout d'abord les étapes histologiques des processus inflammatoires, avant de détailler les processus physiopathologiques et les médiateurs chimiques impliqués.

2.1. Processus histologiques

Le processus inflammatoire implique localement trois phases morphologiques :

- Une phase précoce vasculo-sanguine (ou phase vasculaire)
- Une phase cellulaire avec recrutement et constitution d'un granulome inflammatoire
- Une phase de détersion et de cicatrisation

Phase vasculo-sanguine

Cette phase vasculo-sanguine est celle à l'origine des signes cardinaux précédemment identifiés de l'inflammation : rougeur, tuméfaction, chaleur et douleur.

La phase vasculo-sanguine est constituée de trois phénomènes :

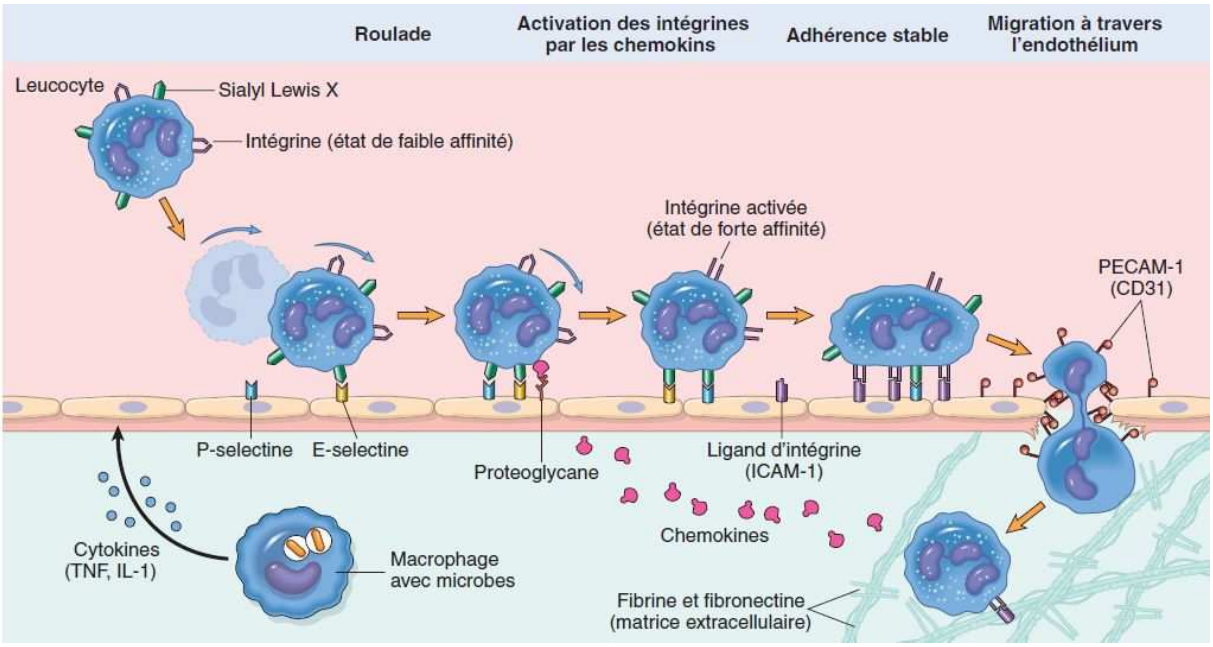
- Une congestion active vasculaire : il s'agit d'une vasodilatation active artériolaire puis capillaire. Les capillaires sont distendus par les hématies et bordées par un endothélium turgescent.

Ce phénomène est responsable de la rougeur et de la chaleur observés cliniquement.

- Un œdème inflammatoire : du fait de la congestion vasculaire, la perméabilité vasculaire augmente. Cela conduit à un exsudat de plasma dans le tissu conjonctif. L'augmentation de la pression sanguine hydrostatique, et la baisse de la pression oncotique de par la fuite protéique aboutit à l'accumulation de protéines plasmatiques dans le liquide interstitiel du tissu conjonctif. L'œdème inflammatoire a plusieurs rôles :
    - Apport local de médiateurs chimiques : immunoglobulines, facteurs de coagulation, éléments du complément
    - Dilution des toxines présentes dans la lésion
    - Limitation du foyer inflammatoire par une barrière de fibrinogène
    - Ralentissement circulatoire par hémoconcentration

  La pression exercée est à l'origine d'une compression des terminaisons nerveuses responsable de la douleur, l'œdème lui-même correspondant à la tuméfaction observée.
- La diapédèse leucocytaire (**Figure 2**) : Elle correspond au passage puis à l'accumulation des leucocytes depuis la microcirculation vers le foyer inflammatoire. Celle-ci est favorisée par l'augmentation de la perméabilité vasculaire d'une part, et par le ralentissement circulatoire d'autre part. Cette traversée active de la paroi vasculaire comporte elle-même plusieurs étapes :
    - La margination des leucocytes à proximité des cellules endothéliales
    - L'adhérence des leucocytes aux cellules endothéliales, mettant en jeu des molécules d'adhésion au niveau de l'endothélium et de la paroi leucocytaire. Au cours de la réaction inflammatoire, on observe une augmentation de l'expression de ces molécules d'adhésion sous l'influence de divers médiateurs.
    - Le passage trans-endothélial des leucocytes. Les leucocytes émettent des pseudopodes qui s'insinuent entre les jonctions intercellulaires des cellules endothéliales puis traversent la membrane basale grâce à une dépolymérisation transitoire provoquée par leurs enzymes. La transmigration des leucocytes est aussi possible grâce à la fragmentation de leur noyau en masses chromatiniennes de faible diamètre.

Figure 2 Diapédèse leucocytaire illustrée ici pour les polynucléaires neutrophiles (Collège Français des Pathologistes (CoPath), 2012)



Au terme de ces étapes, le tissu conjonctif contient un liquide plus ou moins riche en éléments vasculaires, constituant l'exsudat inflammatoire.

Phase cellulaire

Le tissu conjonctif site du foyer lésionnel s'enrichit progressivement en éléments cellulaire constituant le *granulome inflammatoire*. Celui-ci est constitué d'éléments du tissu local (fibroblastes, mastocytes et macrophages résidents) et de cellules issues de la vascularisation (polynucléaires, monocytes et lymphocytes) par diapédèse. La diapédèse leucocytaire concerne dans les premières heures de la réaction inflammatoire essentiellement les polynucléaires (dans les 6 à 24h), puis les monocytes et les lymphocytes (entre 24 et 48h). Ces éléments leucocytaires migrent progressivement vers le foyer lésionnel par chimiotactisme.

Le granulome inflammatoire est le siège d'une multiplication et d'une différentiation cellulaire intense :

- Les cellules phagocytaires augmentent en nombre : les polynucléaires sont recrutés en permanence, les macrophages se multiplient, les monocytes sont activés et se différentient en macrophages.
- Les lymphocytes B sont transformés en plasmocytes, capables de secréter des immunoglobulines spécifiques. Les lymphocytes T sont activés et fonctionnent en coopération avec les lymphocytes B.
- Les fibroblastes sont transformés en myofibroblastes.

La constitution du granulome est dynamique, et se modifie dans le temps, en fonction de l'avancement de la réaction inflammatoire et de la cause de celle-ci.

Le granulome a pour rôles principaux :

- La détersion des débris lésionnels par les macrophages et les polynucléaires. La détersion permet l'élimination progressive des débris tissulaires nécrotiques, des agents pathogènes et de l'exsudat inflammatoire. Celle-ci est effectuée principalement par phagocytose, l'œdème liquidien étant quant à lui drainé vers la circulation lymphatique. Dans certains cas, cette détersion interne peut être complétée par un processus de détersion externe, via la fistulisation à la peau ou dans un conduit naturel des produits de dégradation.
- Le développement d'une réaction immunitaire adaptés par les lymphocytes B et/ou T.
- La sécrétion de médiateurs inflammatoires (cytokines, chimiokines et eicosanoïdes) intervenant dans le recrutement cellulaire, la phagocytose, la défense immunitaire, et la modification de la matrice conjonctive.

Il peut arriver que la phase cellulaire ne conduise pas ensuite à une phase de réparation. L'inflammation dans ce cas se chronicise, conduisant à une dégradation tissulaire progressive.

Réparation et cicatrisation

La réparation tissulaire conduit à une cicatrice lorsque le tissu lésé ne peut se régénérer ou lorsque la destruction tissulaire a été trop importante ou prolongée. La réparation tissulaire comprend les étapes suivantes :

- Bourgeon charnu : constitution d'un nouveau tissu conjonctif, contenant principalement des leucocytes, des fibroblastes et myofibroblastes, ainsi que des néo-vaisseaux sanguins.
- Cicatrice : constituée principalement de tissu conjonctif fibreux
- Renouvellement épithélial : reconstitution de l'épithélium initial par prolifération de cellules saines à la périphérie du foyer lésionnel

2.2. Processus cellulaires et médiateurs circulants

Les étapes de la réaction inflammatoire sont déclenchées, régulées et contrôlées par des médiateurs chimiques re-largués initialement au niveau local par les leucocytes activés au niveau du foyer inflammatoire. L'ensemble des médiateurs se répondant dans ce système complexe et leurs interactions n'ont pas été entièrement élucidés, et, pour beaucoup, dépendent du site initial de l'inflammation, de sa cause, des types de cellules immunitaires impliqués et du stade relatif de la réaction inflammatoire.

Principales familles de médiateurs de l'inflammation

Les multiples médiateurs de l'inflammation intervenant dans les différentes étapes de la réaction inflammatoire appartiennent à des familles de molécules complexes, pour lesquelles l'état des connaissances est en constante évolution et pour lesquelles une classification fonctionnelle n'est pas toujours disponible ou pertinente. Les familles reconnues impliquées dans l'inflammation sont principalement les suivantes :

1. Amines vasoactives

Molécules issues des granulations des cellules immunitaires, libérées par exocytose lors de l'activation des cellules (mastocytes principalement) ayant des propriétés vasoactives favorisant la perméabilité vasculaire. Impliquées dans la phase initiale vasculo-sanguine de l'inflammation, elles regroupent principalement l'histamine et la sérotonine.

2. Eicosanoïdes

Les eicosanoïdes sont une famille de médiateurs de l'inflammation synthétisés à partir des lipides. Les familles d'eicosanoïdes et leurs principales fonctions sont décrites plus bas dans ce document (voir paragraphe Eicosanoïdes, page 52)

3. Cytokines

Les cytokines ont été initialement définies comme des molécules solubles de signalisation cellulaire synthétisées par les cellules immunitaires. Cette définition initiale a été élargie par la découverte progressive que les cellules immunitaires n'étaient pas les seules cellules produisant ces médiateurs.

L'activité des cytokines passe par leur liaison avec un récepteur spécifique, pouvant avoir une activité autocrine (sur la cellule elle-même), paracrine (sur les cellules environnantes) ou endocrine (sur des cellules à distance, *via* un passage sanguin).

Les cytokines interviennent dans de nombreux processus cellulaires, l'inflammation, mais aussi la réponse immunitaire innée et adaptative, la croissance cellulaire et l'hématopoïèse. Les grandes sous-familles de cytokines sont décrites dans le Tableau 1.

Tableau 1 Principales sous-familles de cytokines

Famille	Molécules	Fonctions
Interférons	IFNα, IFNβ, **IFNγ**	Stimulation de la réaction inflammatoire
Interleukines	**IL1** – IL35	Stimulation de la réaction inflammatoire Prolifération des cellules immunitaires
Tumor necrosis factor	**TNFα**, TNFβ	Stimulation de la réaction inflammatoire
Facteurs de croissance de transformation	TGFα, TGFβ	Croissance Différenciation
Facteurs de croissance hématopoïétiques	CSF (colony stimulating factor)	Croissance
Chémokines	CCL, CCXL	Chimiotactisme

Les molécules notées en gras sont celles intervenant plus spécifiquement dans la réaction inflammatoire aiguë.

4. Protéines de la phase aiguë

Les réactifs de la phase aigüe sont des protéines produites au niveau du foie, dont la synthèse est activée par une activité endocrine des cytokines pro-inflammatoires produites au niveau du site inflammatoire. Elles sont définies par le fait que leur concentration plasmatique durant la réaction inflammatoire augmente ou diminue de plus de 25%.

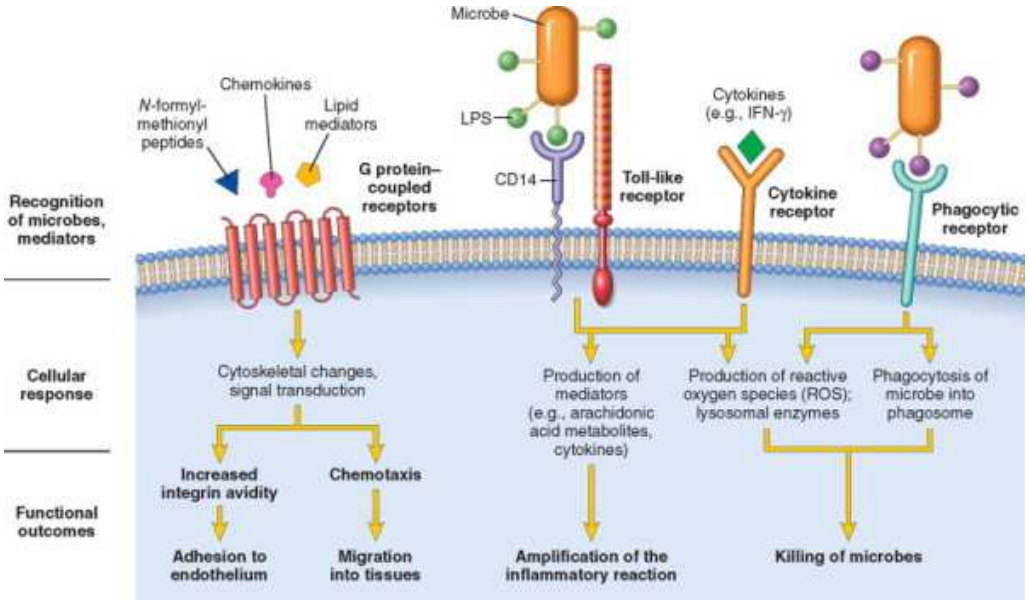
Les principales molécules chez l'homme sont la C reactive protein (CRP), l'orosomucoide, l'haptoglobine (**Figure 7**, Page 22)

Reconnaissance

La reconnaissance des pathogènes, qu'ils soient d'origine endogènes (cellules tumorales) ou exogènes (microbiens) implique initialement le système immunitaire dit inné (Delves et Roitt, 2000), c'est-à-dire les défenses immunitaires ne comprenant pas d'éléments de mémoire : macrophages tissulaires, cellules dendritiques et mastocytes principalement.

Les cellules immunitaires possèdent à leur surface plusieurs types de récepteurs permettant la reconnaissance des éléments peptidiques des pathogènes, et provoquant différentes réaction conduisant à l'activation du leucocyte et à la dégradation du pathogène. (**Figure 3**)

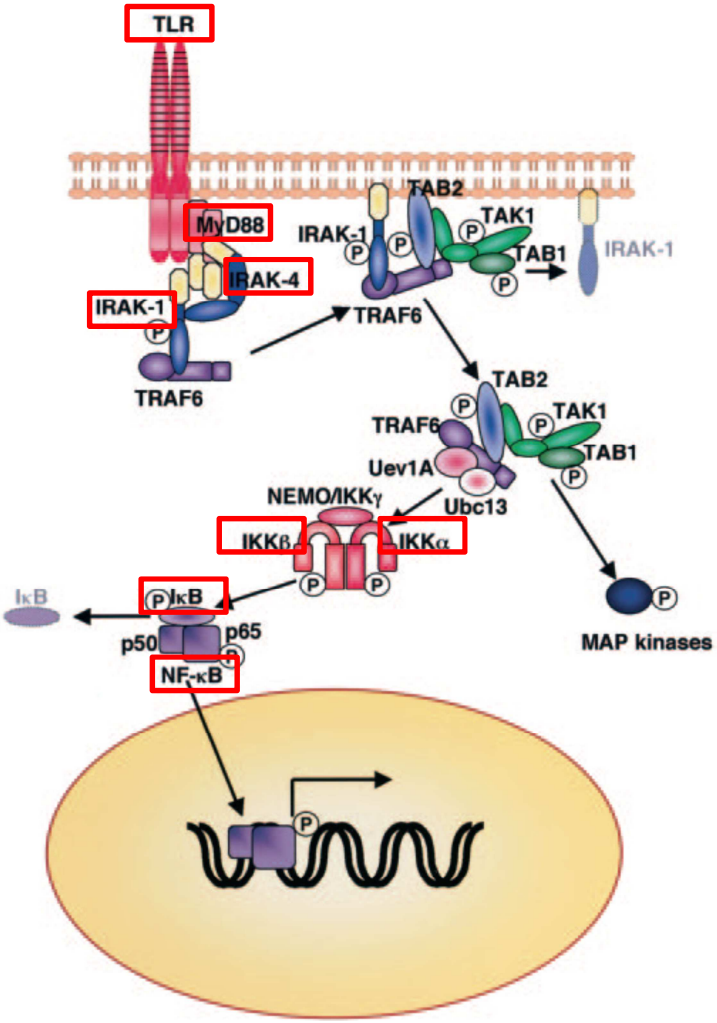
Figure 3 Types de récepteurs présents à la surface des leucocytes, permettant la reconnaissance des agents pathogènes.(Kumar *et al.*, 2013)



La reconnaissance de motifs peptidiques particuliers présentés par les agents pathogènes microbiens (les *Pathogens associated molecular patterns*) implique une famille de récepteurs, les *Toll-like Receptors* (TLR). Initialement identifiés dans les mécanismes de reconnaissance anti-infectieux, les TLR ont aussi été mis en évidence dans les lésions d'athérosclérose (Bjorkbacka, 2006) et dans l'obésité (Kim *et al.*, 2007). Ces récepteurs présentent une homologie intracellulaire avec les récepteurs à l'IL-1. Une fois que la molécule pathogène est liée au TLR, la transduction du signal conduit à l'activation de la cellule immunitaire et à la production par celle-ci de chémokines, cytokines, médiateurs inflammatoires et espèces réactives de l'oxygène et de l'azote.(Akira, 2003, Cohen, 2002, De Bandt, 2007)

La transduction du signal s'opère par l'association de plusieurs molécules, une fois que la reconnaissance initiale entre le pathogène et le TLR est effectuée. L'activation du TLR conduit au recrutement d'une protéine adaptatrice (la myeloid differentiation protein 88 (MyD88) ou la TIR domain-containing adapter protein (TIRAP)) qui active les kinases dites associées au récepteur de l'IL1 (IRAK). Ces dernières vont s'associer à d'autres complexes protéiques pour finalement activer des protéines kinases, dont la protéine kinase IκB (IKK). Le complexe IκB maintient au niveau cytoplasmique le facteur de transcription Nuclear Factor κB (NFκB) dans une forme inactive. L'activation de la protéine kinase IκB conduit au clivage du complexe protéique et à l'activation du facteur de NFκB. ainsi qu'à sa migration au niveau du noyau. NFκB induit par la suite la transcription de nombreux médiateurs de l'inflammation, dont les cytokines IL1 et IL6.(**Figure 4**)

Figure 4 Transduction du signal issu de la reconnaissance par les TLR (Akira, 2003)

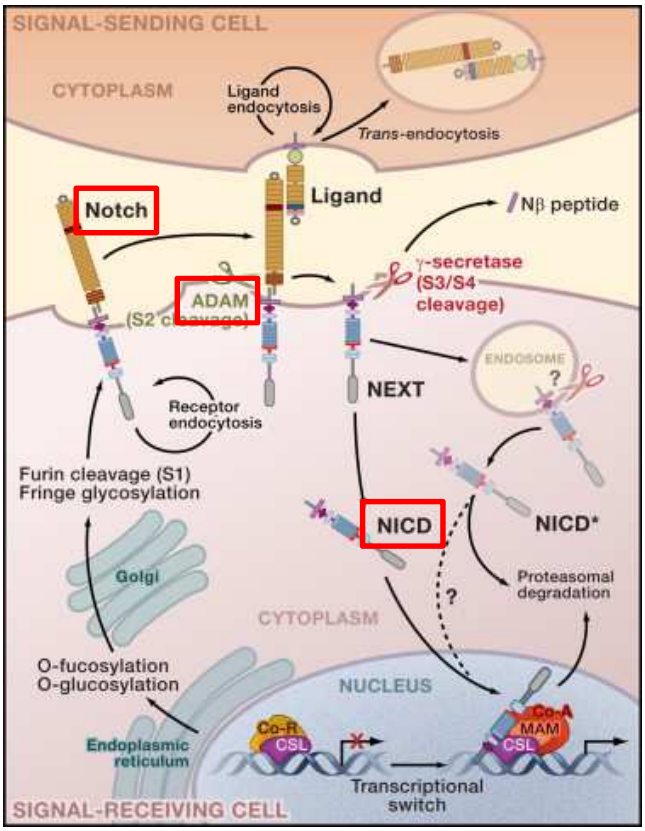


L'activation du TLR conduit au recrutement des protéines adaptatrices MyD88 et IRAK. Ce complexe protéique active à son tour une série d'autres complexes, conduisant enfin à l'activation de la protéine kinase IκB (IKK), clivant le complexe IκB et activant le facteur de transcription NFκB. Celui-ci migre au niveau nucléaire pour induire la transcription de nombreux facteurs pro-inflammatoires.

En dehors des récepteurs TLR, la transduction du signal inflammatoire dans les macrophages activés peut aussi être effectuée par la voie des récepteurs Notch. La voie de signalisation Notch est une voie juxtacrine nécessitant un contact direct entre cellules et déterminant des fonctions cellulaires telles que la croissance, la différentiation et la survie dans de nombreux types cellulaires et de nombreux tissus de l'organisme.(Quillard et Charreau, 2013) Les récepteurs et ligands Notch sont des protéines transmembranaires. Quatre récepteurs Notch (Notch 1-4) et cinq ligands spécifiques Notch (Jag1, Jag2, Dll1-3) ont été identifiés chez les mammifères. En dehors de ces ligands spécifiques, d'autres ligands ont démontré pouvoir activer la voie de signalisation Notch. Le domaine extracellulaire du récepteur Notch lui confère sa propriété de reconnaissance du ligand, et la partie intracellulaire intervient dans la cascade de transduction du signal. L'interaction entre le ligand et le récepteur conduit à une succession de clivages

protéolytiques aboutissant finalement à l'activation d'un facteur de transcription spécifique (CSL) conduisant à la transcription de gènes cibles. (**Figure 5**)

Figure 5 Voie de signalisation Notch (Kopan et Ilagan, 2009)



L'interaction entre le ligand et le récepteur Notch expose mécaniquement le site de clivage ADAM, qui est clivé par une désintégrine et une métalloprotéinase en un complexe NICD. Celui-ci est clivé par un complexe γ-secrétase, ce qui conduit à sa translocation au niveau nuvléaire. NICD se conjugue alors avec le facteur de transcription CSL, ce qui conduit à l'expression des gènes cibles

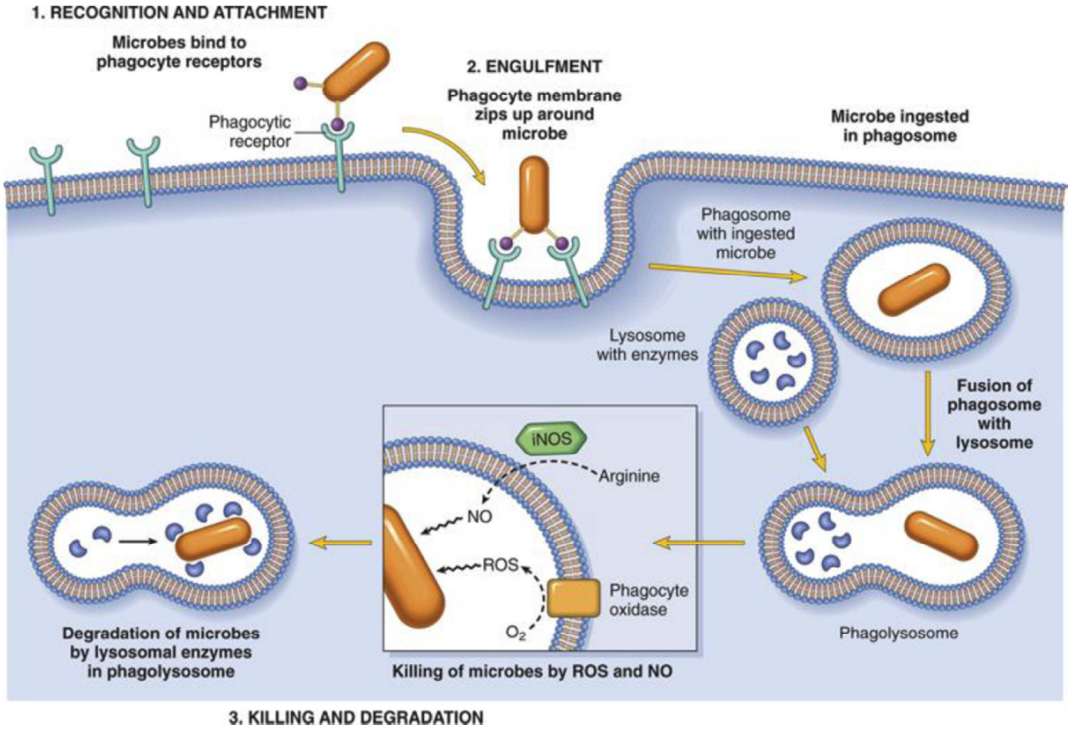
Récemment, les ligands de la famille Notch ont été mis en évidence dans les relations entre macrophages. Il a été montré que la présence des récepteurs Notch augmentait lors de l'activation des macrophages et que cette voie de signalisation pouvait conduire à la production de facteurs pro-inflammatoires.(Fung *et al.*, 2007)

Phagocytose

Parallèlement à la transduction du signal à la suite de la reconnaissance par les récepteurs, l'association entre un ligand et certains récepteurs solubles appelés opsonines (en particulier molécule du complément C3 et immunoglubulines G) permet une reconnaissance et une phagocytose par les cellules immunitaires de l'ensemble du pathogène. (Stuart et Ezekowitz, 2008) Cette liaison entre récepteur et ligand initie la phagocytose par l'internalisation de l'élément en question dans des vacuoles d'endocytose (**Figure 6**). Celles-ci fusionnent au sein de la cellule avec des lysosomes pour former le phagolysosome. Au sein de celui-ci, l'action d'enzymes et la libération de molécules chimiques toxiques vont permettre la dégradation de la

particule internalisée : anion peroxyde, radicaux hydroxyles (radicaux libres oxydants), acide hypochloreux, oxyde nitrique, protéines et peptides cationiques antimicrobiens et lysozyme.

Figure 6 Processus de phagocytose. (Kumar et al., 2013)



Recrutement - Intensification

Ces étapes initiales conduisent à la production de médiateurs circulants pro-inflammatoires, *via* de multiples mécanismes (voir aussi **Figure 3**) (Nathan, 2002) :

1. La reconnaissance des pathogènes via les récepteurs TLR et les récepteurs Notch conduisent à la transcription de gènes cibles aboutissant à la production de médiateurs inflammatoires libérés au niveau extracellulaire, puis dans la circulation générale (TNFα, IL1, eicosanoïdes).
2. Le système nerveux autonome participe par des mécanismes directs et indirects à la régulation de la réponse inflammatoire, décrivant un système comparable à un arc réflexe. (Tracey, 2002) Dans un système conduisant majoritairement à une inhibition de l'inflammation, certains mécanismes en revanche participent à l'intensification de la réponse inflammatoire. Il a été constaté par exemple que les neurones pouvaient libérer des peptides bioactifs en réponse à la douleur (Steinhoff *et al.*, 2000, Tracey, 2002). Les cellules du système nerveux central sont capables de synthétiser les cytokines inflammatoires tels que le Tumor Necrosis Factor α (TNFα) et l'interleukine 1 (IL1), qui peuvent participer directement dans la communication neuronale. Ces cytokines sont capables d'activer la production de glucocorticoïdes. (Tracey, 2002)

3. Les débris cellulaires provenant des cellules lysées ou apoptotiques libèrent dans l'espace extracellulaire des protéines constitutionnellement intracellulaires qui activent la production de cytokines. Il s'agit des protéines de choc thermique (heat shock protein), de facteurs de transcription et de peptides mitochondriaux.
4. La reconnaissance d'éléments pathogènes par les fragments du complément (agissant comme récepteurs au niveau de la membrane des macrophages, voir le paragraphe relatif à la phagocytose) provoquent leur clivage depuis des formes inactives en des fragments biologiquement actifs.

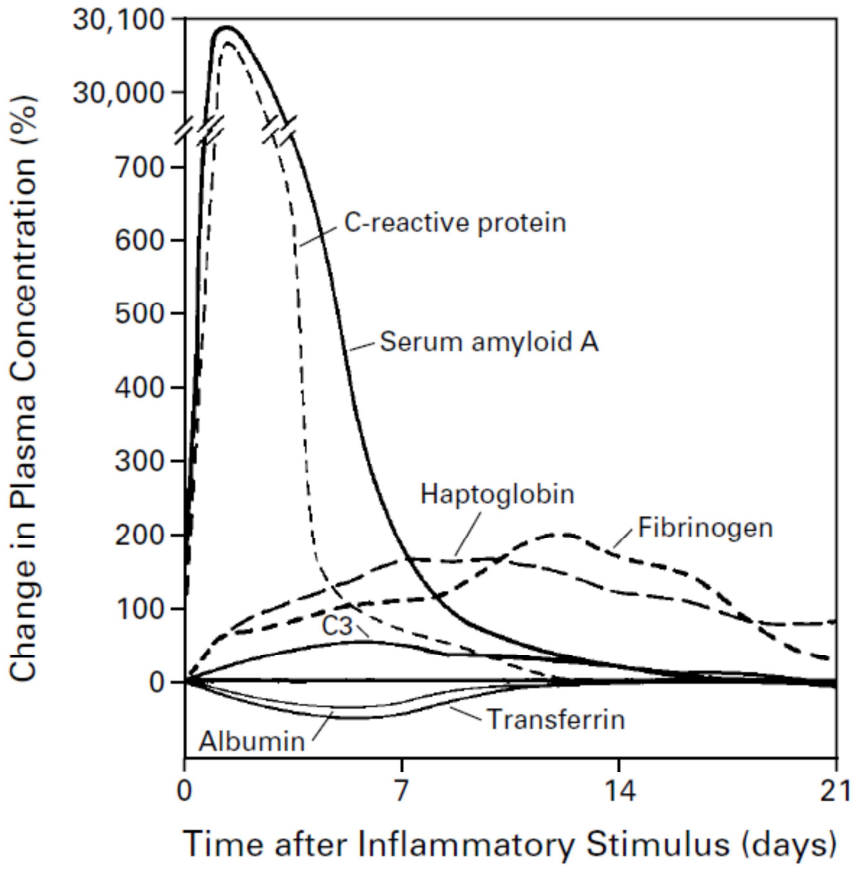
Ce relargage au niveau extracellulaire local de médiateurs pro-inflammatoires provoque une amplification du processus inflammatoire et l'implication d'acteurs issus de la circulation générale :

- Les fragments du complément solubles activés (C3a, C4a et C5a en particulier) provoquent la dégranulation des mastocytes périvasculaires, libérant de l'histamine, des eicosanoïdes, des tryptases, cytokines et chimiokines. L'histamine est un puissant vasoconstricteur, provoquant la contraction des cellules musculaires lisses et augmentant la perméabilité vasculaire.
- Les médiateurs libérés par les cellules immunitaires localement provoquent une régulation positive de molécules d'adhésion au sein des cellules vasculaires, les sélectines (Delves et al., 2000). La L-sélectine, à la surface des cellules vasculaires circulantes reconnaît les structures de Sialyl-Lewis à la surface des cellules immunitaires. Cette reconnaissance provoque une modification des protéines présentes à la surface des polynucléaires : la L-sélectine est rapidement éliminée de la surface cellulaire, remplacée par d'autres molécules d'adhésion, comme la E-sélectine ou les intégrines (dont ICAM). La liaison entre ces molécules assure la diapédèse leucocytaire expliquée plus haut dans le recrutement des cellules immunitaires circulantes (**Figure 2**).
- La libération de cytokines pro-inflammatoires (principalement l'IL6, le TNFα, l'IL1β et l'interféron γ) produites par les cellules immunitaires active la régulation de l'expression génétique d'autres médiateurs inflammatoires (Gabay et Kushner, 1999), amplifiant ainsi la réponse inflammatoire. Ces réactions ont lieu tant au niveau local qu'à distance si ces cytokines entrent dans la circulation systémique. L'IL6, en particulier, active au niveau hépatique la production de CRP

La production de ces médiateurs inflammatoires par les cellules inflammatoires dépend principalement de l'activation de facteurs de transcriptions spécifiques, dont les facteur de transcription-clés sont ceux de la famille du NFκB (Li et Verma, 2002).

Durant cette phase d'intensification, les concentrations de certains médiateurs augmentent au niveau sanguin (protéines de la phase aiguë). Cette augmentation systémique est normalement transitoire, le temps nécessaire à l'élimination du pathogène (**Figure 7**).

Figure 7 Modification des concentrations plasmatiques de certaines protéines de la phase aiguë de l'inflammation après un stimulus inflammatoire d'intensité modérée.(Gabay et al., 1999)



Par ailleurs, de par leur fonction de cellules présentatrices d'antigènes, les cellules phagocytaires permettent une activation complémentaire du système immunitaire adaptatif (lymphocytes B et T). Les étapes de leur activation et de leur régulation ne seront pas détaillées, car dépassant le cadre de ce travail.

Résolution

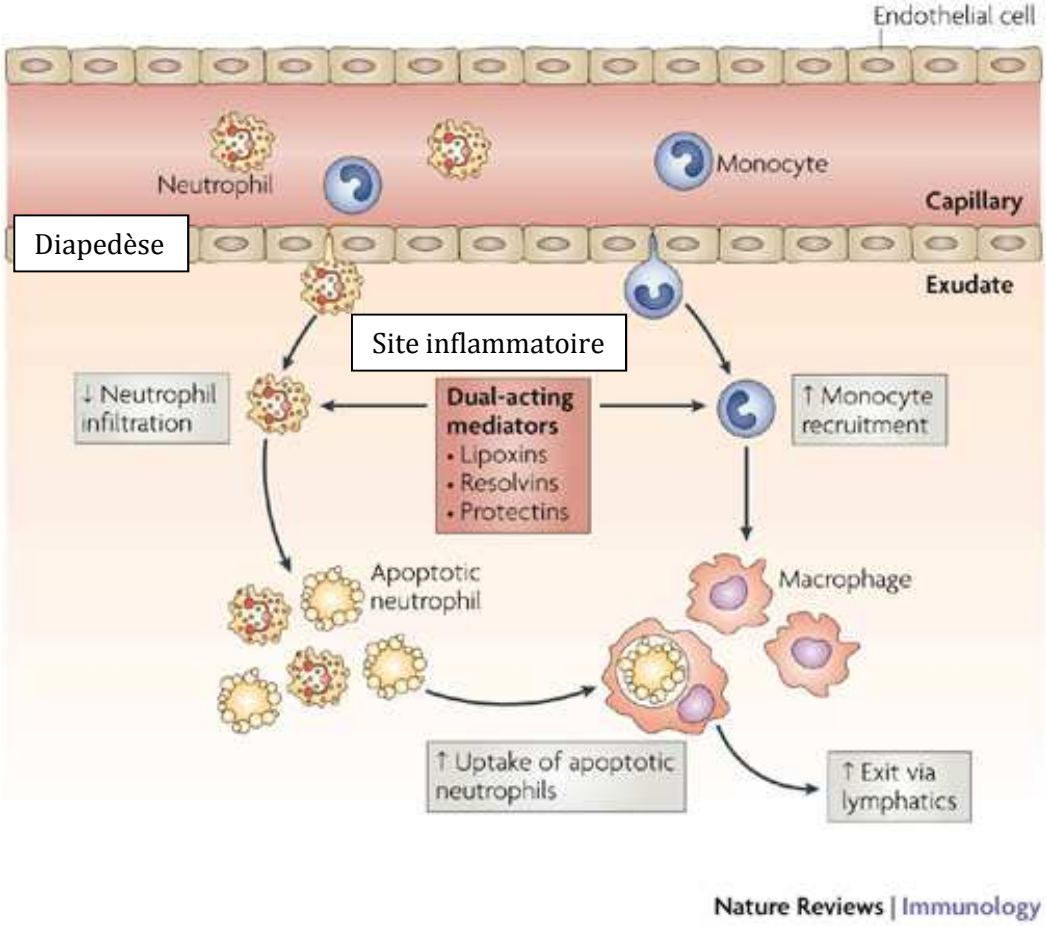
Le processus de résolution agit comme un programme actif et coordonné conduisant à la production de médiateurs intracellulaires spécialisés, à la mort par apoptose des macrophages activés et nettoyage progressif des cellules mortes par les phagocytes (via le recrutement de monocytes dits 'non-phlogistiques'), eux-mêmes éliminés via la circulation lymphatique.(Serhan *et al.*, 2008) La résolution de l'inflammation implique des signaux *anti-inflammatoires* (bloquant l'action des médiateurs pro-inflammatoires ou inhibant leur production) et *pro-résolution de l'inflammation* (processus actifs impliqués dans l'activation de la phagocytose des cellules

apoptotiques et l'évacuation des cellules immunitaires du foyer inflammatoire). L'initiation de signaux de résolution de l'inflammation semble être entamée très tôt dans le processus inflammatoire.(Serhan et Savill, 2005)

Plusieurs mécanismes sont mis en jeu dans la résolution de l'inflammation :

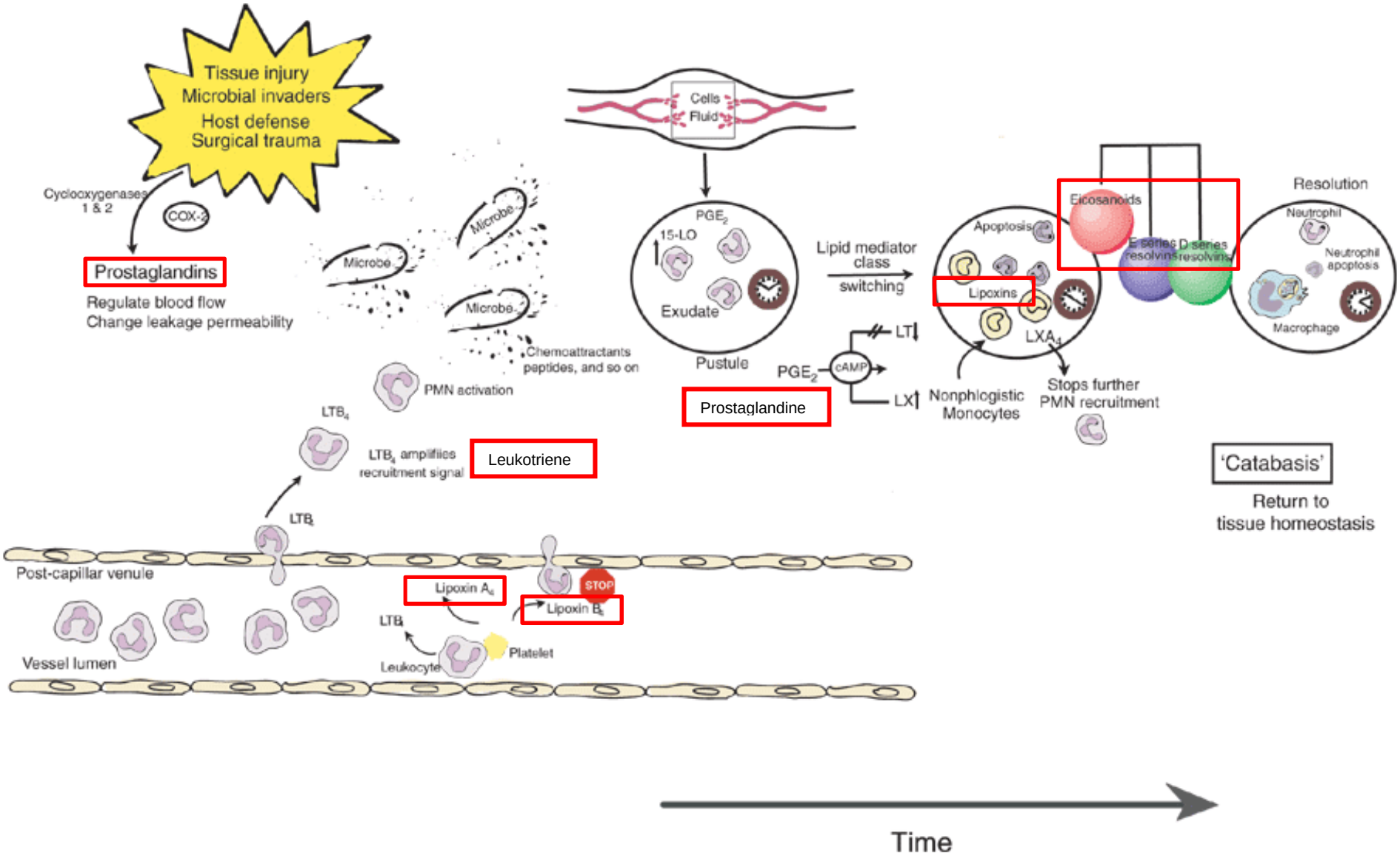
- Le système nerveux parasympathique se comporte comme un 'réflexe anti-inflammatoire', libérant de l'acétylcholine au niveau du foyer inflammatoire, pouvant inhiber spécifiquement la production de cytokines pro-inflammatoires par les macrophages.(Tracey, 2002)
- La production de médiateurs par les macrophages se modifie depuis des médiateurs pro-inflammatoires (prostaglandine E2 en particulier) vers des médiateurs anti-inflammatoires et activateurs de la résolution de l'inflammation : lipoxines, résolvines et protectines.(Serhan et al., 2005) (**Figure 9**). Ce phénomène a été décrit comme le 'lipid-mediator class switching'. Les eicosanoïdes pro-inflammatoires semblent activer directement la transcription d'enzymes permettant la production de ces médiateurs**.** Les médiateurs anti-inflammatoires retardent l'entrée de nouveaux neutrophiles au niveau du foyer inflammatoire, diminuent la perméabilité membranaire et activent la phagocytose des polynucléaires apoptotiques. **(Figure 8)**

Figure 8 Etapes de la résolution de l'inflammation au niveau du site inflammatoire (Serhan et al., 2008)



La résolution histologique de l'inflammation correspond à la diminution des neutrophiles du site inflammatoire. Ce processus programmé est régulé activement à de multiples niveaux par la modification des profils de cellules recrutées au niveau du site inflammatoire (diminution du recrutement des neutrophiles pour des monocytes), stimulation de la phagocytose des cellules apoptotiques par les macrophages et promotion de l'excrétion des macrophages par les lymphatiques.

Figure 9 Séquence temporelle de la réaction inflammatoire.(Serhan et al., 2005)



3. Inflammation et maladies chroniques

La réaction inflammatoire est en principe un processus essentiellement transitoire et bénéfique, permettant l'élimination d'un facteur d'agression de l'organisme.

La régulation des processus inflammatoire procède d'une balance fine entre mécanismes pro- et anti-inflammatoires, de cinétiques décalées. Néanmoins, dans certaines conditions, les processus inflammatoires persistent, conduisant à des dommages tissulaires progressifs. Ces réponses inflammatoires non régulées ou inappropriées sont caractérisées par une hyper-expression de molécules d'adhésion par les cellules endothéliales et les leucocytes, et par le maintien dans la circulation sanguine de formes solubles circulantes de ces molécules d'adhésion (ICAM-1 et VCAM-1), de cytokines pro-inflammatoires (IL6, TNFα) et de protéines de la phase aiguë (CRP en particulier).

Les maladies inflammatoires chroniques telles que la polyarthrite rhumatoïde, le lupus ou la sclérose en plaque en sont l'archétype, l'inflammation étant reconnue comme l'élément physiopathologique principal. Néanmoins, il a été récemment mis en évidence que l'inflammation était l'un des éléments intervenant précocement et accompagnant le développement d'autres pathologies, telles que le cancer (Coussens et al., 2002, Mantovani *et al.*, 2008), les maladies métaboliques (Hotamisligil, 2006) et cardio-vasculaires (Hansson, 2005, Libby, 2002). Par ailleurs, la découverte que le tissu adipeux pouvait être le siège d'une production endogène de médiateurs inflammatoires a participé à l'élaboration du concept selon lequel l'obésité et le diabète auraient une composante inflammatoire. (Hotamisligil, 2006) Seront développés ici plus largement les éléments reliant inflammation, athérosclérose, obésité et cancer.

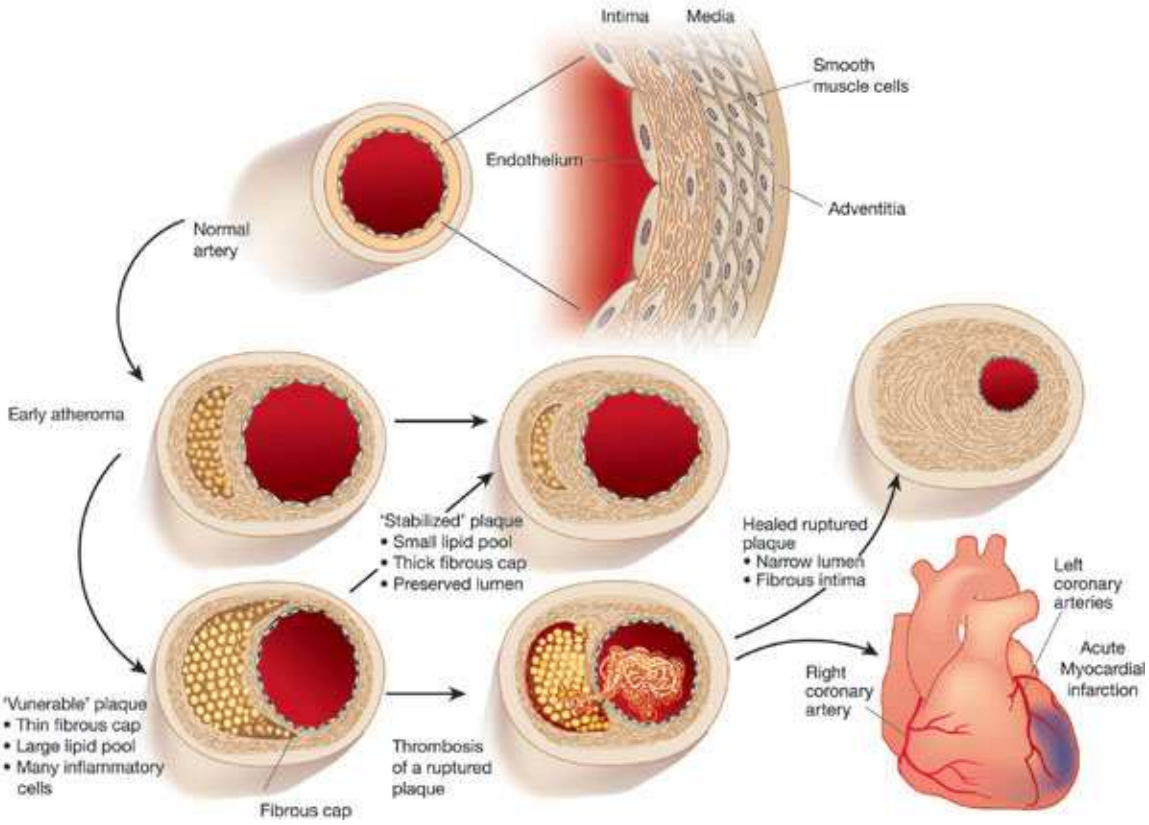
3.1. Inflammation et athérosclérose

La physiopathologie de l'athérosclérose est envisagée à l'heure actuelle comme un processus dynamique de réponse à l'agression (*Response to injury hypothesis*).(Ross, 1999) Selon cette hypothèse, la constitution de l'athérome correspondrait à une série de mécanismes d'agression et de réparation de l'endothélium vasculaire impliquant des mécanismes inflammatoires.

La lésion d'athérome est l'une des manifestations les plus apparentes dans la physiopathologie de l'athérosclérose. Celle-ci correspond à un épaississement localisé et asymétrique de la paroi vasculaire, l'intima. La région centrale de la plaque d'athérome est constituée de cellules spumeuses et de lipides extracellulaires, entourés d'une chape fibreuse constituée de cellules musculaires lisses et d'une matrice riche en collagène.(Hansson, 2005) Le développement de la plaque d'athérome, depuis l'accumulation des lipides jusqu'à une forme complexe conduisant

potentiellement à des manifestations cliniques est un processus dynamique, pour lequel de nombreux facteurs inflammatoires ont été mis en évidence.(Libby, 2002) (**Figure 10**)

Figure 10 Formation de la plaque d'athérome (Libby, 2002)

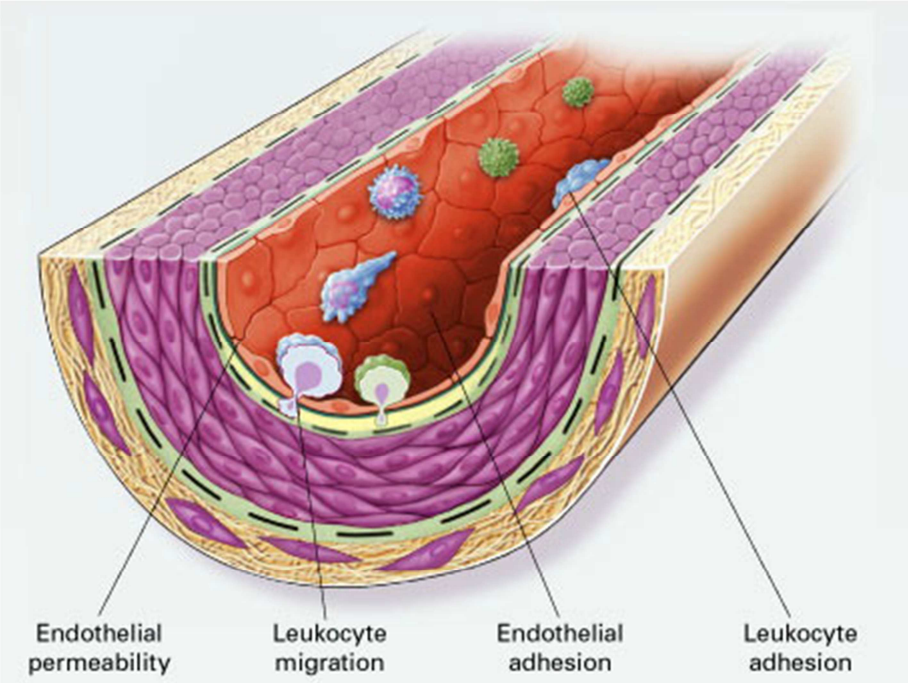


Inflammation et initiation de la lésion athéromateuse

Selon l'hypothèse de la réaction à l'agression (*Response to injury hypothseis),* les premières étapes fondamentales conduisant à la formation d'une plaque d'athérome impliquent une altération de l'endothélium vasculaire, induisant une réponse inflammatoire.

L'altération de la fonction endothéliale comporte une augmentation de la perméabilité vasculaire, une augmentation de la capacité d'adhésion des leucocytes et des plaquettes et une altération de l'expression des gènes (**Figure 11**).(Ross, 1999) Les éléments conduisant initialement à cette dysfonction endothéliale ne sont pas encore complètement connus. Les facteurs déclenchant les mieux connus sont les turbulences hémodynamiques et l'hypercholestérolémie.

Figure 11 Mécanismes impliqués dans la dysfonction endothéliale (Ross, 1999)



Les modifications intervenant au niveau endothélial correspondent à une augmentation de la perméabilité aux lipoprotéines, une régulation positive de facteurs d'adhésion aux leucocytes, une régulation positive de molécules d'adhésion vasculaires, et une migration des leucocytesau niveau de la paroi artérielle (developpé plus loin)

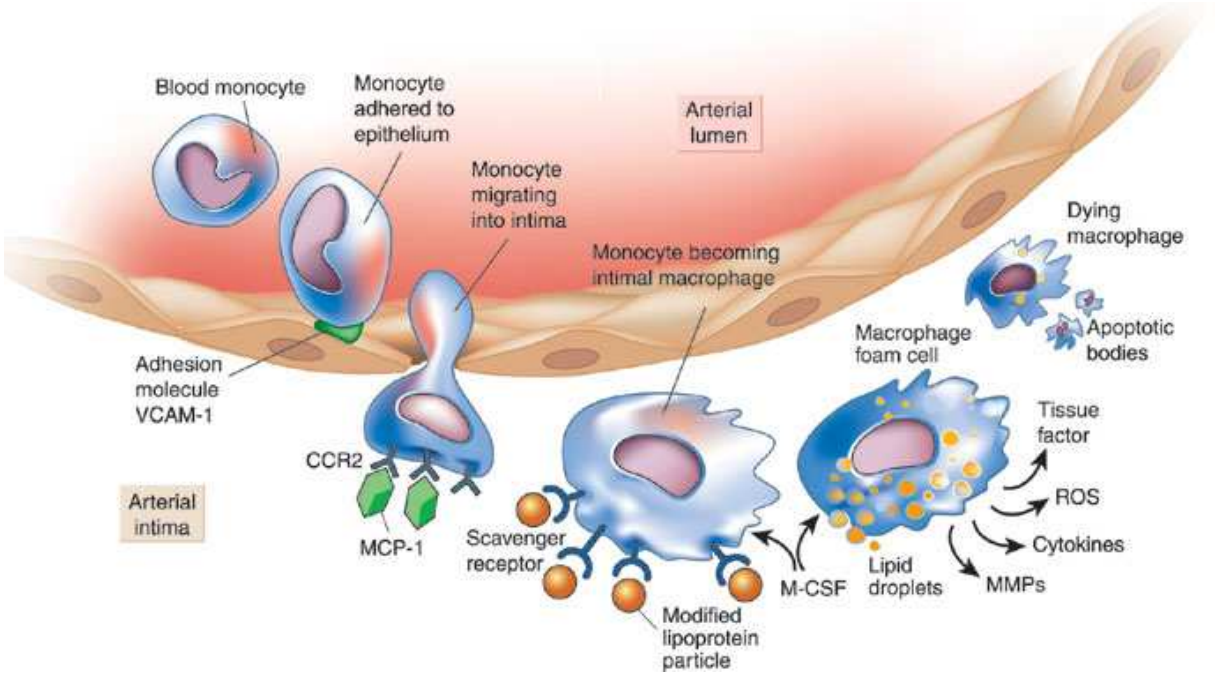
L'effet des turbulences hémodynamiques est illustré par la constatation que les plaques d'athérome sont localisées dans des zones de flux vasculaire turbulent, comme les ostium des artères et les bifurcations artérielles.(Chatzizisis *et al.*, 2007)

En cas d'hypercholestérolémie en, les particules de LDL sanguin en excès sont capturées au niveau intimal par des processus de transcytose.(Stocker et Keaney, 2004) Durant ce processus, les phospholipides ainsi que certains éléments des lipoprotéines contenus dans les LDL sont oxydés. Ces éléments oxydés conduisent à l'activation de la transcription de facteurs d'adhésion vasculaires (en particulier VCAM-1) au niveau endothélial.(Stocker et al., 2004) Par ailleurs, certaines molécules de recrutement et d'adhésion sont spécifiquement produites au niveau de la lésion athéromateuse, comme le monocyte chemoattractive protein 1 (MCP-1). L'expression de ces molécules est responsable de phénomènes de recrutement et de diapédèse leucocytaire au niveau du site vasculaire de la lésion. (Libby, 2002)

Les monocytes ayant été recrutés au niveau de la lésion d'athérome se différentient en macrophages. Ceux-ci expriment des récepteurs scavengers, le scavenger receptor A (SRA) ou le CD36, qui permettent l'internalisation des esters de cholestérol et leur accumulation au sein de gouttelettes cytoplasmiques. Les macrophages chargés en esters de cholestérol sont connus sous

le nom de « cellules spumeuses » (foam cells) et sont caractéristiques des premiers stades de développement des lésions d'athérosclérose.(**Figure 12**)(Libby, 2002)

Figure 12 Diapédèse leucocytaire et formation des cellules spumeuses au niveau de l'intima artérielle.(Libby, 2002)



Inflammation et progression de la lésion athéromateuse

La lésion athéromateuse initiale (strie lipidique), constituée principalement de cellules inflammatoires et de particules lipidiques, se modifie progressivement : elle augmente de taille et une chape fibreuse constituée principalement de cellules musculaires lisses l'entoure. C'est ce type de lésion qui est principalement à l'origine de la symptomatologie athéromateuse, qu'il s'agisse d'angine de poitrine (par rétrécissement artériel chronique) ou infarctus (par thrombose au niveau de la plaque).

La croissance de la plaque d'athérome a longtemps été perçue comme un phénomène continu, lié à la sécrétion de facteurs de croissance par les macrophages présents dans la lésion, qui stimuleraient la prolifération ininterrompue des cellules musculaires lisses. Néanmoins, la lésion athéromateuse aurait plutôt tendance à croître par à-coups *via* différents phénomènes de déstabilisation de la plaque. Cette rupture de plaque serait associée à une thrombose localisée, conduisant à l'expansion de la lésion athéromateuse d'un seul tenant.(Libby, 2002)

La déstabilisation des plaques peuvent être la conséquence de trois mécanismes, eux-mêmes étroitement liés aux processus inflammatoires (Libby, 2002) :

- Desquamation endothéliale : une desquamation endothéliale localisée conduit à la mise à nu de facteurs thrombogènes, conduisant à une activation plaquettaire et à la formation

d'un micro-thrombus. Si ces épisodes seraient fréquents et plutôt asymptomatiques, ils seraient néanmoins responsables de 25% des accidents coronaires. Certains médiateurs inflammatoires seraient associés à l'apoptose endothéliale d'une part, et à la dégradation de la matrice extracellulaire sur laquelle reposent les cellules endothéliales d'autre part (*via* l'activation de métalloprotéinases).

- Rupture de microvaisseaux : les cellules inflammatoires présentes au sein de la plaque d'athérome favorisent la néo-angiogenèse via la production de facteurs de croissances (comme le Vascular endothelial growth factor VEGF), ce qui conduit à la mise en place de microvaisseaux au sein des lésions athéromateuses. Néanmoins, ces vaisseaux sont fragiles, et susceptibles de se rompre. Une telle rupture intra-plaque conduit à une hémorragie, puis à une thrombose in situ. La thrombine secrétée lors de ces épisodes stimule la prolifération des cellules musculaires lisses, favorisant ainsi la constitution d'une plaque plus épaisse.
- Rupture de la chape fibreuse : la chape fibreuse entourant le cœur lipidique de la plaque d'athérome peut aussi se fissurer, permettant aux facteurs de coagulation d'entrer en contact avec les produits hautement thrombogènes du cœur lipidique. Ce phénomène est estimé être à l'origine de 75% des évènements coronaires. Néanmoins, il semblerait qu'il ne conduise pas systématiquement à une thrombose étendue à l'ensemble de la lumière artérielle, mais qu'il puisse entraîner uniquement une thrombose murale. Une fois la phase pro-coagulante passée, la résorption du thrombus conduit à l'accumulation de débris fibreux, conduisant une fois de plus à l'augmentation de la taille de la plaque, par augmentation de la chape fibreuse. Les médiateurs inflammatoires (IL-1β, TNFα) conduisent à la surexpression par les cellules présentes dans la lésion athéromateuse de gélatinases, responsables de la dégradation du collagène présent dans la chape fibreuse, et à la diminution de la production de collagène de remplacement par les cellules musculaires lisses, conduisant *in fine* à la fragilisation et à la potentielle fissuration de la chape fibreuse.

Ainsi les processus inflammatoires interviennent à tous les niveaux dans la constitution et la progression des lésions d'athérome. Le fait d'avoir une CRP augmentée a été reconnu comme étant un nouveau marqueur prédictif cardiovasculaire, même si l'intérêt de son dosage en routine pour le dépistage du risque cardiovasculaire n'a pas encore été démontré.(Pearson *et al.*, 2003)

3.2. Inflammation et obésité

Diabète et syndrome métabolique se développent tous deux sur un terrain prédisposant, la surcharge pondérale et l'obésité. L'obésité est caractérisée par un développement excessif du tissu adipeux, sous-cutané ou viscéral. Si ce tissu adipeux a longtemps été considéré uniquement comme un lieu de stockage de l'énergie sous forme de lipide, cette conception s'est complexifiée, jusqu'à le considérer comme une glande endocrine à part entière.(Hutley et Prins, 2005)

Concernant l'inflammation, il a tout d'abord été montré que le tissu adipeux était capable de produire du TNFα dans des modèles murins d'obésité.(Hotamisligil *et al.*, 1993) Cette première découverte a été suivie d'autres, montrant que le profil d'expression d'autres biomarqueurs d'inflammation était modifié dans le tissu adipeux en cas d'obésité.(Calder *et al.*, 2009, Wellen et Hotamisligil, 2005) En particulier, les adipocytes seraient capables de secréter des cytokines pro-inflammatoires, telles que l'IL1 ou l'IL6. Cette expression de médiateurs pro-inflammatoire serait à la fois une réponse à des stimuli habituels de l'inflammation, mais serait aussi fonction de la taille de l'adipocyte. Les concentrations circulantes en TNFα et IL6 sont d'ailleurs augmentées dans l'obésité.

En dehors de la production de médiateurs inflammatoires directement par les adipocytes, le tissu adipeux des sujets obèses est le siège d'une multiplication importante des macrophages.(Greenberg et Obin, 2006) Ceux-ci ont pour principale fonction la détersion des cellules adipocytaires apoptotiques, dont le nombre augmente de façon importante dans l'obésité. Ces macrophages, qui produisent eux aussi des médiateurs inflammatoires, seraient responsables d'environ 50% de la sécrétion de TNFα par le tissu adipeux. Par ailleurs, la surcharge pondérale est associée à une augmentation des acides gras circulants, eux-mêmes responsables d'une activation des voies de transduction pro-inflammatoires. En effet, il a été montré que certains acides gras saturés pouvaient conduire à l'activation des récepteurs TLR, et ainsi à l'activation d'une cascade de signalisation conduisant à la production de cytokines pro-inflammatoires.(Lee *et al.*, 2001)

Cet état pro-inflammatoire serait directement lié à la régulation de la multiplication et de la différentiation adipocytaire en période d'excès d'apport énergétique (phase de constitution de l'obésité) :

- L'augmentation du contenu en triglycérides des adipocytes aurait pour effet de stimuler la production de leptine. Celle-ci augmenterait l'expression de molécules d'adhésion (comme ICAM-1) et participerait au recrutement de monocytes depuis la circulation sanguine vers le tissu adipeux. (Neels et Olefsky, 2006)

- La sécrétion de TNFα par les cellules adipocytaires serait liée à l'augmentation de taille de la cellule, et aurait un rôle dans la régulation de cette taille adipocytaire, et dans l'apoptose.(Sethi et Hotamisligil, 1999)

Par ailleurs, de multiples voies de signalisation inflammatoires activées lors de l'obésité participent au développement de l'insulino-résistance :

- La sécrétion de TNFα par les adipocytes aurait un effet autocrine direct, en favorisant la phosphorylation du récepteur à l'insuline, ce qui inhibe la voie de signalisation efférente.(Sethi et al., 1999, Wellen et al., 2005)
- Les cytokines inflammatoires activent les kinases du groupe JNK (sérines/thréonines kinases) qui elles aussi ont pour conséquence la phosphorylation du récepteur à l'insuline, et l'inhibition de la voie de signalisation efférente.

D'une façon générale, les voies métaboliques et les voies de l'inflammation partagent de nombreux facteurs de transcription et médiateurs communs (NFκB, TNFα entre autres). Certains travaux ont montré que les systèmes immunitaires et métaboliques auraient évolué de façon divergente, mais proviendraient d'une origine commune, ce qui expliquerait les nombreuses similitudes retrouvées dans les voies de signalisation impliquées dans les deux systèmes.(Hotamisligil, 2006)

3.3. Inflammation et cancer

La présence de cellules immunitaires au sein des tumeurs a été constatée dès le XIXème siècle.(Balkwill et Mantovani, 2001) En revanche, l'hypothèse que l'inflammation pouvait être directement reliée au développement des cancers s'est développée beaucoup plus tard, au vu de l'accumulation d'éléments de preuve concordants. Ce constat initial de la présence de cellules immunitaires au sein de pratiquement toutes les tumeurs, mêmes en l'absence de pathologie inflammatoire sous-jacente a été repris et les recherches visant à comprendre les mécanismes cellulaires et biochimiques associant oncogénèse et inflammation se sont développées.(Coussens et al., 2002, Mantovani et al., 2008)

Par ailleurs, les études sur des modèles animaux, ainsi que des études épidémiologiques ont mis en évidence le fait qu'un certain nombre de pathologies inflammatoires chroniques sont associées directement à une augmentation du risque de cancer.

- Infections microbiennes : par exemple, l'infection par *Helicobacter pilori* est responsable du développement de cancers gastriques (Parsonnet *et al.*, 1991) ; l'infection par *Papillomavirus* est responsable du développement de cancers du col de l'utérus (Munoz

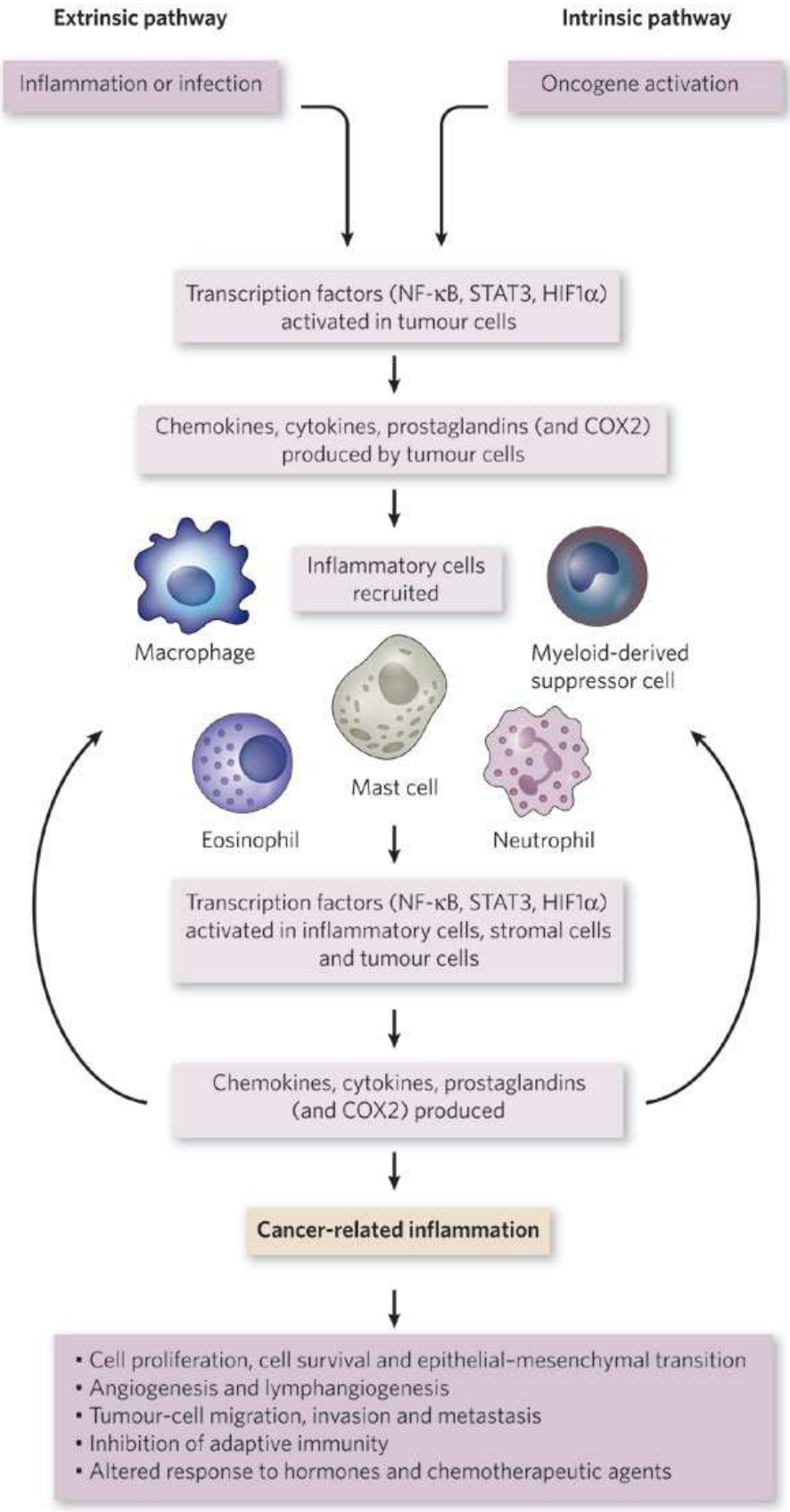
et al., 2003). Les infections seraient responsables de 15% des cancers, pour un total de 1,2 millions de cas par an dans le monde.(Balkwill et al., 2001)

- Maladies auto-immunes : les maladies inflammatoires chroniques de l'intestin (MICI) sont associées à un risque accru de cancer colorectal et de lymphomes intestinaux.(Franks et Slansky, 2012)
- Enfin, le fait que les traitements par anti-inflammatoires non stéroïdiens diminuent l'incidence et la mortalité dans certains cancers a renforcé l'hypothèse d'une relation forte entre inflammation et cancer.(Flossmann et Rothwell, 2007)

L'étude des mécanismes mis en jeu entre cancer et inflammation a permis de mettre en évidence des voies de signalisation à l'origine de l'environnement inflammatoire présent dans le cancer. Il s'agit essentiellement de deux voies : une voie extrinsèque (où l'inflammation chronique initiale stimule l'oncogenèse) et une voie intrinsèque (où les anomalies génétiques sont à l'origine du développement tumoral et de l'inflammation) (**Figure 13**).(Mantovani et al., 2008)

Ces deux voies de signalisation se rejoignent pour aboutir à la constitution d'un environnement inflammatoire de la tumeur, comprenant de nombreuses cellules immunitaires, et produisant de façon continue des médiateurs pro-inflammatoires et angiogéniques.

Figure 13 Voies de signalisation impliquées dans le développement de l'inflammation associée au cancer (Mantovani et al., 2008)



Voie intrinsèque

La présence de cellules et de médiateurs inflammatoires au sein de tumeurs pour lesquelles aucune hypothèse d'origine inflammatoire initiale pouvait être proposée a permis l'émergence de l'hypothèse que les anomalies génétiques à l'origine du développement tumoral pouvaient potentiellement aussi intervenir dans l'inflammation.(Mantovani et al., 2008)

Plusieurs mécanismes oncogénétiques ont été étudiés dans ce sens et ont montré que les anomalies génétiques conduisant à la genèse tumorale pouvaient aussi stimuler directement l'inflammation, via le recrutement de cellules immunitaires ou l'expression de cytokines pro-inflammatoires.

Par exemple, le réarrangement du chromosome sur lequel est située le gène de la protéine kinase RET est un évènement suffisant pour induire le développement d'un carcinome papillaire de la thyroïde.(Borrello *et al.*, 2005) L'activation de RET stimule également directement la transcription de facteurs impliqués dans l'inflammation : IL-1β, cyclo-oxygénase 2 (impliquée dans la production de prostaglandines) ainsi que de nombreuses chemokines impliquées dans le recrutement et l'adhésion des cellules immunitaires.(Borrello et al., 2005)

D'autres oncogènes ont montré des activités pro-inflammatoires similaires : les oncogènes RAS activent une voie de signalisation de l'inflammation, l'oncogène MYC peut stimuler la production de IL-1β.(Mantovani et al., 2008)

Voie extrinsèque

Une inflammation chronique localisée correspond à un micro-environnement pouvant aussi stimuler le développement tumoral. En effet, l'inflammation se caractérise par la présence d'une prolifération cellulaire intense, de nombreuses cellules immunitaires, des facteurs de croissance, et de médiateurs altérant potentiellement l'ADN, l'ensemble de ces éléments pouvant stimuler la néoplasie.(Balkwill, 2004, Coussens et al., 2002)

Parmi les médiateurs excrétés pouvant causer des dommages à l'ADN, les composés réactifs de l'oxygène et de l'azote sont de bons candidats (le peroxynitrite en particulier a un fort pouvoir mutagène). La prolifération de ces composés peut conduire à des mutations, délétions ou réarrangements chromosomiques conduisant au développement tumoral.

Le facteur de transcription NF-κB, reconnu comme facteur de transcription clé dans la régulation de l'inflammation, a été aussi identifié comme promoteur tumoral dans certaines circonstances. Tout d'abord, l'activation de NF-κB peut résulter de certaines mutations génétiques potentiellement pro-oncogéniques. Ensuite, NF-κB peut stimuler l'expression de molécules

inflammatoires, mais aussi la survie cellulaire *via* l'expression de gènes anti-apoptotiques.(Karin, 2006)

Par ailleurs, les cellules immunitaires (en particulier les macrophages associés aux tumeurs) recrutées au niveau du site tumoral potentialisent le développement néoplasique par la sécrétion de facteurs angiogéniques et lymphogéniques.(Coussens et al., 2002)

Ainsi, un certain nombre de mécanismes ont été mis en évidence, liant inflammation et cancer. Néanmoins, l'étendue des interactions entre ces voies reste encore relativement peu connue.

4. Nutrition et Inflammation – mécanismes en jeu

De nombreux nutriments incorporés au niveau cellulaire interviennent directement ou indirectement lors de la réponse inflammatoire, en tant que précurseurs de médiateurs inflammatoires ou cofacteurs enzymatiques. Parmi ces mécanismes, les plus connus sont ceux mettant en jeu les acides gras polyinsaturés et les antioxydants.

4.1. Médiateurs lipidiques de l'inflammation

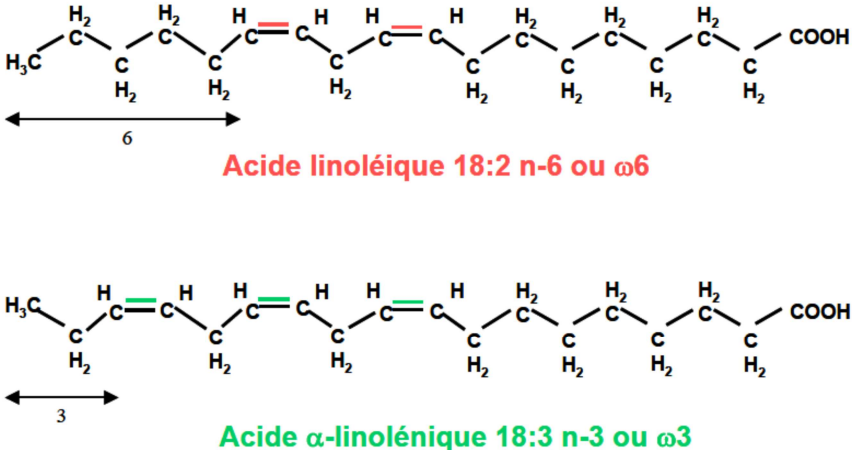
Certains des médiateurs impliqués dans les processus inflammatoires sont des médiateurs d'origine lipidique, dont les précurseurs sont les acides gras polyinsaturés (PUFA).

Acides gras polyinsaturés

Structure biochimique

Les acides gras (AG) sont composés d'un radical constitué par une chaine linéaire d'atomes de carbone, de longueur variable, sur chacun desquels sont fixés en principe un à deux atomes d'hydrogène (un seul en cas de double liaison entre deux atomes de carbone). Une des extrémités de la chaine carbonées est constituée d'un groupe méthyle (CH_3), l'autre étant constituée d'un groupe carboxyle (COOH), porteur de la fonction acide (**Figure 14**). Les PUFA sont des acides gras comportant plusieurs doubles liaisons carbone-carbone. La nomenclature des AG repose sur le nombre de carbones et de doubles liaisons carbone, ainsi que sur la position de la double liaison carbone-carbone la plus proche du groupe méthyle. La notation se fait de la façon suivante : **Cx : y n-z** (ou **ω-z**) où **x** représente le nombre d'atomes carbone, **y** le nombre de doubles liaisons et **z** la position de la double liaison la plus proche du groupe méthyle.

Figure 14 Structure biochimique des PUFAs



Les PUFAs ayant un intérêt en nutrition humaine sont de deux séries, en fonction de la position de la double liaison carbonée la plus proche du groupement méthyle : la série n-3 (connue aussi sous la terminologie oméga-3) et la série n-6 (oméga 6). Ces deux chaines sont respectivement issues de l'acide α-linolénique 18 :3 n-3 ALA et l'acide linoléique 18 :2 n-6 LA (**Figure 14**).

Les principaux PUFAs présents dans l'alimentation humaine sont présentés dans le **Tableau 2.**

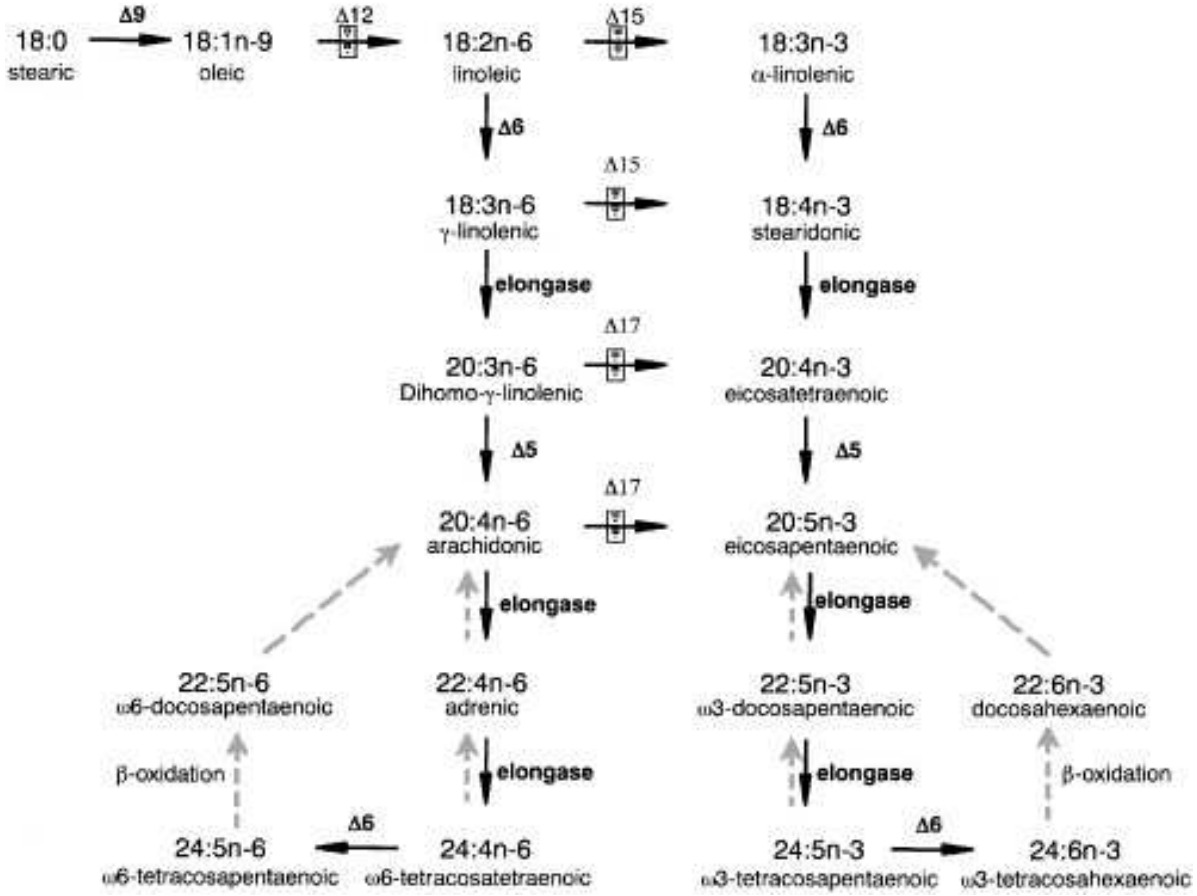
Tableau 2 Principaux PUFAs en alimentation humaine

Série		Nom
N-3	18:3	Acide α-linolénique ALA
	20:5	Acide eicosapentaénoique EPA
	22:5	Acide docosapentaénoique DPA
	22:6	Acide docosahexaénoique DHA
N-6	18:2	Acide linoléique LA
	20:4	Acide arachidonique AA

Sources - biosynthèse

L'ALA et le LA sont des acides gras essentiels car ils sont indispensables au bon fonctionnement de l'organisme, mais ne peuvent être synthétisés par l'être humain. En effet, les enzymes permettant de créer les doubles liaisons en position 3 et 6 du groupement méthyle ne sont pas présentes dans les espèces supérieures. En revanche, la conversion depuis l'ALA et le LA en AA et EPA et DHA sont possibles chez l'être humain (**Figure 15**).

Figure 15 Biosynthèse des PUFAs chez l'humain.(Pereira *et al.*, 2003)



Δ Désaturase ; les désaturases indiquées avec un symbole ⊠ ne sont pas présentes chez l'homme

Ces conversions impliquent des réactions enzymatiques de désaturation et d'élongation (**Figure 15**). Il n'existe pas chez l'homme de possibilité de conversion depuis la série n-3 vers la famille n-6 ou inversement. Néanmoins, si la conversion depuis l'ALA et le LA vers les PUFAs à longue chaine est possible chez l'homme, elle a été mesurée à un niveau extrêmement faible *in vivo*.(Plourde et Cunnane, 2007) En effet, les études *in vivo* montrent que moins de 5 % de l'ALA sont convertis en EPA et moins de 0,5 % en DHA.(Brenna, 2002) En revanche, les concentrations circulantes de DPA sont moins corrélées aux apports alimentaires en DPA qu'aux niveaux circulants d'EPA, suggérant ainsi que le DPA circulant serait davantage issu d'une conversion depuis l'EPA circulant que des apports alimentaires.(Mozaffarian et Wu, 2012) Ces nouvelles connaissances relatives aux taux de conversion des AG a conduit en 2011 à une révision des recommandations et à la création d'une recommandation concernant l'apport minimal nécessaire en DHA. En ce qui concerne l'EPA, aucune recommandation n'a été émise.(ANSES, 2011)

La grande majorité des AG retrouvés dans l'organisme est donc d'origine alimentaire. Les principaux groupes contributeurs dans l'alimentation aux apports en acides gras polyinsaturés

sont les huiles végétales (n-3, n-6 et n-9) et les poissons gras (n-3) et les produits d'origine animale (n-6). (**Tableau 3**)

Tableau 3 Principaux groupes contributeurs aux apports en PUFA dans l'étude SU.VI.MAX en pourcentage de l'apport énergétique total (travaux personnels)

Groupes alimentaires	18:3n-3 ALA	20:5n-3 EPA	22:5n-3 DPA	22:6n-3 DHA	18:2n-6 LA	20:4n-6 AA
Beurre	8,47	-	-	-	1,60	-
Gâteaux, biscuits et pâtisseries	7,98	0,25	1,55	3,03	5,56	6,94
Fromage	10,83	-	-	-	2,11	1,99
Crèmes dessert, glaces	1,04	0,25	0,93	1,59	0,90	3,57
Œufs	1,28	0,73	4,18	7,72	2,30	17,37
Fruits	5,49	-	-	-	1,45	-
Poissons gras	1,23	36,09	23,75	38,88	0,45	5,96
Poissons maigres	0,11	17,78	5,89	18,23	0,02	2,54
Margarine	2,93	-	-	-	3,31	-
Viande	6,20	10,20	20,84	2,68	2,91	14,81
Noix	4,25	-	-	-	3,38	-
Huile d'olive	6,09	-	-	-	4,04	-
Abats	0,18	2,10	5,38	1,71	0,18	4,33
Pizza, quiche, tartes salées	3,07	2,44	2,06	2,37	2,70	2,88
Volaille	2,51	2,89	8,96	2,86	2,83	15,87
Charcuterie	5,20	5,56	17,91	4,18	6,60	17,61
Féculents	4,11	0,05	0,03	0,08	4,91	0,70
Produits de la mer	0,08	16,44	4,76	11,71	0,02	3,30
Légumes	7,35	-	-	-	1,35	-
Huiles végétales pauvres en n-3	3,66	-	-	-	40,14	-
Huiles végétales riches en n-3	3,67	-	-	-	1,04	-

Données obtenues à partir d'un échantillon de sujets issus de l'étude SU.VI.MAX, ayant au moins 3 enregistrements de 24h effectuées dans les deux premières années suivant l'inclusion dans l'étude en 1994-1996. Les cellules grisées correspondent aux groupes contribuant à plus de 5% de l'apport total.

Fonctions biologiques

Les AG sont les principaux constituants des catégories des lipides plus complexes : triglycérides, sphingolipides et phospholipides.

Les triglycérides sont constitués d'une molécule de glycérol, à laquelle sont rattachés trois acides gras. Les triglycérides sont principalement la forme de stockage des lipides dans l'organisme, au sein des vacuoles lipidiques des adipocytes. Les AG sont utilisés comme substrat énergétique par β-oxydation.

Les sphingolipides sont dérivés de la molécule de sphingosine, résultant de l'amidification d'un acide gras sur une sphingosine (liaison de la fonction carboxyle de l'AG sur le -NH2 de la sphingosine). Ce sont des constituants des membranes cellulaires ; ils participent à la signalisation cellulaire

Les phospholipides sont constitués d'une molécule de glycérol, de deux acides gras et d'une molécule d'acide phosphorique. Cette dernière peut être plus ou moins associée à des molécules non lipidiques (choline par exemple). Les phospholipides ont pour principale propriété d'avoir un pôle hydrophile et un pôle hydrophobe, et sont à ce titre les principaux constituants des doubles membranes lipidiques cellulaires. En fonction des acides gras qui sont attachés aux phospholipides, ceux-ci ont des propriétés physico-chimiques et structurales différentes. L'abondance variable des PUFAs dans les phospholipides peut donc intervenir dans la fluidité de la membrane et dans l'activité des protéines qui lui sont associées.

Enfin, les AG peuvent sont des précurseurs de la synthèse de médiateurs intra- et extracellulaires (cf. infra).

Eicosanoïdes

Sous-familles et propriétés

Les eicosanoïdes (du grec eicosa=vingt, car dérivant des acides gras à 20 atomes de carbone) constituent la principale famille des médiateurs inflammatoires lipidiques.(Funk, 2001) Elle est composée de plusieurs sous-familles, dont les principales sont les suivantes :

- Prostaglandines

Les prostaglandines ont été les premiers éléments moléculaires identifiés dans la famille des eicosanoïdes. Leurs propriétés ont été découvertes dans les années '30, et leur structure complète élucidée dans les années '60. La plupart des prostaglandines ont des propriétés **pro-inflammatoire**.

Une fois synthétisées, ces médiateurs sont relargués dans l'espace extracellulaire par des transporteurs spécifiques. Leur demi-vie étant extrêmement courte (de l'ordre de quelques secondes à quelques minutes), leur périmètre d'activité est restreint autour de la cellule les ayant produites. Les récepteurs des prostaglandines sont principalement situés sur les membranes des cellules endothéliales et musculaires lisses péri-vasculaires. Les propriétés principales des prostaglandines sont la modulation du tonus vasculaire. Dans l'inflammation, elles interviennent dans la phase de recrutement, en augmentant la perméabilité vasculaire.(Funk, 2001) Les prostaglandines ne sont pas produites uniquement dans les cellules inflammatoires, mais dans la majorité des cellules de l'organisme. Elles peuvent donc avoir des actions multiples en fonction de l'environnement cellulaire dans lequel elles se situent. Par exemple, au niveau utérin, les prostaglandines peuvent provoquer des contractions utérines, déclenchant ainsi le travail.

- Leucotriènes

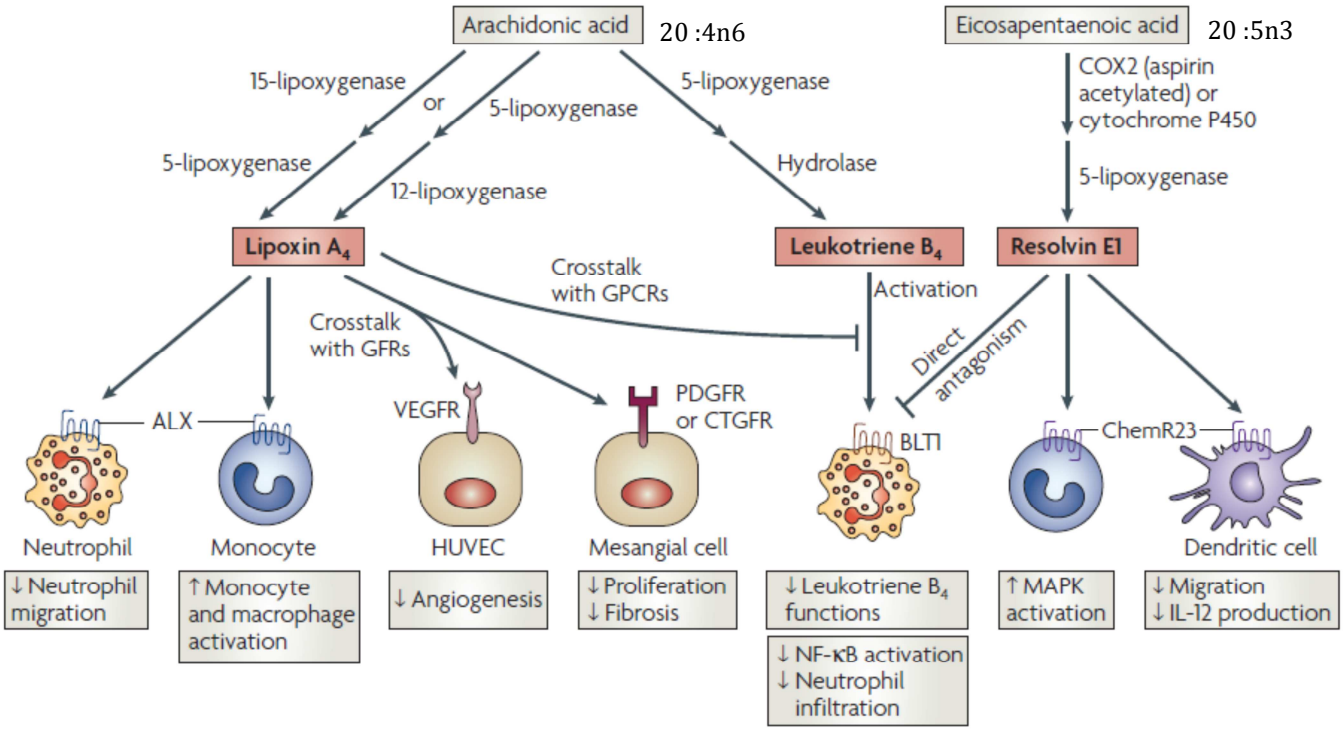
Contrairement aux prostaglandines, les leucotriènes sont principalement synthétisés dans les cellules inflammatoires, plus particulièrement monocytes, macrophages et polynucléaires

neutrophiles. La plupart des leucotriènes ont des propriétés **pro-inflammatoires**. Ils sont excrétés de la cellule par des transporteurs spécifiques, et agissent localement, comme les prostaglandines. Les leucotriènes ont pour propriété de réguler à la hausse la production de molécules d'adhésion cellulaire au niveau de l'endothélium et de favoriser le chimiotactisme des polynucléaires neutrophiles. Ils participent ainsi au recrutement de nouvelles cellules inflammatoires au niveau du foyer inflammatoire par chimiotactisme.(Funk, 2001)

- Lipoxines

Les lipoxines ont été découvertes plus récemment. Elles sont synthétisées par les cellules inflammatoires et les cellules endothéliales principalement.(Serhan et al., 2008) Elles agissent principalement sur les cellules immunitaires et endothéliales (**Figure 16**). Contrairement aux sous-familles précédentes, elles ont des propriétés **anti-inflammatoires** et **pro-résolution de l'inflammation**. Parmi leurs propriétés **pro-résolution de l'inflammation**, les lipoxines peuvent stopper la diapédèse leucocytaire, de favoriser la migration par chimiotactisme de monocytes non-phlogistiques (impliqués dans la phagocytose principalement de cellule apoptotiques), de réduire la fibrose tissulaire.

Figure 16 Mécanismes d'action de la lipoxine A1 et de la résolvine.(Serhan et al., 2008)



Biosynthèse

La production des eicosanoïdes nécessite comme substrats des acides gras polyinsaturés dont les chaines ont au moins 20 atomes de carbones. Parmi les acides gras composant les

phospholipides membranaires répondant à ces contraintes, l'acide arachidonique (20 :4 n-6, AA) est proportionnellement le plus important à la surface des cellules inflammatoires, et est donc leur précurseur principal.

La cascade enzymatique conduisant à la production d'eicosanoïdes est initiée par les cyclo-oxygénases (COX) et lipoxygénases (LOX). (**Figure 17**) L'expression de ces enzymes dépend des types cellulaires, des stimuli reçus ainsi que de la temporalité de la réaction inflammatoire (cf. plus haut paragraphe Résolution de l'inflammation, page 34). L'activation initiale de la lipoxygénase nécessite la présence d'un radical oxydant peroxyde. (Stocker et al., 2004) La production des médiateurs lipidiques de l'inflammation est donc étroitement liée à la balance oxydante de la cellule.

L'AA est le principal substrat de ces enzymes, mais il a été montré que certains PUFAs de la famille des n-3 (acide eicosapentaenoique 20 :5n-3 EPA et acide docosahexaenoique 22 :6n-3 DHA) pouvaient aussi être utilisés comme substrats par les COX et la LOX-5, produisant ainsi des eicosanoïdes possédant une structure conformationnelle distincte (**Figure 18**), et ayant des propriétés pro-inflammatoires atténuées par rapport aux eicosanoïdes provenant de l'AA, voire même des propriétés anti-inflammatoires.(Calder, 2006)

Figure 17 Biosynthèse des eicosanoïdes à partir de l'acide arachidonique. (Calder et al., 2009)

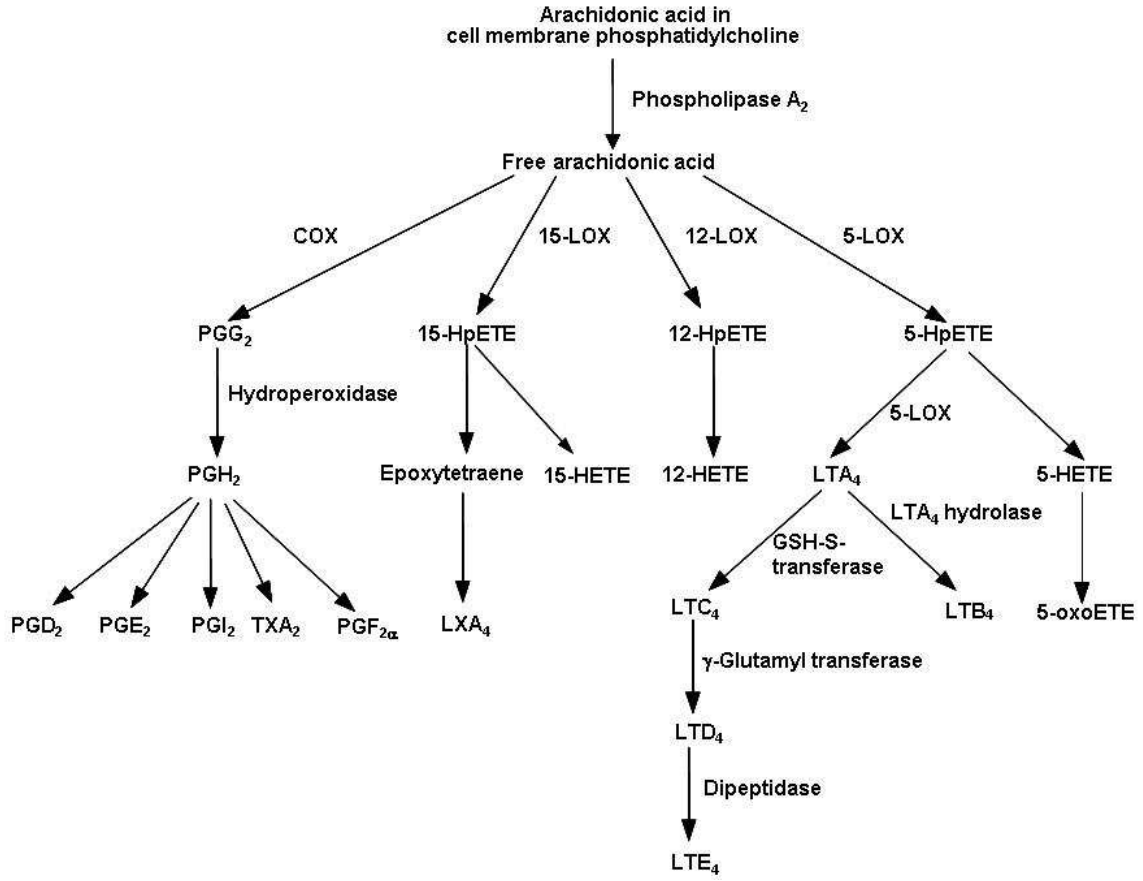
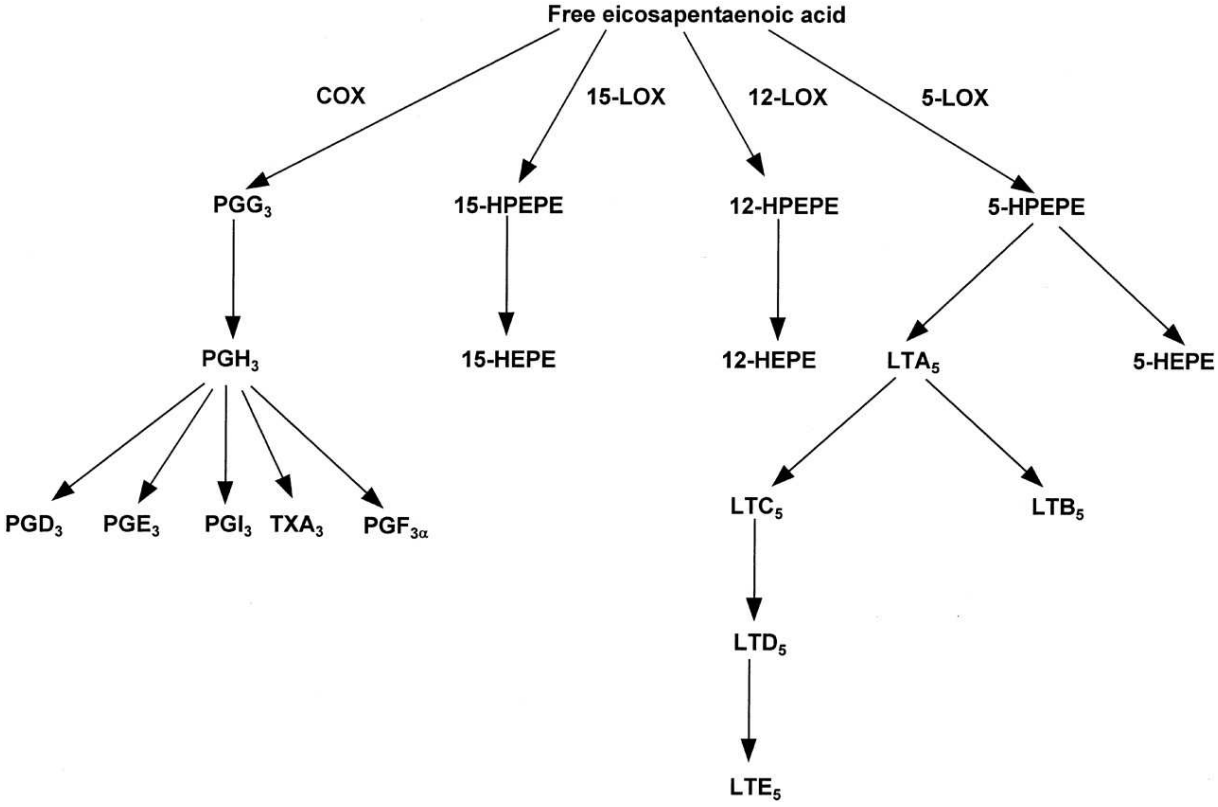


Figure 18 Biosynthèse des eicosanoïdes à partir de l'acide eicosapentaenoique. (Calder et al., 2009)



Protectines et résolvines

En dehors de leur rôle de substrat pour les enzymes des cascades des eicosanoïdes, les PUFAs de type n-3 sont les précurseurs de médiateurs spécifiques, impliqués uniquement dans la **résolution de l'inflammation**, identifiées récemment, les protectines et résolvines.

Sous-familles et propriétés

- Résolvines

Les résolvines sont synthétisées à partir de l'EPA et du DHA, en deux séries, résolvines E et D. La résolvine E1 est capable de stopper la migration trans-endothéliale des polynucléaires neutrophiles et de diminuer l'activation du facteur de transcription NFκB. Les résolvines D ont-elles aussi des propriétés d'interruption du recrutement des neutrophiles.(Serhan et al., 2008)

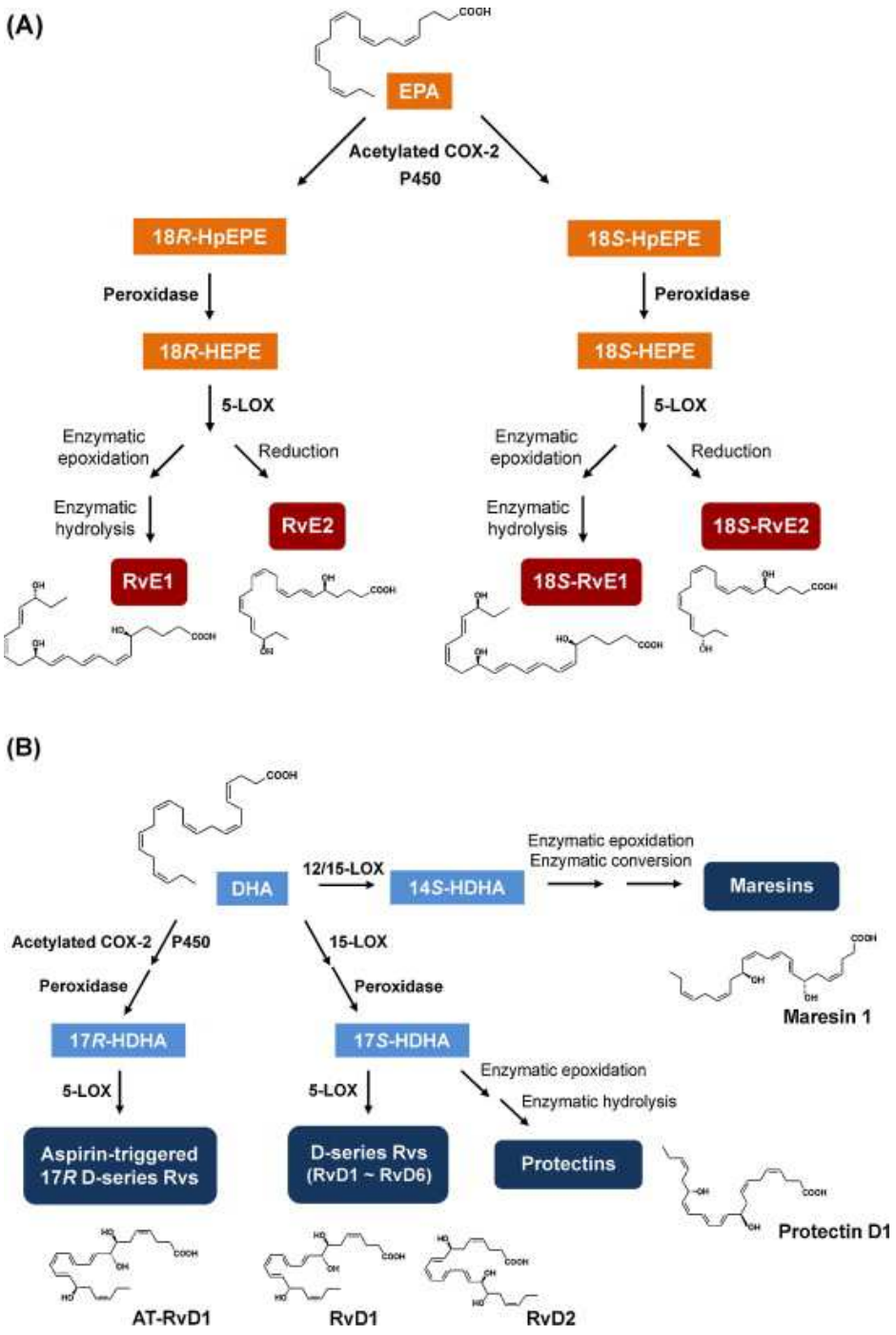
- Protectines

Les protectines sont synthétisées à partir du DHA dans les cellules de l'immunité innée. Les protectines interviennent dans la régulation du recrutement leucocytaire, de la synthèse des cytokines et chémokines (TNFα et interféron γ) et dans la promotion de l'apoptose des lymphocytes au niveau du site inflammatoire.(Serhan et al., 2008)

Biosynthèse

Comme indiqué précédemment, les résolvines et protectines sont synthétisées à partir des PUFA n-3, via des cascades enzymatiques similaires aux eicosanoïdes, impliquant les COX et LOX (**Figure 19**).

Figure 19 Biosynthèse des résolvines et protectines à partir de l'EPA et du DHA. (Lee et Surh, 2012)



(A) L'EPA est converti en résolvines des séries R et E à partir de la COX2 et la LOX5

(B) le DHA donne par acétylation de la COX2 par l'aspirine des résolvines de la série D. Via les LOX, les familles générées sont les résolvines D, les protectines et les marésines.

Lipides alimentaires et médiateurs lipidiques de l'inflammation

Les médiateurs lipidiques de l'inflammation étant issu des AG présents dans les membranes cellulaires des cellules immunitaires, la balance entre les différents PUFA au sein de ces membranes est l'un des éléments régulant la formation d'eicosanoïdes provenant de l'une ou l'autre série n-3 ou n-6. En effet, les modèles animaux ont montré une corrélation positive entre les quantités d'AA présent dans les membranes cellulaires et la production d'eicosanoïdes.(Peterson *et al.*, 1998)

Constitution des membranes cellulaires

A l'état basal, l'AA est proportionnellement plus représenté dans les membranes cellulaires que les autres PUFAs, en particulier n-3.(Calder, 2006)

Néanmoins, la proportion relative entre PUFAs des séries n-3 et n-6 au sein des membranes cellulaires peut être modifiée en fonction de leurs apports alimentaires respectifs. (Calder et al., 2009)

Plusieurs études chez l'animal et chez l'homme ont montré qu'une alimentation riche en AA permet d'augmenter la proportion d'AA présent dans les membranes des cellules immunitaires. (Peterson et al., 1998, Thies *et al.*, 2001b) Par ailleurs, le LA pouvant servir de substrat pour la formation d'AA, les apports en LA seraient aussi associés aux quantités d'AA présentes dans les cellules.(Calder et al., 2009)

De même, une alimentation riche en EPA et en DHA chez l'animal et chez l'homme conduisent à une augmentation de la proportion de ces AG dans les phospholipides constitutionnels des membranes cellulaires. L'incorporation de ces AG dans les membranes se fait en partie au détriment de l'AA.(Calder, 2006)

Production d'eicosanoïdes

L'incorporation d'AA dans les membranes cellulaires modifie la capacité de la cellule à produire des eicosanoïdes. La supplémentation en AA chez l'homme conduit à une augmentation de la production en prostaglandine E2 (PGE2) et de leukotriène B4 (LTB4) par des cellules immunitaires stimulées.(Kelley *et al.*, 1998, Thies *et al.*, 2001a)

L'incorporation d'EPA et/ou de DHA a pour conséquences, quant à elle de diminuer la production d'eicosanoïdes dérivés de l'AA (PGE2, LTB4 et LTE4) par un mécanisme de compétition simple : le DHA et EPA étant incorporés au détriment de l'AA en partie, celui-ci est moins disponible pour la production d'eicosanoïdes.(Healy *et al.*, 2000, Rees *et al.*, 2006) Par ailleurs, la supplémentation en huiles de poisson, riches en PUFAs n-3 conduit à l'augmentation

de la production d'eicosanoïdes alternatifs (**Figure 18**), LTB5, LTE5.(Lee *et al.*, 1985, Vonschacky *et al.*, 1993)

Production de cytokines inflammatoires

Malgré la modification de la proportion des AG présents dans les membranes cellulaires et la modification de la production des eicosanoïdes, il n'a pour l'instant pas été montré que l'apport alimentaire d'AA pouvait modifier la production de cytokines inflammatoires. (Kelley et al., 1998, Thies et al., 2001a)

En revanche, la supplémentation en EPA et/ou DHA peut altérer la production de cytokines inflammatoires par les cellules immunitaires. Des études in vitro et ex-vivo chez l'homme ont montré que le DHA et l'EPA pouvaient inhiber la production d'IL1β et de TNFα par les monocytes, et la production d'IL-6 et d'IL-8 par les cellules endothéliales.(Caughey *et al.*, 1996, Endres *et al.*, 1989, Lee et al., 1985, Meydani *et al.*, 1991, Trebble *et al.*, 2003, Vonschacky et al., 1993) In vivo dans des modèles animaux, une alimentation riche en huiles de poissons permet de diminuer la production d'IL1β, d'IL-6 et de TNFα par les macrophages. (Kelley *et al.*, 1999)

Chez l'homme, les résultats sont plus mitigés.(Calder, 2006) Certaines études ont retrouvé des résultats similaires à ceux des modèles murins, la supplémentation en PUFAs n-3 conduisant à une diminution de la production de cytokines par les macrophages activés, en particulier TNFα. D'autres en revanche n'ont pas permis d'établir d'association. La dose nécessaire à l'obtention de résultats positifs ainsi que la balance entre les différentes composantes (EPA et DHA) sont probablement partiellement en cause. (Calder, 2006)

4.2. Nutriments antioxydants

Les réactions oxydantes font partie intégrante de la réaction inflammatoire, tant au niveau de la réponse inflammatoire par les cellules immunitaires qu'en amont, au moment du déclenchement de celle-ci.

Le stress oxydant est défini comme une rupture d'équilibre entre oxydants et antioxydants en faveur des premiers.(Stocker et al., 2004) La biochimie sous-tendant ces processus correspond à des réactions d'oxydo-réduction au niveau des composés biochimiques cellulaires, conduisant à la libération au niveau moléculaire d'espèces réactives de l'oxygène, (dont l'anion superoxyde O_2^-) ou d'oxydants secondaires (comme le peroxyde d'hydrogène H_2O_2). De plus, les réactions oxydantes ont la propriété de se propager le long des chaines carbonées, avec une multiplication des radicaux libres formés.

(1) LH + ·OH → L· + H_2O
(2) L· + O_2 → LOO·
(3) LOO· + LH → L· + LOOH

Exemple de réaction oxydante en chaine de péroxydation lipidique. Un radical hydroxyl (·OH) absorbe un atome d'hydrogène au niveau latéral de la chaine carbonée d'un acide gras (LH) (1). Le radical centré sur le carbone obtenu (L·) se conjugue rapidement à l'oxygène (O_2) pour former un lipide contenant un radical peroxyl (LOO·) (2)qui peut lui-même propager la chaine de réaction en réagissant avec un autre acide gras pour générer un autre radical L· et un lipide comprenant un radical hydroperoxyde (LOOH) (3)

Les réactions impliquant l'oxygène dans la cellule sont contrôlées afin d'empêcher la libération de réactifs oxydants. En dehors de cette production liée au bon fonctionnement cellulaire, certaines enzymes possèdent la capacité d'effectuer des réactions d'oxydo-réduction. La production régulée de réactifs oxydants pourrait intervenir dans la signalisation cellulaire.(Stocker et al., 2004) En particulier, la production de réactifs oxydants participe à la signalisation cellulaire conduisant à la mort cellulaire par apoptose. Enfin, au-delà d'un certain niveau, ou en réponse à certains stimuli, la génération de réactifs oxydants conduit à la destruction des membranes cellulaires, et par la suite à des dommages tissulaires. Par ailleurs, il est à noter que les premières réactions de formation des médiateurs lipidiques de l'inflammation sont des réactions contrôlées d'oxydo-réduction d'origine enzymatique par les cyclo-oxygénases (COX, voir la biosynthèse des eicosanoïdes page 55).

L'organisme, afin de contrer les effets des réactifs oxydants produits, possède un certain nombre de mécanismes de protection, régulant ainsi avec précision la balance oxydante. Ces mécanismes pour certains font intervenir des enzymes spécifiques, et pour d'autres résultent de réactions spontanées non enzymatiques.

Certains nutriments essentiels participent à ces réactions, plus particulièrement les vitamines C, E et les caroténoïdes soit directement, soit en tant que cofacteurs enzymatiques. Les propriétés de ces oxydants sont aussi décrites dans le manuscrit « Antioxidant status and risk of elevated C-reactive protein 12 years later », page 88.

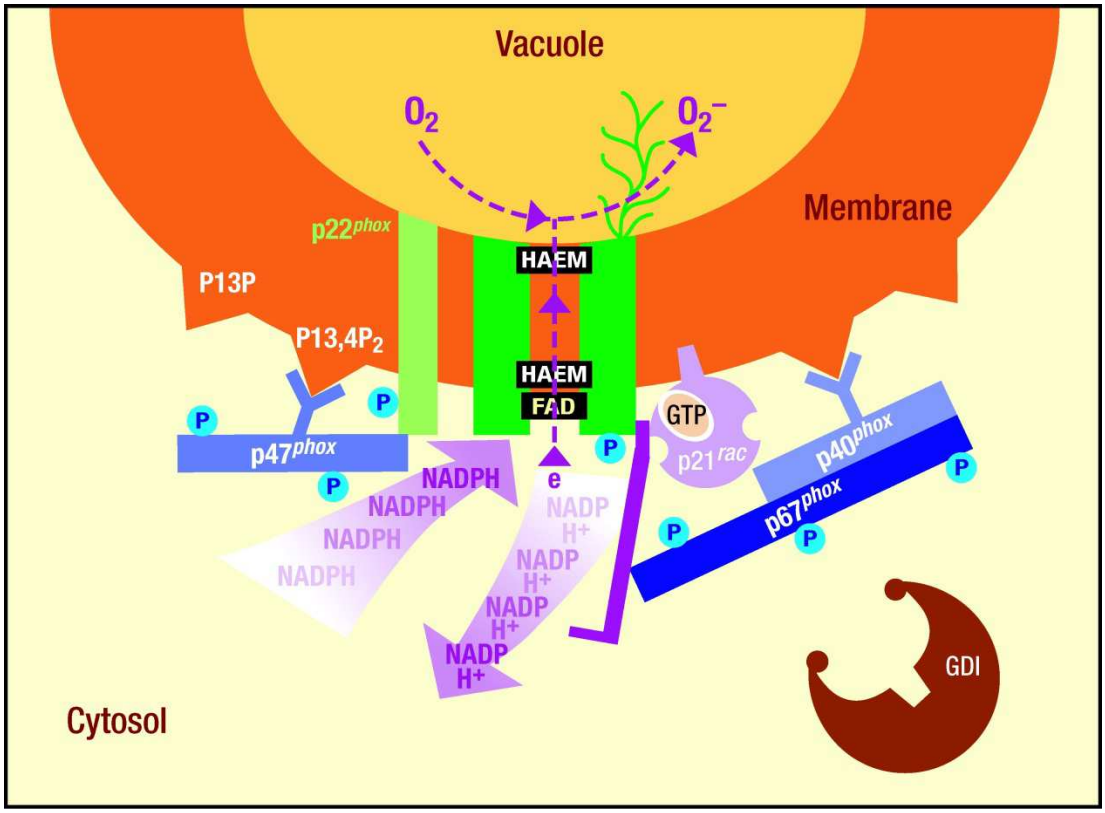
Réactifs oxydants dans l'inflammation

Radicaux libres oxydants et réponse inflammatoire

Les radicaux libres oxydants sont des produits toxiques pour les agents pathogènes. Leur production par les cellules immunitaires au niveau du foyer inflammatoire participe donc à l'élimination de celui-ci. Ces réactions interviennent à un niveau très précoce de la réponse inflammatoire.

Les réactifs oxydants peuvent être produits par les cellules immunitaires capables de phagocytose (macrophage, monocytes et polynucléaires) au sein des phagolysosomes (cf. paragraphe Reconnaissance-Phagocytose page 27) dans des réactions intracellulaires enzymatiques. Ces cellules possèdent en effet le matériel enzymatique nécessaire à la production de radicaux oxydants au sein de la vacuole de phagocytose. Le principal complexe enzymatique impliqué est la nicotinamide adenine dinucleotide phosphate (NADPH) oxydase, composée de 4 sous-unités. Après endocytose et fusion des lysozymes en phagolysosomes, les sous-unités s'assemblent au niveau de la membrane de la vacuole de phagocytose, et l'activation du complexe enzymatique prend moins de 20 secondes.(Segal, 2005, Segal, 2008) Le complexe enzymatique a par la suite la capacité de réduire l'O_2 en superoxyde à l'intérieur de la vacuole de phagocytose, via un transfert membranaire d'électrons (**Figure 20**). Par suite de réactions non enzymatiques, les ions superoxydes produits peuvent être transformés en oxydants secondaires : peroxyde d'hydrogène (H_2O_2), radical hydroxyle ($HO^{\cdot}$).(Segal, 2005)

Figure 20 Fonctionnement de la NADPH oxydase au niveau de la membrane des vacuoles de phagocytose. (Segal, 2005)



La NAPDH oxydase étant aussi présente à la membrane externe des phagocytes, les radicaux oxydants sont aussi relargués dans l'espace extracellulaire lorsque le macrophage est activé. Enfin, la NADPH oxydase est aussi présente à la surface d'autres types cellulaires, en particulier les cellules endothéliales, qui participent ainsi à la production de réactifs oxydants.(Stocker et al., 2004)

Par ailleurs, lors d'une agression induisant une réponse immuno-inflammatoire, les réactifs oxydants présents dans le milieu extra-cellulaire contribuent à l'augmentation du stress oxydant au niveau du foyer inflammatoire.

Radicaux libres oxydants et induction de la réponse inflammatoire

Les radicaux libres oxydants ont été reliés à certaines voies de signalisation cellulaires, impliquées dans la vasodilatation artérielle, l'agrégation plaquettaire et surtout l'apoptose cellulaire.

Par ailleurs, les réactifs oxydants pourraient être à l'origine de l'activation directe du facteur de transcription NFκB.(Stocker et al., 2004) Ce mécanisme relie directement la production de radicaux libres oxydants à la régulation de la réponse inflammatoire, étant donné que le facteur de transcription NFκB régule l'expression de gènes responsables de la production de cytokines inflammatoires (cf. paragraphe Recrutement – intensification page 32)

Les acides gras présents dans les membranes cellulaires peuvent faire l'objet d'une réaction d'auto-oxydation, la fréquence de celle-ci dépendant de l'environnement oxydant et du nombre de doubles liaisons carbone-carbone présente dans leurs chaines. Les PUFAs sont donc plus exposés à ce risque d'auto-oxydation. Par ailleurs, les premières réactions contrôlées d'oxydo-réduction conduisant à la production d'eicosanoïdes peuvent échapper à ce contrôle, et conduire à la propagation des réactions oxydantes le long des chaines carbonées lipidiques.

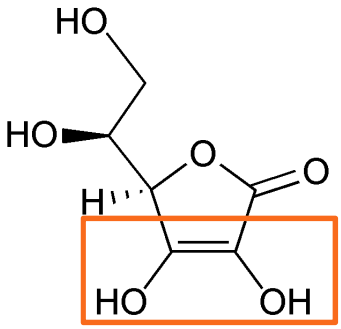
Vitamine C

Structure

L'acide ascorbique ou vitamine C est une vitamine hydrosoluble dérivée des oses qui présente sur ses carbones 2 et 3 une fonction ène-diol.(**Figure 21**) L'organisme humain ne peut synthétiser cette vitamine, étant dépourvu de l'enzyme nécessaire à sa biosynthèse (la gulonolactone oxydase).

La molécune, asymétrique possède deux énantiomères, dont la forme L est la forme naturelle. Elle est présente de façon ubiquitaire dans les liquides biologiques, et, à pH physiologique se présente majoritairement sous sa forme d'ascorbate.

Figure 21 Structure moléculaire de l'acide ascorbique



Fonction ène-diol

Sources

La vitamine C ne pouvant être synthétisée par l'être humain, son origine est essentiellement alimentaire.

Les aliments sources de vitamine C sont essentiellement les fruits et légumes, y compris sous forme de jus (**Tableau 4**). Le stockage et la cuisson affectent considérablement le contenu en vitamine C des aliments.

Tableau 4 Liste des aliments ayant le plus fort contenu en vitamine C – issu de la table de composition des aliments Nutrinet-Santé(Etude Nutrinet-Santé, 2013)

Aliment	Vitamine C (mg) pour 100 g
acérola	1677,6
pomme cajou	252
cassis	187
poivron rouge cru	162
poivron jaune cru	120
poivron vert cru	120
radis noir	114
pétales de riz et blé complet natures (type Spécial K)	100
pétales de riz et blé complet aux fruits (type Spécial K)	90
kiwi	83,2
pétales de riz et blé complet au chocolat (type Spécial K)	81
poivron cuit	74,4
chou romanesco cuit	72,6
litchi frais	71,5
paprika en poudre	71,1
goyave	69,17
chou rave cru	62

Fonctions biologiques

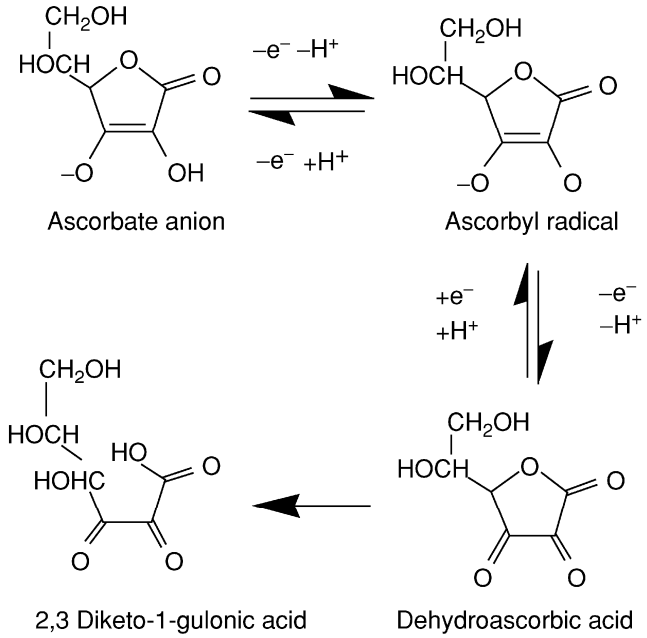
Activité antioxydante

La vitamine C intervient in vivo comme un puissant antioxydant hydrosoluble au niveau intra- et extracellulaire.

L'acide ascorbique est considéré comme la première ligne de défense antioxydante, pouvant réparer ou annuler les réactifs oxydants. (Frei *et al.*, 1989) L'oxydation d'un seul proton conduit à la formation du radical ascorbyl. L'oxydation de deux protons conduit à la formation de l'acide déhydroascorbique. (**Figure 22**) Le radical ascorbyle et l'acide déhydroascorbique peuvent être réduits de nouveau en acide ascorbique via des systèmes enzymatiques dépendant du glutathion, restaurant ainsi le pool de vitamine C disponible dans l'organisme.

L'acide déhydroascorbique peut être hydrolysé de façon irréversible en acide 2,3 dicéto-L-gulonique, dégradé par la suite en acide oxalique et acide thréonique. (**Figure 22**)

Figure 22 Métabolisme de l'acide ascorbique



La vitamine C est très efficace dans la neutralisation des réactifs oxydants primaires (espèces réactives de l'oxygène et de l'azote : radical hydroxyle, anion superoxyde, monoxyde d'azote, et oxygène singulet) et partiellement dans l'élimination des réactifs oxydants secondaires (hypochlorite, radical peroxyle, anion peroxynitrite).(Frei et al., 1989, Stocker et al., 2004) Sa forte activité antioxydante lui permet de prévenir la peroxydation des lipides membranaires en réduisant les réactifs oxydants en phase aqueuse avant l'initiation de la peroxydation des lipides au niveau membranaire. Son activité de réduction de l'acide hypochloreux (activateur de l'α-1-protéinase des neutrophiles) permet de prévenir la protéolyse.(Calder et al., 2009)

Par ailleurs, la vitamine C a la capacité de réduire le radical tocopheroxyle (α-TO·) dans sa forme initiale (α-TOH), ce qui permet d'épargner la vitamine E dans les micelles et les membranes cellulaires.(Halpner *et al.*, 1998, Niki *et al.*, 1995)

Autres fonctions

En dehors de ses capacités antioxydantes faisant partie prenante de la réponse inflammatoire, la vitamine C interviendrait dans la régulation de la synthèse des molécules d'adhésion cellulaires et des prostaglandines.(Horrobin, 1996, Stocker et al., 2004)

Par ailleurs, l'ascorbate joue un rôle de coenzyme pour de nombreuses enzymes engagées dans la formation du collagène, de la carnitine, de neurotransmetteurs et des corticostéroides.

Vitamine E

Structure

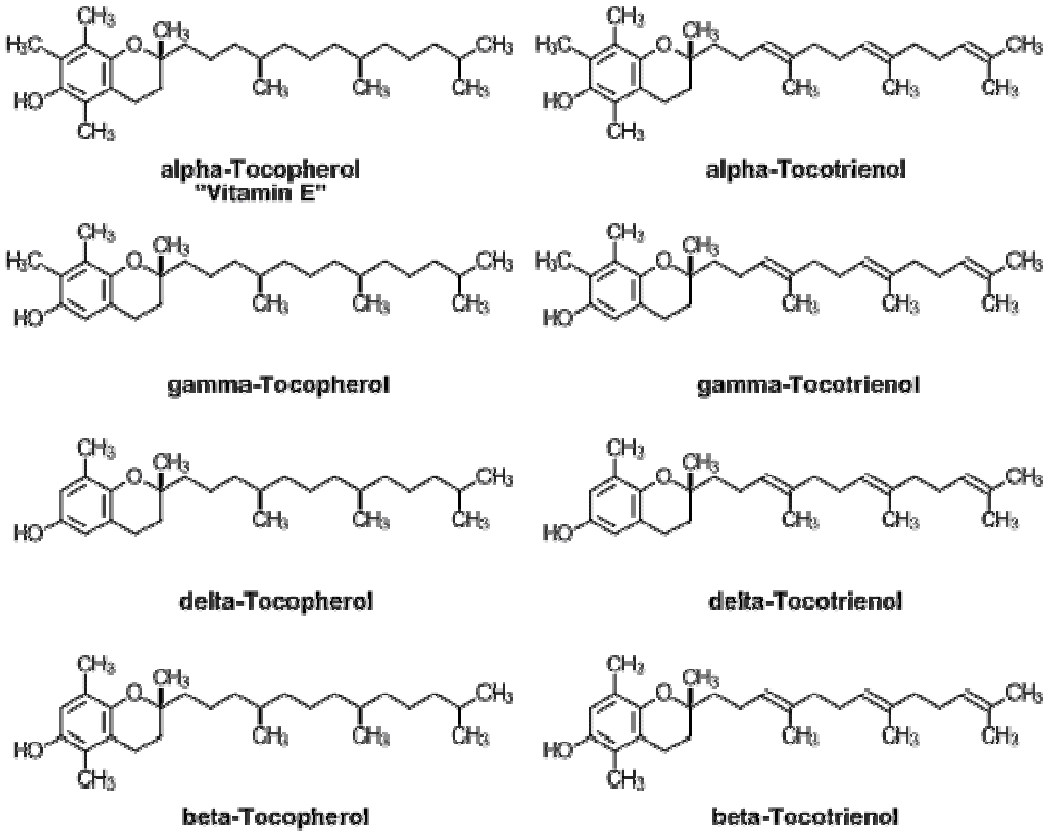
La vitamine E est une vitamine liposoluble comprenant plusieurs composés, les tocophérols (α- à δ-tocophérol) et les tocotriénols (α- à δ-tocotriénol).(**Figure 23**) Ces composés comprennent un noyau chromanol et une chaîne latérale à 16 atomes de carbone. Cette chaine latérale est saturée dans les tocophérols, et présente trois doubles liaisons dans les tocotriénols. Le noyau chromanol présente 1 à 3 groupements méthyle et un groupement hydroxyle, exerçant l'activité antioxydante. Les formes α- à δ- diffèrent par le nombre et la position des groupements méthyles au niveau du noyau chromanol. Ces différences sont responsables de différences dans l'hydrophobicité et de la reconnaissance cellulaire des molécules.

La forme la plus active de la vitamine E est l'α-tocophérol.

La vitamine E étant liposoluble, elle est présente principalement dans les membranes cellulaires et dans les lipoprotéines.

Comme l'acide ascorbique, la vitamine E ne peut être synthétisée dans l'organisme humain.

Figure 23 Structure biochimique de la vitamine E



Sources

La vitamine E est largement présente dans l'alimentation. Les formes de vitamine E présentes dans l'alimentation sont l'α- et le γ-tocophérol. La principale forme alimentaire de vitamine E est le γ-tocophérol, présent à de fortes concentrations dans les huiles végétales et dans les germes de plantes. (**Tableau 5**)

Tableau 5 Principaux groupes contributeurs aux apports alimentaires en Vitamine E – travaux personnels

	Vitamine E (% de l'apport total)
Beurre	2,57
Gâteaux, biscuits et pâtisseries	5,13
Fromage	1,98
Fruits	6,29
Margarines	7,12
Huile d'olive	5,64
Pizza, quiche, tartes salées	2,34
Féculents	2,19
Soupes	3,28
Légumes	6,81
Huiles végétales	34,68

Données obtenues à partir d'un échantillon de sujets issus de l'étude SU.VI.MAX, ayant au moins 3 enregistrements de 24h effectuées dans les deux premières années suivant l'inclusion dans l'étude en 1994-1996

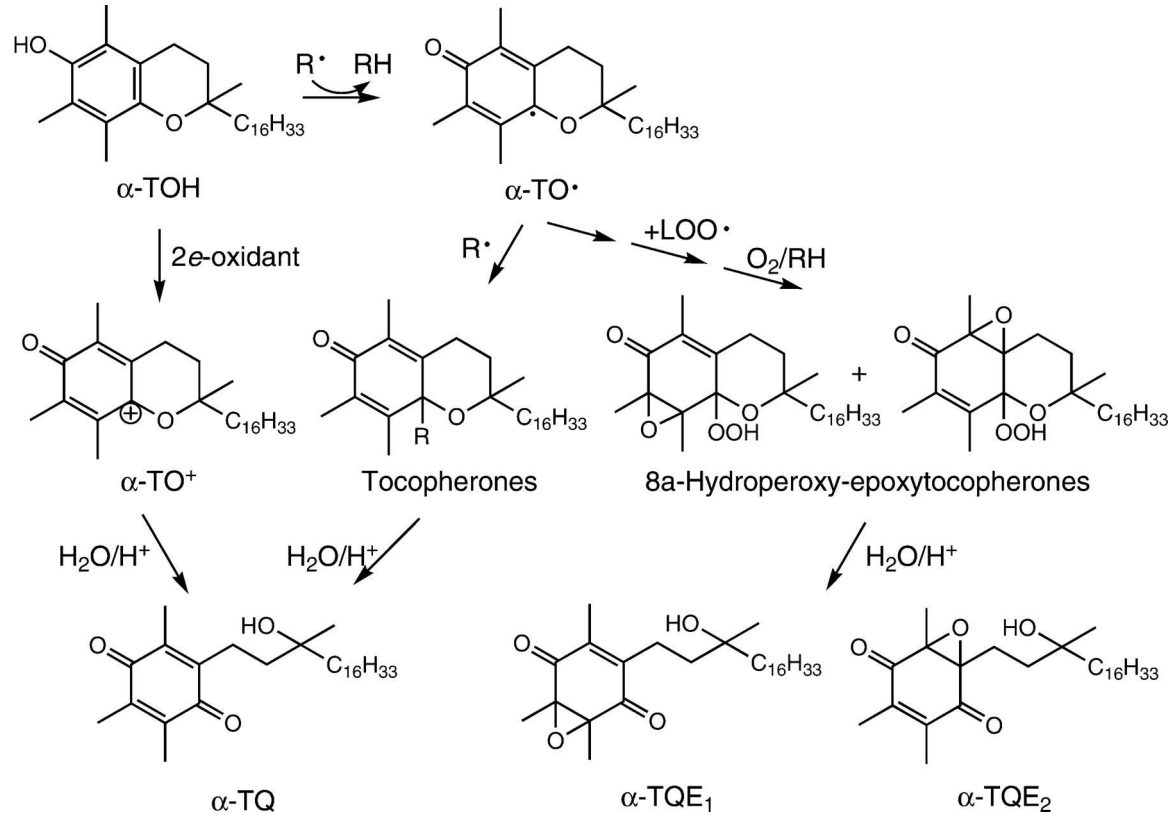
Fonctions biologiques

Activité antioxydante

La réaction d'oxydo-réduction produite par l'α-tocophérol avec un radical oxydant conduit à la formation d'un radical tocopheroxyle (α-TO·) et d'un radical stable non oxydé. L' α-TO· peut en principe réagir une deuxième fois avec une composé radicalaire, conduisant à la formation d'un radical à nouveau non oxydé et des composés quinone ou quinone époxydes, en fonction des radicaux réactifs.(**Figure 24**)

Par ailleurs, la vitamine E peut réagir avec l'oxygène singulet ($^1\Delta gO_2$) et avec certains réactifs oxydants secondaires (hypochlorite et peroxynitrite).

Figure 24 Métabolisme de la vitamine E. (Horrobin, 1996)



TQ : tocopherylquinone TQE : tocopherylquinone epoxyde

La forme α-TO· peut à nouveau être réduite en α-TOH par une réaction avec l'ascorbate, les ubiquinoles, la bilirubine, l'α-tocopheryl hydroxyquinone, l'acide caféique, l'épinéphrine et le 2-hydroxyestradiol.

La vitamine E est un puissant antioxydant, permettant de stopper en particulier les réactions de peroxydation lipidique, de par sa localisation au niveau membranaire. Sa présence au niveau des lipoprotéines permet de prévenir les réactions de peroxydation lipidique dans les LDL et VLDL.

Autres fonctions

En dehors de son activité strictement anitoxydante, la vitamine E diminue l'expression de récepteurs scavengers participant à la formation des cellules spumeuses. (Calder et al., 2009) Par ailleurs, la vitamine E diminue la libération de cytokines pro-inflammatoires, de molécules d'adhésion vasculaire dans les monocytes et d'enzymes de la réaction inflammatoire (protéine kinase C, cyclo-oxygénase).(Azzi, 2004)

Caroténoïdes

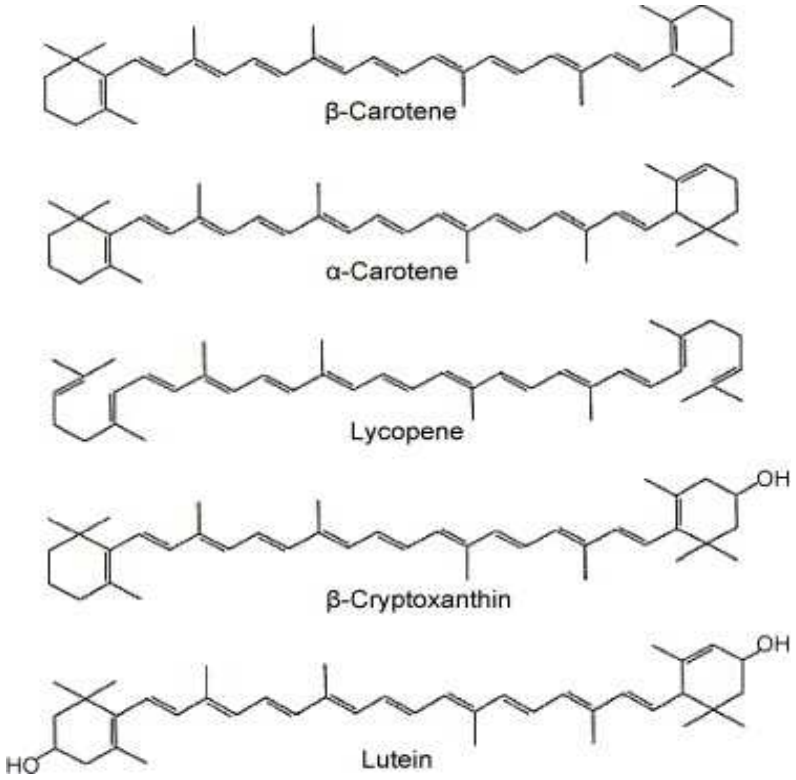
Les caroténoïdes composent une famille complexe comprenant plus de 600 composés. Ce sont des pigments colorés responsable en particulier de la coloration orangée à rouge des fruits et

légumes. Ils ne peuvent être synthétisés par l'organisme humain et proviennent donc de l'alimentation. Ils sont divisés en deux grandes familles : les carotènes (hydrocarbonés uniquement) et les xanthophylles (comprenant des atomes d'oxygène).(Paiva et Russell, 1999)

Structure

Les caroténoïdes sont composés d'un squelette carboné de 40 atomes (**Figure 25**). Ils présentent une structure commune comprenant une structure poly-isoprénoïde, une longue chaine de doubles liaisons carbone conjuguées avec une certaine symétrie autour de la double liaison centrale.(Rao et Rao, 2007) Leurs différences résident principalement dans la cyclisation des groupes terminaux et l'introduction de fonctions alcool.

Figure 25 Structure biochimique des principaux caroténoïdes présents dans l'alimentation humaine. (Rao et al., 2007)



Sources

Parmi les 600 formes de caroténoïdes retrouvés dans les végétaux, seulement une quarantaine sont présents dans l'alimentation humaine. Près de 90% des caroténoïdes de ces derniers sont représentés par l'α-carotène, le β-carotène, le lycopène, la β-cryptoxanthine, la lutéine et la zéaxanthine.(Rao et al., 2007) Les caroténoïdes sont peu altérés par les procédés de conservation ou de cuisson traditionnels (micro-onde, cuisson vapeur ou dans l'eau bouillante), en revanche, ils peuvent être altérés par oxydation lors à température extrême. Les caroténoïdes sont retrouvés principalement dans les végétaux (**Tableau 6**).

Tableau 6 Liste des aliments les plus riches en β-carotène – table de composition des aliments Nutrinet-Santé(Etude Nutrinet-Santé, 2013)

Aliment	β-carotène (µg) pour 100 g
piment rouge	14844
jus de carotte	12376
patate douce	10476
carotte crue	7260
carottes râpées assaisonnées	6534,34
épinard	6288
persil frais	5372
romaine	5230
épinards crus	4600
fines herbes fraîches	4516
blette	3652
cresson	3415
carotte	3340
herbes de provence	3150,33
sarriette deshydratée	3084
purée de carottes	2892,53
mâche	2665
tarte aux épinards	2385,25
laitue	2319
frisée	2289

Fonctions biologiques

Activité antioxydante

Les caroténoïdes ont des propriétés antioxydantes, dans la mesure où ils sont capables de capturer l'oxygène singulet. Cette réaction conduit à la formation de caroténoïdes excités, qui ont la capacité de dissiper l'énergie acquise par une série d'interactions rotationnelles et vibrationnelles avec le solvant, conduisant au retour à une forme stable du composé.(Paiva et al., 1999) L'activité antioxydante des caroténoïdes dépend de leur nombre de doubles liaisons carbone-carbone et de leur terminaison cyclique ou acyclique. Le lycopène, par exemple, qui comprend 11 doubles liaisons conjuguées carbone-carbone et deux doubles liaisons non conjuguées, fait partie des caroténoïdes les plus efficaces. Par cette activité de piégeage de l'oxygène singulet, les caroténoïdes sont capables de prévenir les réactions de peroxydation au niveau des membranes lipidiques.

Activités immunomodulatrices

Le β-carotène inhibe l'activation du facteur de transcription NF-κB au sein des macrophages activés *via* l'inhibition de la dégradation du complexe IκB.(Bai *et al.*, 2005) Cette inhibition conduit à une réduction de la synthèse de cytokines pro-inflammatoires telles que le TNFα et l'IL1-β. Enfin, il stimule les capacités phagocytiques des cellules immunitaires.

Pro-vitamine A

Certains caroténoïdes peuvent être transformés dans l'organisme en rétinol, et ont donc ainsi une activité pro-vitamine A. Il s'agit de l'α-carotène, du β-carotène (source principale) et de la β-cryptoxanthine. Cette transformation a lieu dans l'intestin, selon deux mécanismes : un clivage central produisant deux molécules de rétinol à partir du β-carotène, ou un clivage excentrique, donnant lieu à une molécule de rétinol et à des produits de clivage des caroténoïdes. L'efficacité de la conversion du β-carotène en vitamine A dépend de son apport alimentaire, des apports élevés conduisant à une moindre conversion.

Autres antioxydants

D'autres antioxydants participent aux défenses de l'organisme. En dehors de leur activité d'oxydo-réduction des réactifs oxygénés, ils participent principalement à la régulation de l'immunité acquise, par la régulation de la production, maturation et activité des lymphocytes.

Zinc

Le zinc est un ion métallique présent de façon ubiquitaire au niveau cellulaire et extracellulaire. Il participe à de nombreux processus physiologiques, dont la structure et la stabilisation des membranes cellulaires. Il intervient dans la maturation des cellules immunitaires, lymphocytes principalement.(Prasad, 2009) Il participe à la stabilisation de facteurs de transcription et est un coenzyme pour de nombreuses métalloprotéinases et des enzymes participant aux réactions antioxydantes.(Foster et Samman, 2012) Le déficit en zinc est associé à une altération de la fonction immunitaire.

Dans l'inflammation, la distribution du zinc est modifiée, principalement en faveur du compartiment cellulaire, où le zinc participe à la production protéique et à des réactions antioxydantes. Cette modification de la distribution du zinc pourrait être médiée par les cytokines inflammatoires (TNFα, IL1, IL6).

Chez l'homme, la supplémentation en zinc produit une diminution de la production d'ARN de cytokines pro-inflammatoires, ex vivo et dans des modèles cellulaires.(Foster et al., 2012, Prasad, 2009) A l'inverse, un environnement déficient en zinc conduit à une surproduction de cytokines.(Foster et al., 2012)

Sélénium

Le sélénium est un oligoélément participant en tant que co-enzyme aux défenses antioxydantes.(Rayman, 2000, Stocker et al., 2004) En particulier, le sélénium participe en tant que cofacteur de réduction pour les glutathione peroxydases, permettant de réduire les peroxydes (hydrogène ou peroxydes lipidiques). Par la réduction des produits intermédiaires dans la voie de production des COX et des LOX, le sélénium peut moduler la production des eicosanoïdes.(Rayman, 2000)

La carence en sélénium est responsable d'une altération de la fonction immunitaire, principalement des lymphocytes.

Tableau 7 Principales actions des principaux nutriments antioxydants en rapport avec l'inflammation

Antioxydant	Activité
Vitamine C	Neutralisation des réactifs oxydants primaires de l'oxygène et de l'azote
	Prévention de la peroxydation des lipides membranaires
	Réduction du radical α-tocophéroxyle dans sa forme initiale active α-tocophérol
	Régulation de la synthèse de molécules d'adhésion cellulaires et prostaglandines
Vitamine E	Neutralisation des réactifs oxydants
	Prévention de la peroxydation des lipides membranaires
	Régulation de l'expression des récepteurs scavengers
	Régulation de la libération de cytokines inflammatoires, de molécules d'adhésion vasculaire et d'enzymes (protéine kinase C, COX)
Caroténoïdes	Neutralisation des réactifs oxydants
	Prévention de la peroxydation des lipides membranaires
	Régulation du facteur de transcription NFκB
	Régulation de la production de cytokines pro-inflammatoires
	Stimulation des capacités phagocytiques des cellules immunitaires

4.3. Alimentation dans sa globalité

Au-delà de l'effet de certains nutriments spécifiques dont les propriétés biochimiques ont été étudiées dans le cadre de l'inflammation, la prise en compte de l'alimentation dans sa globalité représente une approche complémentaire dans l'étude des relations entre nutrition et inflammation. En effet, les nutriments ne sont pas consommés seuls, mais sous forme d'aliments comprenant des combinaisons complexes de nutriments comportant des interactions et des synergies potentielle, au sein de repas.(Hu, 2002a) Par ailleurs, les corrélations existantes entre nutriments au sein d'un même aliment rendent difficile la possibilité de séparer complètement leurs effets propres au sein d'études. Enfin les effets propres de chaque nutriment peuvent être trop faibles pour être détectables, mais l'effet combiné de ceux-ci au sein de profils alimentaires peuvent en revanche être interprétables.

L'étude du comportement alimentaire dans sa globalité a enfin comme avantage de permettre une interprétation directe, pouvant donner lieu à de recommandations nutritionnelles

Deux types de méthodes ont été développées afin d'évaluer les effets des apports alimentaires dans leur globalité : des méthodes dites *a priori* et d'autres dites *a posteriori*.

Méthodes a priori

Les méthodes dites a priori prennent en compte des hypothèses issues de la littérature scientifique sur les relations entre la nutrition et les maladies, catégorisant ainsi les aliments comme favorables ou défavorables à partir de critères prédéfinis. Ils proposent la construction de scores, représentant l'adéquation des sujets soit à des recommandations nutritionnelles (recommandations nutritionnelles françaises du Programme National Nutrition Santé pour le PNNS-GS, recommandations américaines pour le Dietary Guidelines for Americans Adherence Index (DGAI) et le Healthy Eating Index (HEI), par exemple) soit à un type d'alimentation supposée bénéfique pour la santé (alimentation méditerranéenne). Des points positifs sont attribués pour les groupes alimentaires considérés comme bénéfiques, et des points négatifs pour les groupes alimentaires considérés comme néfastes. L'une des limites de ces scores est le fait qu'ils ne tiennent pas forcément compte des apports énergétiques des individus, et peuvent donc donner lieu à des scores élevés pour des individus ayant des apports supérieurs à leurs besoins énergétiques. Par ailleurs leur construction fait souvent appel à des systèmes de pondérations des composantes (nombre de points par item, pénalités et/ou bonus) qu'il convient de justifier.

L'établissement des points du score étant réalisé a priori, ces méthodes sont reproductibles quelle que soit la population d'étude, et permettent une comparaison entre populations.

Méthodes a posteriori

Les méthodes dites a posteriori reposent sur des méthodes statistiques permettant de prendre en compte les corrélations entre les apports alimentaires observées au niveau de la population d'enquête. Celles-ci peuvent permettre de construire des scores dans la population ou de catégoriser les individus en groupes distincts (clusters) en fonction de leur alimentation. Elles reposent sur des méthodes telles que l'analyse en composante principale et l'analyse factorielle en ce qui concerne les scores, et l'analyse en cluster pour la catégorisation des individus. (Hu *et al.*, 1999, Hu, 2002b)

L'analyse en composante principale maximise la variation expliquée dans les apports en groupes alimentaires au niveau de l'échantillon sélectionné. Une nouvelle méthode statistique, la régression des rangs réduits (RRR), permet de construire des scores alimentaires (sur des groupes alimentaires) spécifiquement associés à certains éléments de réponse (soit nutriments soit biomarqueurs intermédiaires de risque).(Hoffmann *et al.*, 2004a) Cette procédure maximise la variation expliquée dans les éléments de réponse, permettant ainsi de tester certaines hypothèses mécanistiques de la relation entre nutrition et pathologies.

Etant construits à partir des corrélations entre aliments observées dans la population d'étude, les scores dérivés de ces méthodes ne sont pas entièrement reproductibles entre populations.

Alimentation dans sa globalité et inflammation

Cet aspect a été plus longuement détaillé dans un article « Dietary patterns and risk of elevated C reactive protein concentrations 12 years later », page 140.

Plusieurs études ont évalué les relations entre alimentation dans sa globalité et inflammation chronique. Les études réalisées dans ce domaine sont pour la plupart de méthodologie transversale et utilisent des méthodes d'analyse factorielle. De plus, l'inflammation n'y est pas toujours étudiée en tant que telle, mais en tant que l'un des facteurs de risque cardiovasculaire. Le Tableau 8 reprend les éléments de la littérature relatifs aux relations étudiées entre profils alimentaires construits selon plusieurs méthodologies et la CRP.

Dans l'ensemble, ces études montrent que les profils alimentaires 'Sains' (Healthy) riches en fruits et légumes et en poissons, et pauvres en charcuterie sont négativement associés aux paramètres inflammatoires. Inversement, les profils alimentaires de type 'Western' riches en produits transformés et en viandes et pauvres en fruits et légumes sont positivement associés aux paramètres inflammatoires.(Tableau 8)

Une récente revue de la littérature sur ces éléments conclue de façon similaire que les profils alimentaires de type 'Western', étaient positivement associés aux biomarqueurs inflammatoires,

alors que les scores a priori, et les profils alimentaires de type 'Healthy' étaient négativement associés aux biomarqueurs inflammatoires. Les résultats retrouvés par les études étaient en effet concordants, malgré les limites inhérentes à la comparaison directe des scores obtenus par méthodes a posteriori.(Barbaresko *et al.*, 2013)

Tableau 8 Résultats synthétiques des études portant sur l'association entre alimentation prose dans sa globalité et CRP

Auteur, date (référence)	N	Population	Méthode mesure exposition alimentaire	Exposition	Résultat
Scores a priori					
Cavicchia, 2009 (Cavicchia *et al.*, 2009)	494	Echantillon Italien	Score a priori pro-inflammatoire, élaboré à partir d'une revue de la littérature		Association négative S
Chrysohoou, 2004 (Chrysohoou *et al.*, 2004)	3042	Etude ATTICA		Score méditérranéen	Association négative S
Carter, 2010 (Carter *et al.*, 2010)	13197	Etude Nhanes		Score méditérranéen	Association négative S Hommes ≥45 ans, NS Hommes<45 ans et femmes
Analyse en composante principale					
Fung, 2001 (Fung *et al.*, 2001)	466	Health professionals follow-up study	Fréquentiel alimentaire, 130 items	Profil 'Prudent'	Association NS
				Profil 'Western'	Association positive S
Lopez-Garcia, 2004 (Lopez-Garcia *et al.*, 2004a)	732	Nurse's Health Study	Fréquentiel alimentaire, 116 items	Profil 'Prudent'	Association négative S
				Profil 'Western'	Association positive S
Nettleton, 2008 (Nettleton *et al.*, 2008)	5001	Multiethnic study of atherosclerosis	Fréquentiel alimentaire, 120 items	Profil 'High fat and processed meat'	Association positive S
				Profil 'Vegetables and Fish'	Association NS
				Profil 'Bean, Tomato and refined grain'	Association NS
				Profil 'Whole grain and Fruit'	Association négative S
Esmaillzadeh, 2007 (Esmaillzadeh *et al.*, 2007)	486	Enseignantes de Téhéran	Fréquentiel alimentaire, 168 items	Profil 'Healthy'	Association négative S
				Profil 'Western'	Association positive S
				Profil 'Traditional'	Association NS

S Significatif ; NS Non Significatif

	N	Population	Méthode mesure exposition alimentaire	Exposition	Résultat
Nanri, 2011 (Nanri *et al.*, 2011)	Hommes=3905; Femme=5640	Echantillon japonais	Fréquentiel alimentaire, 46 items	Profil 'Healthy'	Association négative S hommes et femmes
				Profil 'Western'	Association positive NS hommes et S femmes
				Profil 'Seafood'	Association positive S hommes et NS femmes
				Profil 'Bread'	Association négative S hommes et femmes
				Profil 'Dessert'	Association positive S hommes et NS femmes
Centritto, 2009 (Centritto *et al.*, 2009)	7646	Echantillon Italien	Fréquentiel alimentaire, 188 items	Profil 'huile d'olive et légumes"	Association négative S
				Profil 'Pâtes et viande'	Association positive S
				Profil 'œufs et sucreries'	Association positive S
Villegas, 2011 (Villegas *et al.*, 2010)	3646	Echantillon Shanghai	Fréquentiel alimentaire, 81 items	Profil 'Légumes'	Association négative NS
				Profil 'Fruits'	Association négative S
				Profil 'Viande'	Association positive NS
Eilat, 2009 (Eilat-Adar *et al.*, 2009)	1066	Echantillon Esquimos	Fréquentiel alimentaire, 97 items	Profil 'Traditional'	Association négative NS
				Profil 'Western'	Association NS
				Profil 'Purchased healthy'	Association positive NS
				Profil 'Beverages and sweets'	Association négative S
Meyer, 2011 (Meyer *et al.*, 2011)	981	MONICA Echantillon allemand	journal alimentaire 7 jours	Profil 'ACP'-type Western	Association positive S
Nettleton, 2007 (Nettleton *et al.*, 2007)	5089	Multiethnic study of atherosclerosis	Fréquentiel alimentaire, 120 items	Profil 'High fat and processed meat'	Association positive S

	N	Population	Méthode mesure exposition alimentaire	Exposition	Résultat
Pierce, 2007(Pierce *et al.*, 2007)	250x2 (deuxième et troisième générations)	Echantillon de descendants d'immigrants japonais aux USA	FFQ	Profil 'Western'	Association positive S chez les troisième génération
Mikkilä, 2007 (Mikkilae *et al.*, 2007)	1037	Echantillon Finlandais	Rappels des 24h	Profil 'Traditional' Profil 'Health Conscious'	Association positive S Association positive S femmes, NS hommes

S Significatif ; NS Non Significatif

OBJECTIFS

L'objectif général de ce travail de thèse était d'étudier, dans le cadre d'études épidémiologiques prospectives, les relations entre nutrition et inflammation.

Dans le cadre de l'inflammation, ont été étudiés de façon plus approfondie les nutriments pour lesquels un lien fort avec l'inflammation a été montré par des études in vitro et des modèles animaux, à savoir les nutriments antioxydants (via leur statut biologique) ainsi que les acides gras polyinsaturés (n-3 et n-6)

Nous nous sommes aussi intéressés à la mesure de l'exposition nutritionnelle dans sa globalité *via* des méthodes a posteriori, telle que la Régression des rangs réduits (RRR).

Le critère de jugement étudié correspondait à l'inflammation de bas grade en tant que telle, mesurée par la concentration sanguine en CRP.

Les données dont ces travaux font l'objet sont issues des études SU.VI.MAX et SU.VIMAX 2. L'étude SU.VI.MAX est un essai randomisé en prévention primaire destiné à évaluer l'impact d'une supplémentation en nutriments antioxydants à doses nutritionnelles sur la survenue de maladies cardio-vasculaires et de cancer, débuté en 1994-1995. Nous avons tout d'abord étudié l'effet de la supplémentation en nutriments antioxydants sur la CRP mesurée 12 années après. Les résultats de ces analyses étant non-concluantes, nous nous sommes tournés vers l'exploration d'autres expositions nutritionnelles sur la CRP.

En premier lieu, nous avons étudié les relations entre les antioxydants et l'inflammation au travers des associations entre le statut en antioxydants et la concentration en CRP 12 années après.

Julia C, Galan P, Touvier M, Meunier N, Papet I, Sapin V, Cano N, Faure P, Hercberg S, Kesse-Guyot E. Antioxidant status and risk of elevated C-reactive protein 12 years later. Soumis à ***Clinical Nutrition***

Puis, nous avons étudié les relations entre les apports alimentaires en acides gras polyinsaturés des familles n-3 et n-6 et la concentration en CRP mesurée plusieurs années après, ainsi que le potentiel effet modulateur des apports en vitamine E dans cette relation

Julia C, Touvier M, Meunier N, Papet I, Galan P, Hercberg S, Kesse-Guyot E. Long-term associations between PUFAS intakes and C-Reactive Protein plasma levels and interaction with vitamin E intake. Accepté pour publication dans ***Journal of Nutrition***

Dans un troisième temps, nous avons pu étudier les relations entre un pattern alimentaire spécifiquement associé aux apports en nutriments pro- et anti-inflammatoires (établi par RRR) et la concentration en CRP 12 années après la mesure de l'exposition nutritionnelle.

Julia, C., Meunier, N., Touvier, M., Ahluwalia, N., Sapin, V., Papet, I, Cano, N., Hercberg, S., Galan, P, Kesse-Guyot, E. Dietary patterns and risk of elevated CRP concentrations 12 years later. ***British Journal of Nutrition****. 2013 Jan 10:1-8. [Epub ahead of print]*

Ces trois publications sont les publications principales rattachées à ce travail de thèse.

Deux autres publications en premier auteur, réalisées lors de mon internat de santé publique, ont développé mon intérêt pour l'épidémiologie nutritionnelle d'une part, et pour la thématique de la relation entre nutrition et inflammation d'autre part. C'est à partir de ces travaux initiaux que mon travail de thèse sur les approches épidémiologiques de la relation entre nutrition et inflammation a pu être développé. Elles sont présentées dans les Publications Annexes à ce document (Page 143).

Ainsi, j'ai aussi travaillé sur les relations entre le score d'adéquation aux recommandations du PNNS et la présence d'un syndrome métabolique au sein de l'Etude Nationale Nutrition Santé (ENNS).

Julia, C., Vernay, M., Salanave, B., Deschamps, V., Malon, A., Oleko, A., Hercberg, S., Castetbon, K.Nutrition patterns and metabolic syndrom: a need for action in young adults. ***Preventive Medicine****. 2010 Dec;51(6):488-93.*

Par ailleurs, la thématique sur laquelle j'ai travaillé m'a permis de participer à l'étude des relations entre des biomarqueurs pré-diagnostiques d'inflammation, de fonction endothéliale et d'adiposité et des pathologies comme le diabète, le cancer et les maladies cardio-vasculaires

Julia, C., Czernichow, S., Charnaux, N., Ahluwalia, N., Andreeva, A., Touvier, M., Galan, P., Fezeu, L. Relationships between adipokines, biomarkers of endothelial dysfunction and inflammation and risk of type 2 diabetes in the SU.VI.MAX cohort. En cours de Révision ***Diabetes Research and Clinical Practice***

En dehors de cette publication en premier auteur, j'ai pu participer à d'autres travaux de l'équipe portant sur la relation prospective entre biomarqueurs de l'inflammation et survenue de pathologies (cancer et maladies cardiovasculaires).

Touvier M, Fezeu L, Ahluwalia N, Julia C, Charnaux N, Sutton A, Méjean C, Latino-Martel P, Hercberg S, Galan P, Czernichow S. Association between prediagnostic biomarkers of inflammation and endothelial function and cancer risk: a nested case-control study. ***Am J Epidemiol****. 2013 Jan 1;177(1):3-13. doi: 10.1093/aje/kws359. Epub 2012 Nov 20.*

Touvier M, Fezeu L, Ahluwalia N, Julia C, Charnaux N, Sutton A, Méjean C, Latino-Martel P, Hercberg S, Galan P, Czernichow S. Pre-diagnostic levels of adiponectin and soluble vascular cell adhesion molecule-1 are associated with colorectal cancer risk. ***World J Gastroenterol****. 2012 Jun 14;18(22):2805-12.*

Ahluwaliaa; A., Blacher, J., Szabo de Edelenyi F., Faure P., Julia C.; Hercberg, S., Galan P., Prognostic value of multiple emerging biomarkers in cardiovascular risk prediction in patients with stable cardiovascular disease. ***Atherosclerosis*** *2013 (in press)*

RESULTATS

Les résultats de mon travail de thèse sont présentés au travers des articles scientifiques publiés ou en cours de publication.

Chaque article est précédé d'une synthèse reprenant les principaux éléments du travail : contexte, objectifs, matériel et méthodes résultats, discussion, conclusion

Antioxidant status and risk of elevated C-reactive protein 12 years later

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Synthèse

Introduction

Les antioxydants sont des molécules ayant de fortes propriétés anti-inflammatoires.(Calder et al., 2009, Conner et Grisham, 1996, Cunningham-Rundles *et al.*, 2005, Hensley *et al.*, 2000, Sies, 1997) Au-delà de l'effet associé aux apports alimentaires en ces nutriments, il semblait intéressant de s'attacher à étudier les relations entre le statut en antioxydants (mesuré par la concentration sérique de ses marqueurs) et l'inflammation.

Peu d'études observationnelles se sont intéressées à cette problématique et la plupart d'entre elles ont étudié la relation entre statut sanguin en antioxydants et inflammation de façon transversale.(Beydoun *et al.*, 2011, Erlinger *et al.*, 2001, Ford *et al.*, 2003, Kritchevsky *et al.*, 2000, Morris *et al.*, 2010, Singh *et al.*, 2005) Une seule étude a étudié l'effet longitudinal du statut en antioxydants, montrant une relation inverse entre le statut en caroténoïdes et des marqueurs d'inflammation de bas grade.(Hozawa *et al.*, 2007)

Notre objectif était donc d'étudier l'association à long terme entre statut en nutriments antioxydants (β-carotène, vitamine C et α-tocophérol) et le fait d'avoir une CRP plasmatique augmentée (≥3mg/l) plusieurs années après.

Matériel et Méthodes

Les sujets éligibles dans cette étude étaient les sujets inclus dans l'étude SU.VI.MAX ayant poursuivi leur suivi par une inclusion dans l'étude SU.VI.MAX 2 (Hercberg *et al.*, 1998, Hercberg *et al.*, 2004) et pour lesquels les éléments suivants étaient disponibles :

- Mesure d'exposition : présence d'un dosage biologique sanguin de β-carotène, vitamine C et α-tocophérol lors de la visite initiale en 1994-1996.
- Mesure du critère de jugement : présence d'une mesure de la CRP réalisée pour un sous-échantillon lors de l'examen clinique effectué pour l'étude de suivi SU.VI.MAX2 (entre 2007 et 2009)
- Mesure des facteurs d'ajustement : variables socio-démographiques (sexe, âge), de mode de vie (statut tabagique, activité physique) et d'anthropométrie (poids et taille mesurés lors d'un examen clinique à un an de l'inclusion dans l'étude SU.VI.MAX 1995-1997), apports alimentaires (alcool) ainsi que l'affectation initiale dans le groupe placebo ou le groupe de supplémentation de la phase initiale d'essai randomisé de l'étude SU.VI.MAX.

Les sujets ayant des niveaux extrêmes de concentrations en nutriments antioxydants et les sujets ayant une CRP>10mg/l (considérée comme de l'inflammation aiguë) ont été exclus des analyses.(Pearson et al., 2003)

Les quintiles des concentrations en nutriments antioxydants ont été calculés pour la totalité de la population. Les sujets ayant une CRP>3mg/l ont été considérés comme ayant une CRP augmentée.(Pearson et al., 2003)

Les associations entre quintiles de concentrations en nutriments antioxydants et CRP augmentée ont été étudiés par régressions logistiques uni- et multivariés. De potentiels effets modulateurs ont été recherchés pour les facteurs suivants : sexe, groupe de supplémentation pendant la phase d'essai de l'étude SU.VI.MAX initiale, tabagisme, apports en alcool et statut par rapport au surpoids et à l'obésité au début de l'étude.

Des études de sensibilité ont été effectuées :

- Répétition des analyses principales après exclusion des sujets susceptibles d'avoir eu une CRP augmentée à l'inclusion : sujets obèses et diabétiques
- Répétition des analyses principales en prenant en tant que concentrations sanguines de références les concentrations en antioxydants à l'année 6 (plutôt qu'à l'inclusion), afin d'évaluer les relations existant à moyen terme entre concentrations en antioxydants et CRP augmentée

Résultats

Parmi les 6850 sujets inclus dans l'étude de suivi SU.VI.MAX2, 3476 avaient effectué une mesure de CRP. Parmi ceux-ci, 2031 ont été inclus dans l'étude. Après exclusion des sujets ayant des valeurs extrêmes de concentrations en antioxydants, le nombre de sujets disponibles était le suivant :

- 2047 pour l'étude de la relation entre β-carotène et CRP
- 2060 pour l'étude de la relation entre α-tocophérol et CRP
- 1719 pour l'étude de la relation entre vitamine C et CRP

Aucune association n'était retrouvée entre le groupe de supplémentation dans l'essai initial SU.VI.MAX et la CRP augmentée, que ce soit pour l'ensemble des sujets (OR univarié 0.96, IC95% : 0,74-1,22 (P=0,72)), ou pour les analyses en sous-groupes.

Le statut en β-carotène était négativement associé à la CRP augmentée, dans les modèles univariés ($P_{\text{tendance linéaire}}$ <0.001) et multivariés ($P_{\text{tendance linéaire}}$ <0.001). Les statuts en vitamine C et en α-tocophérol n'était pas significativement associés à la CRP augmentée.

La stratification selon des variables en lien avec le statut en antioxydants a montré que l'association entre la concentration en β-carotène et la CRP augmentée était significative chez les femmes seulement ($P_{\text{tendance linéaire}}$ = 0.004, P d'interaction =0.30), chez les sujets dans le groupe de

supplémentation actif pendant la phase d'essai randomisé de l'étude SU.VI.MAX ($P_{tendance\ linéaire}$ = 0.002, $P_{interaction}$ = 0.03), chez les sujets non-fumeurs ($P_{tendance\ linéaire}$ = 0.01, $P_{interaction}$ = 0.51) et chez les sujets ayant un IMC normal à l'inclusion ($P_{tendance\ linéaire}$ = 0.004,$P_{interaction}$ = 0.19).

Les analyses de sensibilité réalisées après exclusion des sujets susceptibles d'avoir une CRP augmentée à l'inclusion (sujets obèses et diabétiques) n'ont pas modifié les résultats.

De plus, les mêmes analyses, répétées en prenant en compte les concentrations en nutriments antioxydants à l'année 6 plutôt qu'à l'année 12 ont donné des résultats similaires. Le fait que les associations observées soient maintenues suggère que nos résultats pourraient refléter des effets à moyen et long terme du statut en β-carotène sur la CRP augmentée.

Discussion

Nos résultats montrent que le statut en β-carotène est négativement associé à la CRP augmentée. Cet effet est dose-réponse et perdure dans le temps. L'association entre β-carotène et CRP augmentée était plus importante chez les femmes, chez les sujets supplémentés pendant l'étude SU.VI.MAX (8 ans), chez les non-fumeurs et chez les sujets dont l'IMC était normal à l'inclusion. Les statuts en vitamine C et α-tocophérol n'étaient pas associés à l'inflammation de bas grade.

Nos résultats sont similaires à ceux de l'étude observationnelle CARDIA/YALTA, qui a montré une association négative à long terme (8 à 15 ans) entre le statut en caroténoïdes total (α- et β-carotène, β-cryptoxanthine, zéaxanthine/lutéine) et des biomarqueurs inflammatoires (CRP, leucocytes, IL6, TNFα). Ces résultats concordants sont en faveur de l'existence d'une association négative à long terme entre le statut en caroténoïdes et l'inflammation de bas grade.(Hozawa et al., 2007)

En dehors de cette étude, les associations entre statut en antioxydants et inflammation dans les études observationnelles ont été analysées à partir de données transversales, sous l'hypothèse initiale que l'inflammation est responsable d'une diminution de la concentration en antioxydants.(Beydoun et al., 2011, Erlinger et al., 2001, Ford et al., 2003, Kritchevsky et al., 2000, Morris et al., 2010, Singh et al., 2005) Nos résultats montrent que la relation inverse est aussi importante, l'inflammation pouvant être la conséquence à long terme de statut bas en β-carotène. En effet, en dehors de leurs propriétés liées à la capture des réactifs de l'oxygène, les caroténoïdes ont des propriétés indépendantes de stimulation de l'immunité.

Nos résultats montrent aussi que cette association est plus importante chez les femmes, chez les non-fumeurs et chez les sujets supplémentés pendant 8 ans pendant la période de l'essai SU.VI.MAX. L'association entre β-carotène et CRP augmentée chez les femmes serait liée à leur niveau de concentration plus élevé en antioxydants.

En ce qui concerne le statut tabagique, certaines études ont montré un effet délétère de la supplémentation en β-carotène sur l'état de santé chez les fumeurs.(Albanes *et al.*, 1996, Goralczyk, 2009, Omenn *et al.*, 1996) Le tabagisme altèrerait le métabolisme du β-carotène et augmenterait le potentiel carcinogène des dérivés du tabac.(Russell, 2004) Enfin, les non-fumeurs ont tendance à avoir des concentrations sanguines plus faibles en antioxydants.(Hercberg et al., 2004) Ces facteurs combinés pourraient expliquer nos résultats stratifiés sur le statut tabagique.

Dans cette perspective, les résultats observés lors de la stratification sur le groupe de supplémentation pourraient être expliqués par le fait que la supplémentation aurait augmenté les concentrations sériques en antioxydants pour l'ensemble de la population, mais n'auraient atteint une concentration protectrice que chez ceux ayant déjà un niveau élevé en β-carotène, avec un effet seuil. Une explication alternative serait que la variabilité dans le statut en β-carotène obtenue par supplémentation est moins importante que celle obtenue par l'alimentation. La supplémentation seule ne permettrait donc pas d'obtenir une modification du statut suffisante pour observer un effet sur l'inflammation. En revanche, la variabilité combinée obtenue par la supplémentation et par l'alimentation expliquerait les résultats.

Les forces de notre étude reposent sur l'inclusion et le suivi longitudinal de sujets avec un suivi de plus de douze ans. De plus, les analyses ont pris en compte les effets de nombreuses variables de confusion potentielles permettant de limiter l'effet de confusion résiduel. Enfin, les analyses stratifiées ont permis de tester d'éventuels modulateurs de l'effet observé entre statut en antioxydants et CRP.

Les points faibles de notre étude résident dans l'absence de dosage de la CRP à l'inclusion, ne permettant pas d'exclure les sujets ayant une CRP augmentée dès l'inclusion. Les résultats des analyses de sensibilité, excluant les sujets susceptibles d'avoir une CRP augmentée confortent néanmoins la stabilité de nos résultats. De plus, nous n'avions pas accès à la CRP ultrasensible, notre dosage ayant un niveau minimal de détection de 1mg/l. Néanmoins, ce niveau de détection a permis de classer les sujets selon leur risque cardiovasculaire.

Conclusion

Nos résultats permettent d'augmenter le niveau des connaissances de la relation entre statut en antioxydants et inflammation de bas grade, en mettant en évidence une association négative entre β-carotène et CRP augmentée. Néanmoins, des études longitudinales prenant en compte l'aspect dynamique de l'inflammation et de la concentration en antioxydants permettraient de confirmer les hypothèses soulevées.

Manuscrit

Abstract

Low-grade inflammation is an independent risk factor for cardiovascular disease, for which nutritional status is a potentially modifiable determinant. Relationships between antioxidant status, having demonstrated consistent anti-inflammatory characteristics, and inflammatory biomarkers could give new insights in cardiovascular diseases prevention. Our objective was to investigate long-term associations between antioxidant nutrients (vitamin C, α-tocopherol, β-carotene) status and elevated blood C-reactive protein (CRP) in a population-based cohort. Differential effects were examined in subgroup analyses. Subjects included in the SU.VI.MAX trial study who had available data on baseline (1994-1995) blood nutrient concentrations and CRP measurements 12 years later (follow-up examination 2007-2009) were included. Associations between baseline β-carotene, α-tocopherol and vitamin C circulating concentrations and elevated CRP (>3mg/l) were investigated in multivariate logistic regression models. Subgroup analyses were performed according to gender, supplementation group of the initial trial, smoking status and alcohol intake. Serum α-tocopherol and vitamin C concentrations (Odds ratios (OR) and 95% confidence intervals (95%CI) quintile 5 vs.1: 1.24 (0.81;1.89) P for trend : 0.382; 0.86 (0.55;1.35); P for trend :0.262 respectively) were not associated to elevated CRP concentrations. β-carotene status was inversely associated to elevated CRP: adjusted OR quintile 5 vs. 1: 0.70 (0.46;1.08); P for trend 0.048. Subgroup analyses showed that associations were stronger in women (P for trend across quintiles=0.03), never smokers (P for trend across quintiles =0.02) and subjects in the supplementation group (P for trend across quintiles =0.01). Our results suggest that β-carotene status may be inversely associated with low-grade inflammation in the long-term.

Introduction

Low-grade systemic inflammation is considered a novel and independent risk factor for several chronic diseases, and has been more specifically investigated in the fields of cardiovascular and metabolic diseases.[1,2] Elevated serum levels of C-reactive protein (CRP), reflecting ongoing systemic inflammatory processes, have been found to be related to onset of cardiovascular disease and cardiovascular mortality.[3] Biomarkers of inflammation, have also been reported to be independently related to cardiovascular death after a first event.[4]

In this context, investigating factors involved in low-grade inflammation is of major interest. Indeed, if low-grade inflammation is one of the underlying pathways for cardiovascular disease, identifying modifiable risk factors for it could lead to new avenues in disease prevention.

Micronutrients qualify as a potential modifiable determinant of inflammation. Indeed, they appear to be involved in all stages of the inflammatory response: inflammatory cytokine production, regulation of immune system cell functions and scavenging by-products of activated leucocytes.[5–7]

Among all nutrients involved in inflammatory response, antioxidant micronutrients, which participate in scavenging oxidant species or prevent lipid peroxidation, [8] have consistent anti-inflammatory properties. Generation of free oxygen radicals (e.g. superoxide radicals, hydrogen peroxide, referred as oxidants) is part of the host inflammatory response: they are produced by activated leucocytes at the site of inflammation, damage cell components (cell membranes particularly) and induce production of inflammatory cytokines (through the activation of the transcription factor NF-κB involved in the regulation of inflammatory genes expression by oxidized cell components).[1]

Vitamin C, a hydrophilic vitamin, has a high reducing power, and acts both intra- and extra-cellularly to scavenge reactive oxygen and nitrogen species, even before they initiate lipid peroxidation.[9] Vitamin C also has the ability to spare or regenerate other antioxidants (vitamin E and glutathione) back to their active forms.[10,11] Vitamin E and carotenoids, which act mostly as membrane antioxidants, due to their lipophilic properties, limits the generation of oxygen and nitrogen active species and the propagation of oxidant initiated reactions. [8,12]

Beside their main scavenging properties, anti-oxidant micronutrients are also independently involved in other stages of the inflammatory response: vitamin C modulates prostaglandins synthesis[13]; vitamin E is involved in regulating pro-inflammatory cytokine release, vascular adhesion of monocytes and activities of several inflammatory enzymes (cyclo-oxygenase, protein kinase C among others)[14]; β-carotene mediates the gene expression of the redox-

sensitive transcription factor NF-κB, stimulates phagocytic and bacteria-killing abilities of immune cells, and regulates cytokine production. [15]

Several cross-sectional studies have reported a negative association between circulating concentrations of antioxidant micronutrients and inflammatory biomarkers. Results from the NHANES study consistently reported negative associations in particular between carotenoids circulating concentrations and CRP.[16–19] However, given the cross-sectional nature of the data, directionality of the observed associations was uncertain: either inflammation depressed concentration of some vitamins or poor levels of antioxidant defenses could prospectively promote inflammation. Results from a single long-term study (the CARDIA/YALTA study) support the latter contention. Authors reported that concentrations of circulating carotenoids estimated at baseline were negatively associated to CRP estimated several years later (7 to 15 years later).[20] However, associations with other antioxidant micronutrients than carotenoids were not investigated.

Our objective was to investigate the long-term associations between circulating concentrations of antioxidant micronutrients (vitamin C, α-tocopherol and β-carotene) and elevated CRP. We hypothesized that the observed effect would differ according to factors influencing antioxidant metabolism and baseline status. To this purpose we used data from the French SU.VI.MAX and SU.VI.MAX2 study, a cohort study with a follow-up duration of more than 12 years, and for which nutritional status was evaluated at baseline.

Material and methods

Study population

The study population was selected from participants in the SU.VI.MAX study. The SU.VI.MAX study is a randomized, double-blind, placebo-controlled, primary prevention trial designed to evaluate the effect of a supplementation in antioxidant vitamins and minerals at nutritional doses (120 mg of ascorbic acid, 30 mg of vitamin E, 6 mg of β-carotene, 100 µg of selenium as selenium-enriched yeast), and 20 mg of zinc (as gluconate)) on the incidence of cardiovascular disease and cancer.[21,22] Volunteer subjects aged 35-60 years old for women and 45-60 years old for men were enrolled in 1994-1995 for a planned 8-year intervention. Subjects were excluded if they presented any disease possibly hindering participation in the trial (including prevalent cancer and cardiovascular disease) and if they had any previous regular use of supplementation with the vitamins and mineral provided for the trial. [21,22] Initial results of the SU.VI.MAX trial phase concluded to a beneficial effect of supplementation on cancer incidence and all-cause mortality in men, but not in women. [21,22] In 2007-2009, subjects were enrolled, on a voluntary basis, in an additional follow-up defining the SU.VI.MAX2 study. Subjects

underwent a clinical examination and had blood drawn for biological tests. In the SUVIMAX 2 study, a subsample selected on geographical criteria for operative and logistical aspects were included in the CRP study and had serum CRP measured.

Both the SU.VI.MAX and the SU.VI.MAX2 studies were approved by the Ethics Committee for Studies with Human Subjects of Paris-Cochin Hospital (no. 706 and no. 2364, respectively) and the Comité National Informatique et Liberté (no. 334641 and no. 907094, respectively). Subjects all gave written informed consent to participate in the study.

For the present analysis, all subjects included in the CRP study were eligible. Subjects were excluded if they had a CRP>10mg/l, as such values are considered beyond the range of low-grade inflammation [23] and for outlier values of antioxidant concentrations. Further exclusions were performed for any missing data on any of the covariates investigated.

Data collection

Socio-demographic, and lifestyle variables

Socio-demographic (marital status (single/cohabiting), highest achieved diploma (Primary, secondary, superior)), physical activity (irregular, <1h equivalent walking/day, ≥1h equivalent walking/day) and smoking status (never smoked, former smoker, current smoker) data were obtained through self-administered questionnaire at baseline. Alcohol intake in grams per day was also estimated by using a short, validated semi-quantitative auto-administered dietary questionnaire.

Anthropometric measurements

Anthropometric measurements were taken at a clinical examination one year after inclusion in the SU.VI.MAX study, and are referred as baseline measurements. Weight was measured in subjects in light clothing and with no shoes to the nearest 0.1kg and height was measured to the nearest cm with a wall-mounted stadiometer in the same conditions.

Blood sampling and biological analyses.

Blood samples were obtained in evacuated tubes (Becton Dickinson) before the beginning of the trial phase in subjects after a 12-hours fasting period. All biochemical measurements were performed in a single laboratory. Blood samples were centrifuged immediately, frozen, and kept at -80°C until the determination of concentrations. Vitamin C status was evaluated by serum ascorbic acid concentration using an automated method based on the continuous flow principle, segmented with air bubbles. Total vitamin C (dehydroascorbic acid) was determined by spectrofluorimetry after immediate dilution (1:10) in 10% metaphosphoric acid. All samples were kept frozen at -80°C until the determination. These measurements were made with a

Technicon continuous flow analysis apparatus (Bayer-Technicon) that was equipped with an RF-530 Shimatzu fluorescence detector (Shimatzu Corp). β-Carotene and α-tocopherol concentrations were determined by HPLC by using Arnaud's method [24] with minor modifications. We used an HPLC system (Biotek-Kontron) that included a 525 gradient pump, a 402 Peltier Oven, a 465 autosampler, a Geminyx data station, and a 540 diode-array detector. CVs for blood assays were 9.0% for β-carotene; 3.5% for vitamin C, and 3.5% for α-tocopherol. Outlier values for antioxidant concentrations were excluded, based on the quantile-quantile plot of the log-transformed variable.

Blood samples were drawn from participants in the SU.VI.MAX2 study, immediately centrifugated and frozen at -80°C and stored. CRP concentrations were measured using immunoturbidimetric assay (reagent: Tina quant C-reactive protein (latex) assay), with a detection limit of 1mg/l for CRP. Intra-assay and inter-assay coefficients of variations were of 0.61% and 2.87% respectively. The assay detection limit is above the detection limit of 0.15mg/l cited for high-sensitivity CRP but below the standard clinical laboratory assay of 3-5 mg/l. Subjects exhibiting a CRP value >10mg/l were excluded from the analyses.

Statistical analyses

Baseline body mass index (BMI) was calculated as the weight (in kg) divided by the square of height (in m). Serum levels of vitamin C, β-carotene and α-tocopherol were divided into quintiles in order to investigate dose-response relationships.

CRP was categorized as ≤3mg/l and >3mg/l, and logistic regression models were applied to estimate odd ratios (95% confidence interval) of high levels of inflammation with baseline antioxidant status.[23] We estimated crude and multivariate associations, with two levels of adjustment: (1) gender, age, intervention allocation (supplementation group of the initial SU.VI.MAX trial: active vs. placebo), smoking status (never smoker/former smoker/current smoker), physical activity (irregular/<1h equivalent walking/day / ≥1h equivalent walking /day), alcohol intake; (2) Model 1+ baseline BMI (here hence referred to as fully adjusted model).

Subgroup analyses by baseline characteristics affecting antioxidant status were performed. Tested stratification variables were sex, allocation group (placebo group vs. antioxidant supplementation group), smoking status (never smoker vs. former/current smokers), alcohol intake (cut-off value taken as the sex-specific median alcohol intake) and overweight/obese status at baseline.

Results

Of the 6850 subjects enrolled in the SU.VI.MAX2 study, 3476 were included in the CRP study. Of these, 2953 had had blood α-tocopherol and β-carotene status evaluated and 2350 had had blood vitamin C evaluated. After exclusion of CRP values >10mg/l and outlier values of antioxidant concentrations (13 subjects in the analyses on β-carotene, 1 subject in the analyses on α-tocopherol and 5 subjects in the analyses of vitamin C), 2047 subjects had all covariates available for investigation of relationships between elevated CRP and β-carotene, 2060 subjects had all covariates available for α-tocopherol investigation, while 1719 subjects had all covariates available for vitamin C investigation. (Table 1, characteristics presented for the 2060 subjects with complete data for α-tocopherol; antioxidant concentrations for all subjects with available data)

Subjects having CRP>3mg/l at SUVIMAX 2 (end of the follow-up) study evaluation were more likely to be older and to have had higher baseline BMI (P for comparison <0.001 respectively). They also were more likely to have had lower baseline β-carotene concentrations (P for comparison = 0.006). No associations were found between supplementation group and elevated CRP, either in analyses involving all subjects (crude Odd Ratio (OR) 0.96, 95% confidence interval (95%CI): 0.74-1.22, P=0.72), or in sub-group analyses (data not tabulated).

Crude logistic regression analyses showed a negative association between baseline serum status of β-carotene and elevated CRP (P for trend<0.001). No association was found between serum status of α-tocopherol and vitamin C and elevated CRP in crude logistic regression models. In adjusted models, the negative association between baseline serum β-carotene status and elevated CRP were maintained after adjusting for socio-demographic and behavioral variables (P for trend<0.001) and after further adjustment on BMI, even if weakened (P for trend=0.01). (Table 2) In a sensitivity analysis, we also adjusted on lipoproteins, as proposed by Gross and al.[25] but such an adjustment did not significantly alter results (data not shown).

Sub-group analyses after stratification on variables affecting serum nutrient status showed that the negative associations between baseline serum β-carotene status and elevated CRP were more important in women (P for trend=0.004, P for interaction=0.30), in subjects of the supplementation group (P for trend=0.002, P for interaction=0.03) (Figure 1a), in never smokers (P for trend=0.009, P for interaction=0.51) (Figure 1b) and in subjects with a normal BMI at baseline (P for trend=0.004, P for interaction=0.19) (Online supplemental Figure 1c) in fully adjusted models.

We performed several sensitivity analyses, removing subjects at risk of having had elevated CRP levels at baseline (obese subjects and diabetic subjects), and obtained similar results both for

main analysis and subgroup analysis (data not tabulated). Moreover, in order to also examine medium-term associations, we analyzed the associations between β-carotene circulating concentrations measured at year 6 (corresponding to midway follow-up) and elevated CRP for a subsample of subjects with available data. The observed negative associations were similar to those observed using baseline data, suggesting medium-term as well as long-term protective effect of β-carotene on low-grade inflammation (data not tabulated).

Discussion

Our results support the contention that β-carotene serum status displays a beneficial dose-response effect on low-grade inflammation in the long-term. Other antioxidant vitamins' status, i.e. vitamin C and α-tocopherol, were not associated to low-grade inflammation after adjusting on potential confounders. Associations between high CRP and serum levels of β-carotene appear strongest in women, never smokers and subjects receiving a 8-y antioxidant supplementation at nutritional doses.

Consistent with our results, investigators in the CARDIA/YALTA study showed a long-term association between total carotenoid serum status (α- and β-carotene, β-cryptoxanthine, zeaxanthine/lutein) and inflammatory biomarkers.[20] A significant negative association was found between baseline serum total carotenoid levels and CRP measured 7 years later (adjusted slope (mg/L of CRP by nmol/L of total carotenoid concentration) -0.12, $P<0.01$).[20] Similar results were found when assessing the effect of carotenoid levels at year 7 and inflammatory biomarkers at year 15. Differences between the two studies pertain to the included population: the CARDIA study included adults aged 18-30 years old in 1985-1986, whereas the SUVIMAX population was middle-aged at inclusion. Besides, authors considered total carotenoid concentrations, adding circulating concentrations of α- and β-carotene, β-cryptoxanthine and zeaxanthine/lutein, which have different metabolic properties, while we investigated β-carotene circulating concentrations alone. However, these consistent results on otherwise different populations tend to corroborate the existence of both medium and long-term beneficial effect of high carotenoid circulating concentrations on low-grade inflammation.

To our knowledge, our study is, with Hozawa's and al., the only one investigating the associations between inflammatory biomarkers and serum nutrient status in the long term.

Most studies have indeed investigated these associations in cross-sectional designs, focusing on the effect of inflammation on anti-oxidant status.[16,18,19,26,27] Authors make the case that inflammation, causing free oxygen radicals liberation, acts to lower the anti-oxidant defenses of the body. Our results argue that the associations could be looked at the other way, inflammation being the mid- and long-term consequence of low antioxidant status.

Indeed, beside their role in scavenging oxidant radicals after initiation of the inflammatory response, carotenoids have independent immune-enhancing properties.[1] They stimulate the phagocytic and bacteria-killing ability of peripheral blood neutrophils; they increase the population of specific lymphocyte subsets and lymphocyte cytotoxic activity. B-carotene inhibits inflammatory gene expression by suppressing the activation of NF-κB transcription factor.[15] An alternative explanation is that β-carotene status would reflect high vegetable intake, and probably even a comprehensive healthier dietary behavior, which beneficial effect on health would not be mediated entirely by it. Other vegetable-provided nutrients could provide the anti-inflammatory effect that correlates with β-carotene.

Our results also show that the protective effect of β-carotene status is stronger in women, never smokers and subjects allocated to the active supplementation group during the trial phase of the SUVIMAX study. Results stratified by gender suggest that the negative association between high CRP and β-carotene status would be mostly driven by the higher serum concentrations in β-carotene observed in women. Indeed, women tended to have better nutrient status than men in the SUVIMAX study.[21,22]

Dual relationships have been observed between β-carotene and health status: while some trials investigating effect of β-carotene supplementation in smokers have observed increased risks for adverse health events (ATBC and CARET trials) [28–30], most studies in healthy population underline the protective role of it in disease [20]. Our study pertains to the second case, as healthy volunteers from the general population were selected for the initial SU.VI.MAX trial. Moreover, supplementation in the initial trial phase was at nutritional doses, therefore two to five times lower than what was used in the previously mentioned trials. Smoking alters β-carotene metabolism, as eccentric cleavage β-carotene metabolites can facilitate the binding of smoke derived carcinogens to DNA, [31] and it has been shown that β-carotene supplementation actually leads to adverse outcomes in smokers such as cancer.[20,28–30,32] In in vitro models, it has been demonstrated that beta-carotene may serve as an antioxidant or as a prooxidant, depending on the redox potential of the biologic environment in which it acts.[33] Smokers also tend to have lower serum β-carotene levels than non-smokers.[21,22,34,35] The fact that the preventive effect of β-carotene status was observed only in never smokers is therefore not entirely unexpected.

In this perspective, results pertaining to the interaction with supplementation allocation group, i.e. receiving antioxidant components at nutritional doses or not, would mean that supplementation improved nutritional status of all subjects,[22]but achieved protective effect only in subjects with already high β-carotene status, with a threshold effect. An alternative

explanation is that variability in nutrient status between subjects is far more important than variability induced by supplementation in the individual. Therefore, supplementation alone is not sufficient to induce nutrient status modifications affecting inflammatory status. However, combined variability in nutrient status observed in the supplementation group pertaining to both variability depending on supplementation and variability of initial β-carotene status would explain the results. This hypothesis is strengthened by results from mid-way β-carotene status: distribution of serum β-carotene concentrations tended to be both slightly shifted to higher concentrations and with a larger distribution in supplemented subjects. However, the scale of the distribution span is much larger than the shift between distributions. Alternatively, as supplementation included several antioxidant vitamins and minerals, it can be hypothesized that β-carotene status is the main driver for a beneficial effect on inflammation if nutrient status is otherwise appropriate, as would be observed after supplementation.

Strengths of our study include the use of longitudinal data on subjects from the general French population, with a follow-up of more than 12 years. Main analyses took into account potential confounders pertaining to lifestyle and socio-demographic variables. Moreover, we were able to test effect modifiers in the relationship between nutrient status and elevated CRP.

This study is subject to limitations. We did not collect data on CRP status at baseline, which could undermine our conclusions, as we were not able to exclude subjects with elevated baseline levels of CRP. However, results from the sensitivity analyses we undertook, removing subjects at risk of having had high CRP at baseline using proxy variables showed that our results are stable. Given the large number of comparisons undertaken, an interpretative limitation pertains to the fact that some findings would be due only to chance. However, analyses were hypothesis-driven and consistent with mechanistic knowledge.

Conclusion

Our results provide new insights regarding the relationships between antioxidant status and low-grade inflammation, showing that β-carotene serum status is negatively associated to low-grade inflammation in the medium and long-term. However, these results necessitate confirmation in other cohort and randomized control studies, with a dynamic assessment of both nutrient and inflammatory status.

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Figures and tables

Figure 1a. Association between elevated CRP and β-carotene status (fully adjusted model): stratified analysis (sex, intervention group)

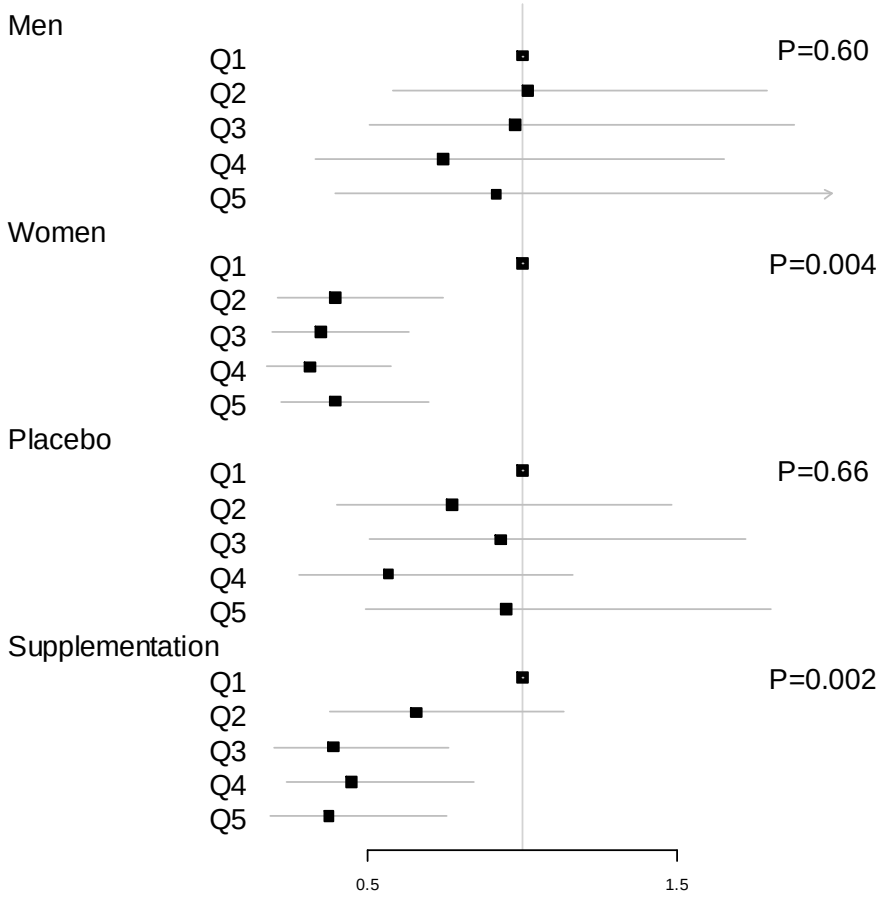

Figure 1b. Association between elevated CRP and β-carotene status (fully adjusted model): stratified analysis (smoking status, alcohol intake)

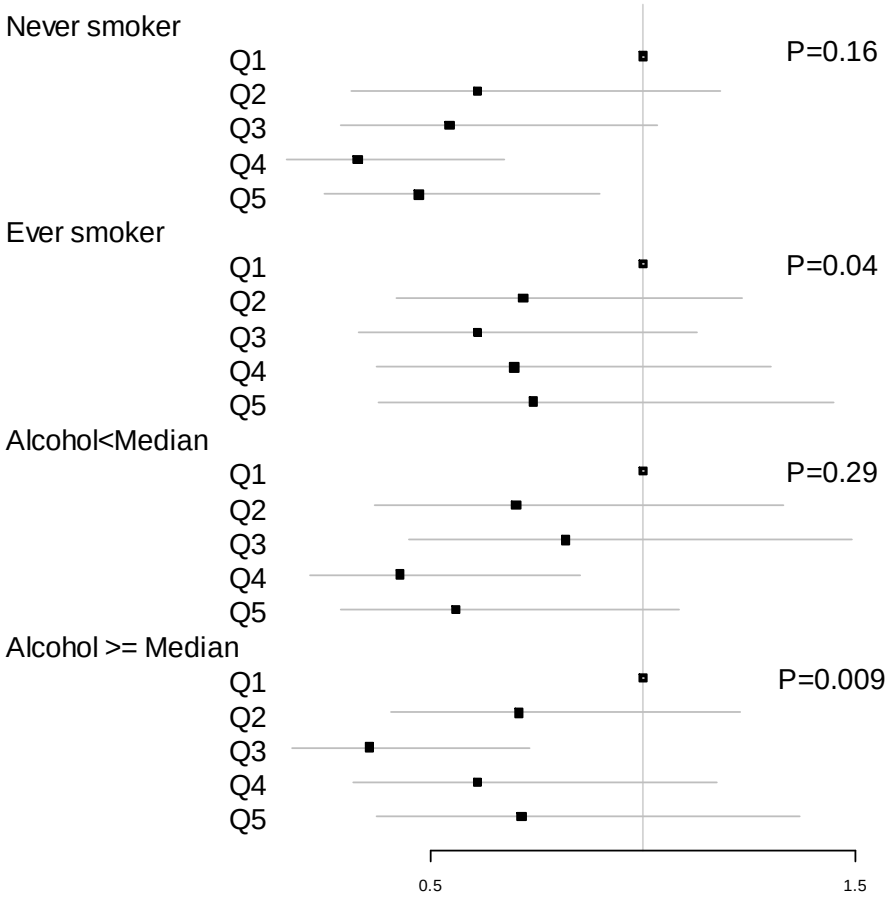

Figure 1c. Association between elevated CRP and β-carotene status (fully adjusted model): stratified analysis (overweight status)

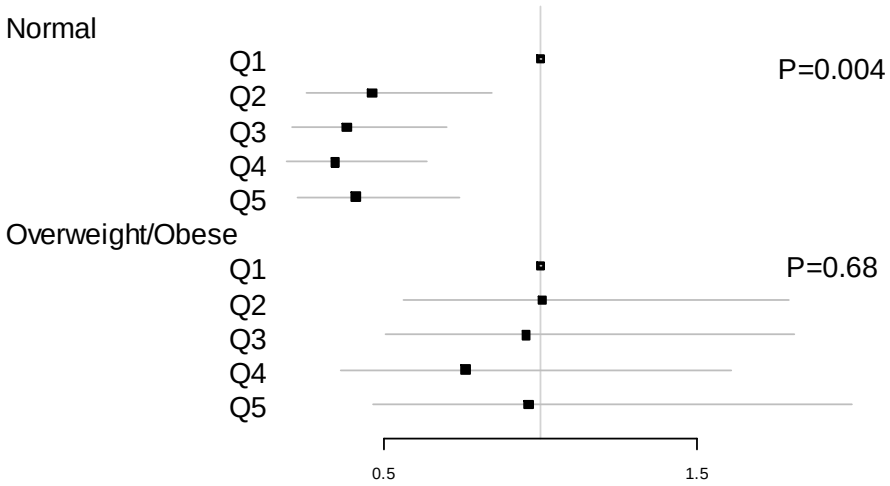

Table 1 Baseline characteristics of included subjects according to CRP status 12 years later

	N[a]		CRP≤3mg/l	CRP>3mg/l	P[b]
Number of cases (%)			1830 (88.8)	230 (11.2)	
Sex[c]	2060	*Men*	851 (46.5)	97 (42.17)	0.214
		Women	979 (53.5)	133 (57.83)	
Baseline age[d]	2060		49.6 ±5.74	50.56 ±6.08	0.025
Supplementation group[c]	2060	*Placebo*	872 (47.65)	113 (49.13)	0.672
		Antioxydants	958 (52.35)	117 (50.87)	
Marital status[c]	2060	*Single*	263 (14.52)	40 (17.54)	0.227
		Cohabiting	1548 (85.48)	188 (82.46)	
Highest achieved diploma[c]	2060	*Primary*	340 (18.64)	53 (23.14)	0.070
		Secondary	694 (38.05)	94 (41.05)	
		University	790 (43.31)	82 (35.81)	
Tobacco use[c]	2060	*Never smoker*	924 (50.49)	107 (46.52)	0.042
		Former smoker	719 (39.29)	87 (37.83)	
		Current smoker	187 (10.22)	36 (15.65)	
Physical activity level[c]	2060	*Irregular*	399 (21.8)	61 (26.52)	0.219
		<1h equivalent walking/day	572 (31.26)	72 (31.3)	
		≥1h equivalent walking/day	859 (46.94)	97 (42.17)	
Baseline BMI (kg/m^2)[d]	2060		23.81 ±3.33	25.5 ±4.14	<0.001
Baseline BMI category[c]	2060	*Normal <25kg/m²*	1249 (68.25)	117 (50.87)	<0.001
		Overweight [25-30[kg/m²	500 (27.32)	78 (33.91)	
		Obese ≥30kg/m²	81 (4.43)	35 (15.22)	
β-carotene (µmol/l) [e]	2048		0.52 (0.34;0.78)	0.44 (0.29;0.69)	0.006
Vitamin E (µmol/l)[e]	2060		30.64 (26.3;35.75)	31.05 (26.57;36.29)	0.14
Vitamin C (µmol/l) [e]	1719		55.83 (43.29;67.05)	53.23 (40.64;65.25)	0.06

[a] N corresponding to number of available observations for the selected variable. Socio-demographic, lifestyle and anthropometric data are presented for the 2060 subjects with available data on vitamin E concentrations. [b] P obtained from Chi-square tests for categorical variables, t-tests for continuous variables (on the log-transformed variable for continuous variables with skewed distributions)

[c] Figures presented are Number of observations and percentages [d] Figures presented are mean ±standard deviation

[e] Figures presented are median (25^{th};75^{th} percentiles) (variables with skewed distribution)

Table 2 Associations between serum levels of β-carotene, α-tocopherol and vitamin C and elevated CRP (logistic regression models)

	Quintiles of micronutrient serum concentrations (Q1-Q5)										
	OR	OR	95% CI[c]	OR	95% CI[c]	OR	95% CI[c]	OR	95% CI[c]	P trend	N
β-carotene (µmol/l)	*(0.08;0.3)*	*(0.3;0.44)*		*(0.44;0.6)*		*(0.6;0.84)*		*(0.84;4.58)*			
Number of cases	67	46		40		33		39			
Crude	1	0.647	(0.43;0.97)	0.552	(0.36;0.84)	0.446	(0.29;0.69)	0.538	(0.35;0.82)	<0.001	2048
Model 1 [a]	1	0.635	(0.42;0.96)	0.51	(0.33;0.79)	0.399	(0.25;0.63)	0.463	(0.3;0.73)	<0.001	2048
Model 2 [b]	1	0.703	(0.46;1.07)	0.602	(0.39;0.94)	0.496	(0.31;0.8)	0.61	(0.38;0.97)	0.01	2048
α-tocopherol (µmol/l)	*(10.23;25.4)*	*(25.4;29.13)*		*(29.15;32.36)*		*(32.39;36.97)*		*(37;85.16)*			
Number of cases	42	42		46		47		53			
Crude	1	1	(0.64;1.57)	1.107	(0.71;1.72)	1.137	(0.73;1.77)	1.297	(0.84;1.99)	0.184	2060
Model 1 [a]	1	0.971	(0.62;1.53)	1.065	(0.68;1.67)	1.078	(0.69;1.69)	1.162	(0.75;1.81)	0.419	2060
Model 2 [b]	1	0.913	(0.57;1.45)	1.026	(0.65;1.62)	1.025	(0.65;1.61)	1.104	(0.71;1.73)	0.533	2060
Vitamin C (µmol/l)	*(9.7;39.64)*	*(39.71;50.86)*		*(50.91;59.78)*		*(59.85;69.38)*		*(69.43;275.35)*			
Number of cases	45	42		39		28		37			
Crude	1	0.906	(0.58;1.42)	0.836	(0.53;1.32)	0.579	(0.35;0.95)	0.793	(0.5;1.26)	0.097	1719
Model 1 [a]	1	0.884	(0.56;1.39)	0.817	(0.51;1.3)	0.55	(0.33;0.92)	0.737	(0.46;1.19)	0.060	1719
Model 2 [b]	1	0.869	(0.55;1.38)	0.868	(0.54;1.39)	0.563	(0.34;0.94)	0.788	(0.48;1.29)	0.121	1724

[a] Model 1 adjusted for sex, age, supplementation group, smoking status, alcohol intake, physical activity
[b] Model 1+ body mass index (BMI) – fully adjusted model
[c] CI confidence interval
[d]Number in parenthesis are minimum and maximum values of nutrients' concentrations in each quintile

Intakes of polyunsaturated fatty acids were inversely associated with plasma C-reactive protein 12 years later in a middle-aged population with vitamin E as an effect modifier

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Synthèse

Introduction

Parmi les facteurs nutritionnels pouvant jouer sur les processus inflammatoires, les acides gras poly-insaturés (PUFAs) sont les plus étudiés, étant donné qu'ils servent de précurseurs à de nombreux médiateurs inflammatoires, dont les eicosanoïdes.(Calder, 1998, Calder, 2006, Simopoulos, 2002) Le substrat principal à la production d'eicosanoïdes est l'acide arachidonique (20 :4n-6 AA), de la série n-6, mais les PUFAs de la chaine n-3 peuvent aussi servir de précurseurs alternatifs, conduisant à la production d'eicosanoïdes, à moindre activité inflammatoire.(Simopoulos, 2002) Enfin, les PUFAs n-3 ont récemment été reconnus comme précurseurs de médiateurs spécifiques intervenant dans la résolution de l'inflammation, les résolvines et protectines.(Serhan, 2007, Serhan et al., 2005)

Les études épidémiologiques tendent à montrer une relation inverse entre apports en PUFAs n-3 et inflammation de bas grade.(He *et al.*, 2009, Lopez-Garcia *et al.*, 2004b, Yoneyama *et al.*, 2007) En ce qui concerne les PUFAs n-6, si les hypothèses mécanistiques suggèrent des effets pro-inflammatoires, les résultats des études épidémiologiques sont contrastés en particulier en ce qui concerne l'acide linoléique (18 :2n-6 LA).(Fritsche, 2008, Johnson et Fritsche, 2012)

Les effets des PUFAs pourraient être modulés par les apports en vitamine E. La vitamine E est un puissant antioxydant,(Singh et al., 2005) et certaines études sur modèles animaux semblent indiquer qu'elle aurait des effets antagonistes de ceux des PUFAs, en particulier dans le cancer.(Cognault *et al.*, 2000, Lhuillery *et al.*, 1997) L'effet modulateur de la vitamine E dans les relations entre PUFAs et inflammation n'a à ce jour pas été étudiés.

Nos objectifs étaient donc d'étudier les associations entre apports en PUFAs des séries n-3 et n-6 et CRP augmentée d'une part, et d'étudier l'effet modulateur potentiel de la vitamine E dans ces relations d'autre part.

Matériel et méthodes

Les sujets éligibles dans cette étude étaient les sujets inclus dans le groupe placebo de l'étude SU.VI.MAX ayant poursuivi le suivi dans l'étude SU.VI.MAX 2 (Hercberg et al., 2004, Kesse-Guyot *et al.*, 2011) et pour lesquels les éléments suivants étaient disponibles :

- Mesure d'exposition : présence d'au moins 6 enregistrements alimentaires de 24 heures dans les deux premières années de suivi (entre 1994 et 1996) de l'étude S.VI.MAX
- Mesure du critère de jugement : présence d'une mesure de la CRP réalisée lors de l'examen clinique effectué pour l'étude de suivi SU.VI.MAX2 (entre 2007 et 2009)

- Mesure des facteurs d'ajustement : variables socio-démographiques (sexe, âge, niveau d'éducation), de mode de vie (statut tabagique, activité physique) et d'anthropométrie (poids et taille mesurés lors d'un examen clinique à un an de l'inclusion dans l'étude SU.VI.MAX 1995-1997 et lors de l'examen clinique de l'étude SU.VI.MAX2 2007-2009, permettant de calculer l'indice de masse corporelle aux deux temps), facteurs alimentaires (apport énergétique, apport en éthanol, profil alimentaire 'Prudent' élaboré à partir des résultats d'une précédente analyse en composante principale sur l'ensemble de la population de SU.VI.MAX (Kesse-Guyot *et al.*, 2009) et nombre d'enregistrements alimentaires disponibles).

Les apports alimentaires en différents PUFAs n-3 (acide α-linolénique 18 :3 ALA, acide eicosapentaénoique 20 :5 EPA, acide docosapentaénoique 22 :5 DPA et acide docosahexaénoique 22 :6 DHA) et n-6 (acide linoléique 18 :2 LA, acide arachidonique 20 :4 AA) ainsi que l'apport combiné en PUFAs n-3 à longue chaine (EPA+DPA+DHA) l'apport total en PUFAs n-3 (EPA+DPA+DHA+ALA) et en PUFAs n-6 (LA+AA) et l'apport en vitamine E ont été calculés à partir des enregistrements de 24 heures.

Les sujets ayant une CRP>10mg/l ont été considérés comme ayant une CRP élevée liée à un évènement aigu et ont été exclus des analyses. Les sujets ayant une CRP>3mg/l ont été considérés comme ayant une CRP augmentée correspondant à une l'inflammation chronique de bas grade.(Pearson et al., 2003)

Les apports en nutriments d'intérêt ont été utilisés en tant que résidus, afin de tenir compte de l'effet de l'apport énergétique (Pearson et al., 2003, Willett et Stampfer, 1986). Les tertiles d'apports en nutriments d'intérêt ont été calculés et l'association entre tertiles de consommation et CRP augmentée a été estimée par régressions logistiques uni- puis multivariées. Les modèles ont été ajustés sur le sexe, l'âge à l'inclusion, le niveau d'éducation, le statut tabagique, l'activité physique, l'IMC à l'inclusion, la modification de l'IMC entre l'inclusion et SU.VI.MAX2, les apports énergétiques, les apports en alcool, le profil alimentaire 'Prudent' et le nombre d'enquêtes alimentaires disponibles.

L'effet modulateur de la vitamine E a été étudié en testant l'interaction entre les tertiles de vitamine E et les tertiles d'apports en PUFAs (en ordinal), puis en conduisant des analyses stratifiées par tertiles de consommation de vitamine E.

Résultats

Parmi les sujets inclus dans le groupe placebo de l'essai SU.VI.MAX, 3235 sujets ont été inclus dans l'étude SU.VI.MAX2. Parmi ceux-ci, 1649 sujets avaient un dosage de CRP disponible, et 899

sujets avaient complété 6 enregistrements de 24h. Après exclusion des sujets ayant un dosage de CRP>10mg/l, 843 sujets étaient disponibles pour analyse.

L'étude des associations entre apports alimentaires en PUFAs et CRP augmentée ont montré une association négative entre CRP augmentée et apport total en PUFAs n-3 (OR du tertile 3 vs. tertile 1: 0.41 (intervalle de confiance (IC) à 95% 0.21;0.77), $P_{tendance\ linéaire}$ =0.01), apport en PUFAs n-3 à longue chaine (OR du tertile 3 vs. tertile 1: 0.55 (IC 95% 0.30;1.00), $P_{tendance\ linéaire}$ =0.05) principalement due à une association négative entre apports en DPA et CRP (OR du tertile 3 vs. tertile 1: 0.44 (IC 95% 0.24;0.83), $P_{tendance\ linéaire}$ =0.01).

Des associations négatives étaient aussi observées pour les apports en PUFAs n-6 totaux (OR du tertile 3 vs. tertile 1: 0.38 (IC 95% 0.21;0.70), $P_{tendance\ linéaire}$ =0.002), principalement par le biais d'une association négative forte entre apports en LA et CRP (OR du tertile 3 vs. tertile 1: 0.41 (IC 95% 0.22;0.75), $P_{tendance\ linéaire}$ =0.004).

Les analyses de sensibilité conduites sur un sous-groupe (n=170) pour lequel la CRP à l'inclusion était disponible et à des niveaux normaux ont montré que les associations entre PUFAs n-3 à longue chaine et CRP augmentée étaient maintenues. Les associations avec les autres PUFAs conservaient la même direction, mais n'étaient plus significatives. Ces résultats suggèrent que les associations observées étaient au moins partiellement de nature longitudinale.

Les interactions entre apports en PUFAs et vitamine E sur le risque de CRP augmentée se distribuaient entre 0.12 (pour le DPA) et 0.90 (pour le DHA). La stratification sur les apports en vitamine E montraient que les associations négatives entre les apports en DPA et PUFAs n-3 à longue chaine étaient significatives uniquement dans le tertile inférieur de consommation de vitamine E. Les associations entre apports en LA et en PUFAs n-6 et CRP augmentée n'étaient pas calculables dans le tertile supérieur d'apports en vitamine E, en raison de la colinéarité entre les apports en LA et apports en vitamine E.

Discussion

Nos résultats confirment l'association négative entre apports alimentaires en PUFAs n-3 et inflammation et contribuent à clarifier les associations avec les PUFAs n-6, montrant que ceux-ci en général, et le LA en particulier sont aussi associés inversement à la CRP augmentée. La vitamine E était un modulateur de l'effet des PUFAs n-3 étant donné que l'association négative entre leur apport et la CRP augmentée n'était significative que pour le tertile inférieur de consommation de vitamine E.

Un certain nombre d'études observationnelles conduites selon des méthodologies transversales au sein de cohortes bien documentées ont montré une association négative entre PUFAs n-3 et

biomarqueurs de l'inflammation.(He et al., 2009, Lopez-Garcia et al., 2004b, Pischon *et al.*, 2003, Poudel-Tandukar *et al.*, 2009) Nos résultats étendent ces conclusions en montrant que ces associations sont maintenues sur le long terme. De plus, notre étude a permis d'étudier directement les associations avec les apports en DPA, généralement exclus de ces études.

En ce qui concerne les PUFAs n-6, leur impact sur l'inflammation est controversé.(Czernichow *et al.*, 2010, Deckelbaum et Calder, 2010, Fritsche, 2008) Dans la mesure où l'AA est le principal précurseur des eicosanoïdes pro-inflammatoires, et que le LA peut servir de précurseur lui-même pour l'AA, leur effet serait plutôt d'ordre pro-inflammatoire.(Simopoulos, 2002) Pourtant, certaines études récentes laissent à penser que les PUFAs n-6 pourraient être précurseurs de médiateurs impliqués aussi dans la résolution de l'inflammation.(Serhan, 2007) Les études chez l'humain ont donné des résultats contradictoires. Si la supplémentation en AA augmente la production de certains eicosanoïdes, elle ne modifie pas les concentrations circulantes d'autres biomarqueurs inflammatoires (CRP en particulier).(Kelley et al., 1998, Thies et al., 2001a) Par ailleurs, une revue de la littérature sur les essais de supplémentation en LA chez des sujets sains ne permettait pas de conclure quant à un effet pro- ou anti-inflammatoire.(Johnson et al., 2012) Enfin, certaines études observationnelles ont montré une relation inverse entre apports en LA et inflammation.(Poudel-Tandukar et al., 2009) Nos résultats vont plutôt dans le sens de ces dernières, montrant une association négative entre apports en LA et CRP augmentée.

Certaines études suggèrent que la vitamine E aurait des effets antagonistes des PUFAs, en particulier dans le contexte de la carcinogenèse. Les acides gras polyinsaturés sont susceptibles de s'auto-oxyder, et l'augmentation des produits de peroxydation lipidique est mesurable dans des modèles cellulaires et animaux.(Chajes *et al.*, 1995, Gonzalez *et al.*, 1993, Lhuillery et al., 1997) L'incorporation d'acides gras n-3 augmente cette peroxydation lipidique, mais dans le même temps augmente aussi l'apoptose et réduit la croissance tumorale dans ces modèles.(Cognault et al., 2000, Lhuillery et al., 1997) En revanche, l'addition de vitamine E dans ce type de protocole renverse les effets observés liés aux PUFAs n-3, et rétablit la croissance tumorale.(Cognault et al., 2000, Lhuillery et al., 1997) Ces éléments suggèrent que la peroxydation lipidique en soi n'est pas toxique, mais permettrait une régulation de la croissance et de l'apoptose, prévenant le développement tumoral.(Larsson *et al.*, 2004)

Les études in vivo n'ont à ce jour pas permis de corroborer ces hypothèses. Aucune interaction significative n'a été observée dans l'étude E3N entre les apports en PUFAs n-3 et la vitamine E dans l'incidence des cancers.(Thiebaut *et al.*, 2005) A notre connaissance, aucune étude ne s'est penchée sur l'interaction entre PUFAs et vitamine E en dehors du cancer.

Nos résultats émettent l'hypothèse d'une interaction entre PUFAs n-3 et vitamine E en dehors de la cancérogenèse, dans l'inflammation. Dans ce cas, on peut émettre l'hypothèse que la peroxydation lipidique dans les cellules du site inflammatoire serait bénéfique car elle conduirait à l'apoptose et à l'élimination des cellules altérées par les processus inflammatoires (comme les cellules spumeuses dans le cas de l'athérosclérose), puis à leur détersion.

L'association entre apports en LA et CRP augmentée n'a pu être étudiée dans le tertile supérieur d'apport en vitamine E, du fait de la colinéarité entre les deux variables. En effet, la vitamine E et le LA partagent les mêmes sources alimentaires, et sont retrouvés principalement dans les huiles végétales.

Notre étude comporte quelques limites. Tout d'abord, la CRP n'a pas été mesurée à l'inclusion, ce qui fait que les associations observées pourraient refléter des relations transversales. Néanmoins, les analyses de sensibilités effectuées chez les sujets avec une CRP normale à l'inclusion montrent que nos résultats reflètent au moins partiellement des effets longitudinaux plutôt que transversaux. De plus, nous n'avons pas eu accès à la CRP ultrasensible, avec une limite de détection à 1mg/l. Néanmoins, cette limite était suffisamment faible pour permettre de classer les sujets en fonction de leur niveau de risque cardiovasculaire.

Les forces de cette étude résident dans l'utilisation d'enregistrements de 24 heures répétés, permettant une analyse détaillée et précise des apports alimentaires, prenant en compte la variabilité intra-individuelle des apports alimentaires et l'évaluation de la stabilité des résultats par des analyses de sensibilité. De plus, les analyses de sensibilité nous ont permis de tester l'hypothèse de relations prospectives entre apports en PUFAs et CRP augmentée.

Conclusion

Nos résultats confortent l'hypothèse d'une relation inverse entre les PUFAs n-3 et n-6 et la CRP augmentée, même à long terme, et indiquent que la vitamine E est un modulateur de cette relation. Les apports en vitamine E devraient donc être examinés dans les études se focalisant sur la relation entre PUFAs et santé.

Manuscrit

Abstract

While n3 polyunsaturated fatty acids (PUFAs) are considered as anti-inflammatory components, the role of dietary n6 PUFAs in inflammation remains controversial. Some mechanistic evidence suggests vitamin E as a potential effect modifier in the relationship between PUFAs and inflammation.

Our objectives were to evaluate the long-term associations between dietary intakes of PUFAs and elevated plasma C reactive protein (CRP), and to investigate potential effect modification by vitamin E.

Subjects in the placebo group of the SU.VI.MAX trial who had available CRP measurements in 2007-09 were included in the study (N=843). Dietary intakes of n3 PUFAs, n6 PUFAs and vitamin E were assessed in 1994-96 with at least 6 dietary records. Logistic regression odds ratios for elevated CRP (>3mg/l) and 95% confidence intervals were estimated for individual PUFA and for total n3 PUFAs and n6 PUFAs intakes. Models were adjusted for socio-demographical, lifestyle, anthropometric and dietary variables. Interactions with vitamin E intakes were also assessed.

Inverse associations were observed between total n3 PUFAs (α-linolenic acid 18:3n3 ALA+ Eicosapentaenoic acid 20:5n3 EPA+Docosapentaenoic acid 22:5n3 DPA+Docosahexaenoic acid 22:6n3 DHA) and n6 PUFA intakes (Linoleic acid 18:2n6 LA+Arachidonic acid 20:4n6 AA) and elevated CRP (OR for tertile 3 vs. tertile 1 of intake: 0.41 (95% CI: 0.21, 0.77), P-trend 0.01 and OR 0.38 (95% CI: 0.21, 0.70), P-trend 0.002 respectively). Stratification on vitamin E intakes showed that inverse associations between n3 PUFA intakes and elevated CRP were significant only in subjects with low intakes of vitamin E.

Our results support the contention that intakes of both n3 PUFAs and n6 PUFAs are inversely associated to plasma CRP concentrations. Vitamin E is a potential effect modifier and should therefore be taken into account in such investigations.

Introduction

Low-grade inflammation has been recognized as an underlying process for several chronic diseases, including cancer and cardiovascular diseases.(1-3) Identification of modifiable risk factors for low-grade inflammation could therefore contribute in understanding and preventing the early stages of chronic diseases.

Polyunsaturated fatty acids (PUFAs) are of utmost interest, as they are precursors for several pro- or anti-inflammatory mediators, including eicosanoids.(4-6) The main substrate for eicosanoids' production is arachidonic acid (20:4n6 AA), but n3 PUFAs have been identified as alternate sources of production for eicosanoids with lesser inflammatory power.(6) They are also precursors for mediators involved in the resolution of inflammation, protectins and resolvins.(7)

Several studies have investigated relationships between n3 PUFAs and inflammation, suggesting a protective effect against low-grade inflammation.(8-10) As to n6 PUFAs however, results are controversial. If mechanistic pathways point them as pro-inflammatory factors, results from human observational studies are more equivocal, especially as regards linoleic acid (18:2n6 LA).(11;12)

Moreover, interaction between PUFAs and vitamin E need to be further investigated. Vitamin E is a potent anti-oxidant, with experimental anti-inflammatory properties.(13) Mechanistic hypotheses would suggest vitamin E and n3 PUFAs to have synergistic anti-inflammatory effects.(14) However, animal models have shown vitamin E and n3 PUFAs to have rather antagonistic effects, in particular in cancer.(15;16) Human studies investigating the interaction between n3 PUFAs and vitamin E intakes in inflammation are therefore of importance. To our knowledge, no epidemiologic study has of yet investigated the potential effect modification by vitamin E in the relationship between PUFAs and inflammatory processes.

Our objectives were therefore: (1) to assess long-term relationships between dietary intakes of n3 PUFAs and n6 PUFAs and elevated plasma status of C-reactive protein (CRP) measured 12 years later and (2) to investigate potential effect modifications by vitamin E intakes in a sample of subjects from the French SU.VI.MAX study.

Material and Methods

Study population

The study population was selected from participants in the SU.VI.MAX study. Subjects not taking any supplements were included in 1994-95 in a randomized, double-blind, placebo-controlled, primary prevention trial designed to evaluate the effect of a planned 8-year supplementation in antioxidant vitamins and minerals at nutritional doses on the incidence of cardiovascular disease and cancer.(17) In 2007-09, subjects were offered to enroll in an additional follow-up study, the SU.VI.MAX2 study.(18)

Both the SU.VI.MAX and the SU.VI.MAX2 studies were approved by the Ethics Committee for Studies with Human Subjects of Paris-Cochin Hospital and the Comité National Informatique et Liberté. All subjects gave written informed consent to participate in the study.

Subjects having had CRP measurements and with CRP values <10mg/L (19) were included in the present study. Subjects having less than six dietary records in 1994-96 and subjects having missing data on covariates were excluded from the analyses.

As vitamin E was included in the supplements used in the trial phase of the SU.VI.MAX study, and as we hypothesize an effect modification by vitamin E intakes, we conducted our analyses only in subjects in the placebo group of the initial SU.VI.MAX trial.

Dietary data assessment

Dietary assessment was carried out via repeated 24h records, as described before.(17) Individual n3 PUFA (α-linolenic acid 18:3n3 ALA, Eicosapentaenoic acid 20:5n3 EPA, Docosapentaenoic acid DPA 22:5n3, Docosahexaenoic acid 22:6n3 DHA) and n6 PUFA (LA and AA), long-chain n3 PUFA (EPA+DPA+DHA), total n3 PUFA and total n6 PUFA and total n3/n6 PUFA intakes ratio were computed.

All nutrient intakes were modeled as residuals, in order to take into account energy intakes.(20) A simplified 'Prudent' dietary pattern (DP) was constructed (21) based on the Prudent DP described in Kesse-Guyot and al. (22) in order to account for potential confusion due to a more general healthy dietary behavior.

Inflammation measurement

Blood samples were drawn from participants in the SU.VI.MAX2 study, immediately centrifuged and frozen at -80°C and stored. Plasma CRP concentrations were measured using immunoturbidimetric assay, with a detection limit of 1mg/l. Intra-assay and inter-assay coefficients of variations were of 0.61% and 2.87% respectively. Subjects with CRP values >3mg/L were considered having elevated CRP. (19)

Covariates

Educational level (primary, secondary, superior), physical activity (irregular, <1h equivalent walking/day, ≥1h equivalent walking/day) and smoking status (never smoked, former smoker, current smoker) data were obtained through self-administered questionnaires at baseline.

Anthropometric measurements were taken at a clinical examination one year after inclusion in the SU.VI.MAX study, and at the clinical examination of the SU.VI.MAX2 study. Weight was

measured in subjects in light clothing and with no shoes to the nearest 0.1kg and height was measured to the nearest cm with a wall-mounted stadiometer in the same conditions.

Baseline glycaemia and serum cholesterolemia were collected after a 12-hour fasting period and measured by an enzymatic method using a Technicon flow analysis device (Technicon DAX-24; Bayer Diagnostic, Pittsburgh, Pa).

Statistics

Body mass index (BMI) was calculated as the weight (in kg) divided by the square of height (in m). Dietary n3 PUFA and n6 PUFA intakes were divided into tertiles. Characteristics of subjects according to plasma CRP status were explored using Chi-square tests for categorical variables (presented as N, percentage) and t-test for continuous variables (presented as Mean, SD). Odd ratios (OR) and 95% confidence intervals (95% CI) of having elevated plasma CRP concentrations according to tertiles of individual PUFA, long-chain n3 PUFAs, total n3 PUFAs, total n6 PUFAs and total n3/n6 PUFA intakes ratio were computed using logistic regression. Models were first crude, then adjusted on sex, baseline age, educational level, smoking status, physical activity, energy intake, alcohol intake, number of dietary records available, baseline BMI, difference between baseline and follow-up BMI, and 'Prudent' DP.

Interactions between PUFAs and vitamin E were tested by adding a cross-term between tertiles of PUFAs and tertiles of vitamin E intakes (coded as ordinal variables) and the analyses were repeated stratifying on vitamin E tertiles (adjusted model only).

In sensitivity analyses, main analyses were repeated in a subset of subjects for whom baseline plasma CRP concentrations had been measured for an ancillary protocol, after exclusion of subjects with elevated baseline plasma CRP concentrations (n=170).

All analyses were performed using SAS software (version 9.3; SAS Institute Inc). Statistical significance was considered for P values <0.05.

Results

In all, 843 subjects corresponded to our criteria and were included in the present analysis.(Figure 1)

Subjects with elevated plasma CRP concentrations were more likely older, with a higher baseline BMI and a higher BMI increase between baseline and follow-up measurements (all P<0,01).(Table 1)

Inverse associations were observed between elevated plasma CRP concentrations and total n3 PUFA intakes (ALA+EPA+DPA+DHA, P-trend 0.01), long chain n3 PUFA intakes (EPA+DPA+DHA,

P-trend=0.05) mainly driven by associations with DPA intakes (P-trend=0.01).(Table 2) Inverse associations were also observed for total n6 PUFA intakes (P-trend=0.002), mainly driven by inverse association with LA intakes (LA+AA, P-trend=0.004).(Table 2) In sensitivity analyses with subjects having normal baseline plasma CRP concentrations, inverse associations with long chain n3 PUFA intakes remained significant. Other associations were maintained in the same direction, but were no longer significant.(data not shown) These results suggest that our main results reflect at least partially longitudinal associations.

Test for interactions with vitamin E ranged between 0.12 (for DPA) and 0.90 (for DHA). Stratification on tertiles of vitamin E showed that inverse associations with DPA intakes and long chain n3 PUFA intakes were significant only in the lowest tertile of vitamin E intake (P-trend across tertiles of PUFA intakes=0.007 and =0.043 respectively). (Table 3) OR for associations between LA intakes and total n6 PUFA intakes and plasma CRP level were not computable in the highest tertile of vitamin E intake, due to the colinearity between LA and vitamin E intakes.

Discussion

Our results strengthen the contention of an inverse association between dietary n3 PUFA intakes and inflammation, and show that n6 PUFAs intakes in general and LA intakes in particular display the same beneficial pattern as n3 PUFA intakes. Vitamin E proved to be an effect modifier of DPA and long chain n3 PUFAs, for which inverse associations were observed only for subjects with the lowest vitamin E intakes.

Some cross-sectional studies have investigated the associations between n3 PUFA intakes (mainly EPA and DHA) and biomarkers of inflammation and demonstrated an inverse association.(8;9;23;24) Our study extends these results to long-term associations. Moreover, we were able to investigate DPA dietary intakes, which were not taken into account in these studies. Effect of dietary PUFAs is thought to be determined by their circulating concentrations. However, circulating levels of DPA do not correlate well with dietary consumption, but to EPA levels, suggesting that circulating DPA is derived from transformation of circulating EPA rather than directly from dietary sources.(25) This could explain in part why most studies focus on EPA and DHA. Our results however show that dietary DPA should also be considered.

As to n6 PUFAs, their effect in inflammation is still debated.(11;26;27) AA is the major precursor for pro-inflammatory eicosanoid production, and as LA itself can serve as a precursor for AA, it is supposed to have similar effects.(6) However, recent research has uncovered possible AA and LA anti-inflammatory properties, as they are also precursor to a range of mediators involved in inflammation resolution.(28)

Human studies investigating AA and LA impact on inflammation have given mixed results: observational cross-sectional studies have given inconsistent results (23;24); supplementation of healthy subjects with AA increased the production of certain eicosanoids but it didn't alter other inflammatory biomarkers (CRP, interleukin 6 and tumor necrosis factor α) (29;30); supplementation trials with dietary LA in healthy subjects show neither pro- or anti-inflammatory effects.(12) Our results suggest a potential anti- rather than pro-inflammatory effect of dietary LA.

Moreover, our results show similar effect sizes for n3 PUFA and n6 PUFA intakes, consistent with results by Poudel-tandoukar et al.(24) This argues for equal balance between n3 PUFA and n6 PUFA intakes in preventing inflammation. However, our results need to be replicated for confirmation.

Vitamin E is a potent antioxidant interrupting lipid peroxidation that has been suggested to have antagonistic effects to PUFAs, in particular in carcinogenesis.(31) N3 PUFAs are able to auto-oxidize, therefore their incorporation induced lipid peroxidation and apoptosis in vitro and in animal models of cancer.(16;32;33) Even so, the n3 PUFAs lead to tumor growth suppression. Adding vitamin E to n3 PUFAs abolished this effect.(16;32;33) This suggests that n3 PUFA induced lipid peroxidation would not be toxic per se but rather act as tumor cell growth and apoptosis regulator.(34)

In vivo human studies have however not been able yet to corroborate such hypotheses. In the E3N prospective cohort, no significant interaction was observed between n3 PUFA intakes and vitamin E on the incidence of cancer.(35) In a 6 weeks double blind trial with n3 PUFA or vitamin E alone or in combination, increased lipid peroxidation was not suppressed by vitamin E supplementation.(36)

Our results corroborate the hypothesis of an interaction between n3 PUFA intakes and vitamin E intakes on inflammatory biomarkers. Significant inverse associations of PUFA intakes were observed for subjects with the lowest intakes of vitamin E. The lack of significance observed in our study could be due to both a lack of power, due to our limited sample size and to the limited power of statistical interaction tests.

We were not able to obtain statistically stable results for LA intakes when stratifying on vitamin E intakes, because of colinearity between the two variables. This colinearity can be explained by the fact that LA and vitamin E share the same food sources, as they are found mostly in vegetable oils (see Supplemental Table 1).

Our study is subject to some limitations. First, plasma CRP concentration was measured with a detection limit as high as 1mg/l. However, this detection limit still allowed us to classify subjects according to cardiovascular risk.(19) Second, plasma CRP concentration was not measured at baseline except for a small sample of subjects. Our results could therefore reflect preexisting associations between PUFA intakes and plasma CRP concentrations. However, the sensitivity analysis yielded similar results, showing that the observed associations reflect at least partially longitudinal associations. Finally, we were not able to investigate associations with serum level of fatty acids, or with other biomarkers of inflammation, which would have strengthened our results.

Strengths of our study include the use of detailed repeated dietary records test of the robustness of the results in sensitivity analysis.

In conclusion, our results support inverse associations between both n3 PUFA intakes and n6 PUFA intakes and elevated plasma CRP concentrations, and that vitamin E intakes should be carefully considered when investigating the effect of PUFA intakes on health outcomes.

Acknowledgements

C.J. developed overall research plan, conducted research, performed statistical analyses, wrote paper and had primary responsibility for final content.

E.KG. participated in project conception, development of overall research plan, critically reviewed the manuscript and supervised research.

M.T. participated to the development of research plan and critically reviewed the manuscript.

EKG, PG, SH designed the SUVIMAX2 study, provided essential material and critically reviewed the manuscript.

IP NM provided essential material and critically reviewed the manuscript

All authors read and approved the final manuscript.

All authors report no conflicts of interests

Abbreviations

(AA, Arachidonic acid)

(ALA, α-linolenic acid)

(BMI, body mass index)

(CI, confidence interval)

(CRP, C-reactive protein)

(DHA, Docosahexaenoic acid)

(DP, Dietary pattern)

(DPA, Docosapentaenoic acid)

(EPA Eicosapentaenoic acid)

(LA, Linoleic acid)

(PUFA, polyunsaturated fatty acid)

(OR, Odd ratio)

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Figure 1: Flow diagram of inclusion in the present analysis

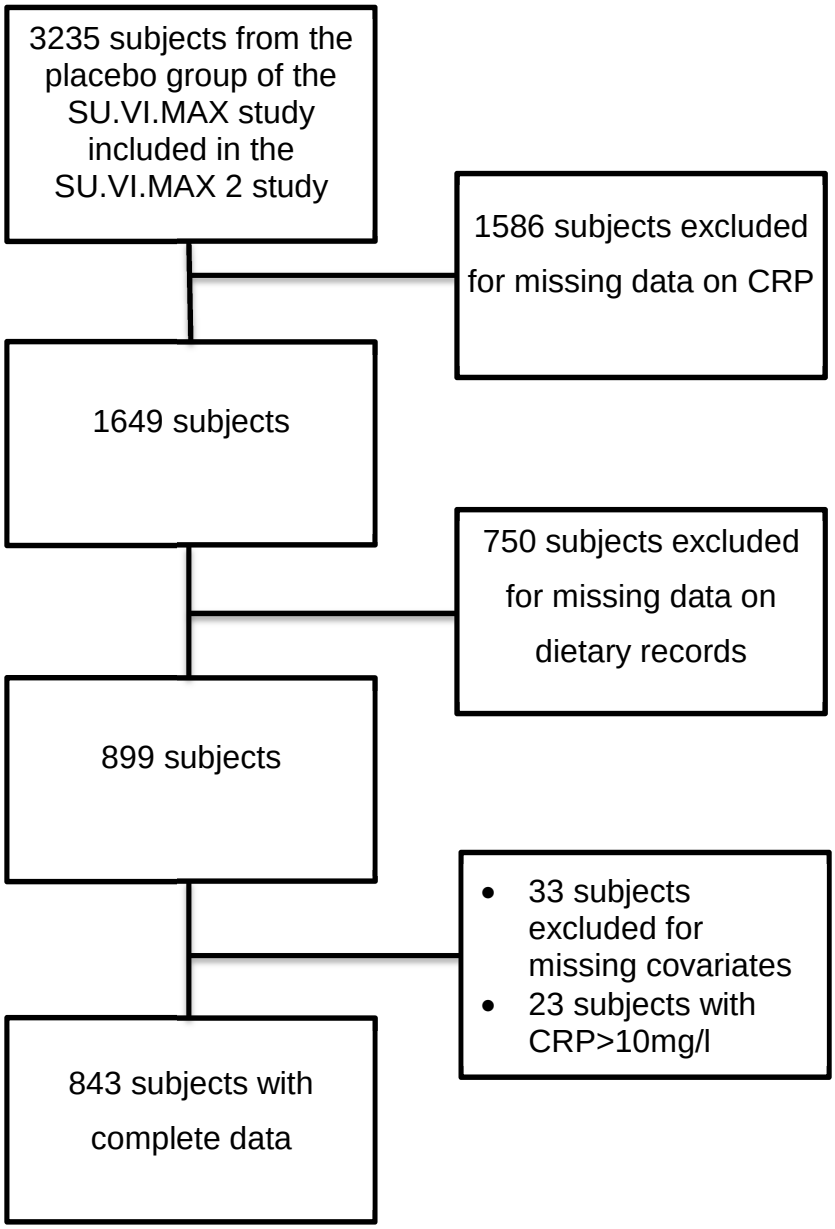

Table 3 Characteristics of the population according to CRP status at SU.VI.MAX2 study (n =843)

	CRP≤3mg/L (n=750)	CRP>3mg/L (n=93)	P value[1]	
Sex, *n(%)*				
Men	359 (47.9)	39 (41.9)		0.28
Women	391 (52.1)	54 (58.1)		
Baseline age, *y*	49.5 ±5.70	51.2 ±6.22		0.02
Educational level, *n(%)*				
Primary	145 (19.3)	20 (21.5)		0.83
Secondary	279 (37.2)	32 (34.4)		
University	326 (43.5)	41 (44.1)		
Smoking status, *n(%)*				
Never smoker	388 (51.7)	51 (54.8)		0.56
Former smoker	282 (37.6)	30 (32.3)		
Current smoker	80 (10.7)	12 (12.9)		
Physical activity, *n(%)*				
Irregular	152 (20.3)	27 (29.0)		0.15
<1h walking/day	235 (31.3)	26 (28.0)		
≥1h walking/day	363 (48.4)	40 (43.0)		
Baseline BMI, *kg/m²*	23.9 ±3.25	25.2 ±4.00		0.002
Final BMI, *kg/m²*	25.0 ±3.59	27.3 ±4.96	<0.001	
Difference in BMI during follow-up, *kg/m²*	1.1 ±1.6	2.1 ±2.2	<0.001	
Dietary pattern[2]	-0.1 ±3.9	-0.4 ±5.4		0.54
Energy intake (alcohol excluded), *kcal/d*	2040 ±544	1990 ±630		0.39
ALA, *g/d*	0.88 ±0.31	0.84 ±0.32		0.17
EPA, *g/d*	0.12 ±0.10	0.12 ±0.15		0.71
DPA, *g/d*	0.06 ±0.04	0.06 ±0.06		0.33
DHA, *g/d*	0.22 ±0.17	0.20 ±0.26		0.38
Long chain n3 PUFA (EPA+DPA+DHA), *g/d*	0.41 ±0.29	0.37 ±0.47		0.46
Total n3 PUFA, *g/d*	1.30 ±0.46	1.20 ±0.70		0.26
LA, *g/d*	11.7 ±4.28	10.8 ±4.66		0.08
AA, *g/d*	0.17 ±0.065	0.17 ±0.10		0.76
Total n6 PUFA, *g/d*	11.9 ±4.31	11.0 ±4.72		0.08
Ratio n3/n6 PUFA, *g/d*	0.11 ±0.04	0.12 ±0.05		0.73
Vitamin E intakes, *mg/d*	13.2 ±4.70	12.4 ±5.01		0.15
Serum Cholesterol, *mmol/L*	6.0 ±0.98	6.0 ±0.99		0.67
Serum Glucose, *mmol/L*	5.7 ±0.66	5.8 ±0.69		0.02

Values are means +/- SD or n (%)

AA, Arachidonic acid ; ALA, α-linolenic acid; CRP, C-reactive protein; DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; EPA Eicosapentaenoic acid; LA, Linoleic acid;

[1]P for test was obtained with Chi square tests for categorical variables and t-tests for continuous variables.

[2] Simplified healthy dietary pattern based on the food groups identified by factor analysis

Table 2 Logistic regression Odd Ratios (95% confidence intervals)of having elevated C-reactive protein according to tertiles of n3 and n6 PUFA intakes.

ALA[1] *g/d*	0.73 (0.68, 0.75)	0.82 (0.80, 0.86)	1.0 (0.93, 1.1)	
	Tertile 1 (n =36 cases)	Tertile 2 (n=29 cases)	Tertile 3 (n=28 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.78 (0.47, 1.3)	0.75 (0.45, 1.3)	0.28
Model 1[2]	1	0.81 (0.46, 1.4)	0.79 (0.44, 1.4)	0.41
EPA[1] *g/d*	0.04 (0.03, 0.05)	0.10 (0.84, 0.12)	0.20 (0.16, 0.26)	
	Tertile 1 (n=36 cases)	Tertile 2 (n=31 cases)	Tertile 3 (n=26 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.84 (0.51, 1.4)	0.69 (0.41, 1.2)	0.18
Model 1[2]	1	0.83 (0.47, 1.4)	0.65 (0.36, 1.2)	0.17
DPA[1] *g/d*	0.03 (0.03, 0.04)	0.06 (0.05, 0.06)	0.09 (0.08, 0.11)	
	Tertile 1 (n=36 cases)	Tertile 2 (n=37)	Tertile 3 (n=20 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	1.0 (0.63, 1.7)	0.52 (0.29, 0.93)	0.032
Model 1[2]	1	0.86 (0.51, 1.4)	0.44 (0.24, 0.83)	0.01
DHA[1] *g/d*	0.07 (0.05, 0.09)	0.17 (0.14, 0.20)	0.36 (0.29, 0.48)	
	Tertile 1 (n=37 cases)	Tertile 2 (n=31 cases)	Tertile 3 (n=25 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.82 (0.49, 1.4)	0.64 (0.38, 1.1)	0.11
Model 1[2]	1	0.77 (0.45, 1.3)	0.65 (0.36, 1.2)	0.16
Long chain n3 PUFA[1] (EPA+DPA+DHA) *g/d*	0.15 (0.11, 0.19)	0.33 (0.28, 0.39)	0.64 (0.53, 0.84)	
	Tertile 1 (n=39 cases)	Tertile 2 (n=30 cases)	Tertile 3 (n=24 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.74 (0.45, 1.2)	0.58 (0.34, 0.99)	0.045
Model 1[2]	1	0.69 (0.40, 1.2)	0.55 (0.30, 1.0)	0.048
Total n3 PUFA[1] *g/d*	0.95 (0.86, 1.01)	1.2 (1.1, 1.3)	1.6 (1.4, 1.8)	
	Tertile 1 (n=39 cases)	Tertile 2 (n=34 cases)	Tertile 3 (n=20 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.85 (0.52, 1.4)	0.48 (0.27, 0.84)	0.011
Model 1[2]	1	0.79 (0.46, 1.4)	0.41 (0.21, 0.77)	0.007
LA[1] *g/day*	8.9 (7.8, 9.6)	11.1 (10.6, 11.6)	14.1 (13.3, 15.8)	
	Tertile 1 (n=39 cases)	Tertile 2 (n=32 cases)	Tertile 3 (n=22)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.80 (0.48, 1.3)	0.53 (0.30, 0.92)	0.023
Model 1[2]	1	0.70 (0.41, 1.2)	0.41 (0.22, 0.75)	0.004
AA[1] *g/day*	0.12 (0.10, 0.14)	0.16 (0.15, 0.17)	0.22 (0.20, 0.26)	
	Tertile 1 (n=31 cases)	Tertile 2 (n=37 cases)	Tertile 3 (n=25 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	1.2 (0.74, 2.0)	0.79 (0.45, 1.4)	0.42
Model 1[2]	1	0.91 (0.52, 1.6)	0.60 (0.33, 1.1)	0.10
Total n6 PUFA[1] *g/d*	9.1 (8.1, 9.8)	11.3 (10.8, 11.7)	14.3 (13.5, 16.0)	
	Tertile 1 (n=41 cases)	Tertile 2 (n=30 cases)	Tertile 3 (n=22 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.70 (0.42, 1.16)	0.50 (0.29, 0.86)	0.011
Model 1[2]	1	0.62 (0.36, 1.06)	0.38 (0.21, 0.70)	0.002

n3/n6 Ratio[1]	0.08	0. 10	0.15	
	Tertile 1 (n=33 cases)	Tertile 2 (n=30 cases)	Tertile 3 (n=30 cases)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Crude	1	0.90 (0.53, 1.5)	0.90 (0.53, 1.5)	0.69
Model 1 [2]	1	0.99 (0.57, 1.7)	0.94 (0.54, 1.6)	0.82

AA, Arachidonic acid ; ALA, α-linolenic acid; CI, confidence interval; CRP, C-reactive protein; DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; EPA Eicosapentaenoic acid; LA, Linoleic acid; OR, Odd ratio; PUFA, polyunsaturated fatty acid

[1]Median intake(interquartile range) of PUFA in each tertile, adjusted on energy intake [2]Model 1 was adjusted for sex, age, educational level (primary, secondary, superior), physical activity (irregular, <1h equivalent walking/day, ≥1h equivalent walking/day), smoking status (never smoked, former smoker, current smoker), energy intake, alcohol intake, number of dietary records available, baseline BMI, difference between baseline and follow-up BMI, and 'Prudent' DP

Table 3 Logistic regression Odd Ratios (95% confidence intervals) of having elevated C-reactive protein according to tertiles of n3 and n6 PUFA intakes, stratified by tertiles of vitamin E intakes

		Tertile 1 of vitamin E consumption		Tertile 2 of vitamin E consumption		Tertile 3 of vitamin E consumption		
Median intake[1], *g/day*		9.84 (8.80, 10.60)		12.59 (11.99, 13.32)		16.28 (15.05, 18.06)		
		OR (95% CI)	P-trend	OR (95% CI)	P-trend	OR (95% CI)	P-trend	P-interaction[2]
ALA	Tertile 1	1		1		1		
	Tertile 2	1.2 (0.51, 2.9)		0.84 (0.28, 2.5)		0.57 (0.15, 2.1)		
	Tertile 3	1.2 (0.44, 3.5)	0.63	0.82 (0.28, 2.4)	0.72	0.72 (0.22, 2.4)	0.72	0.79
EPA	Tertile 1	1		1		1		
	Tertile 2	0.60 (0.25, 1.4)		1.01 (0.32, 3.2)		1.02 (0.33, 3.2)		
	Tertile 3	0.36 (0.12, 1.1)	0.06	0.90 (0.28, 2.8)	0.84	0.70 (0.21, 2.4)	0.57	0.42
DPA	Tertile 1	1		1		1		
	Tertile 2	0.53 (0.23, 1.2)		1.01 (0.35, 3.0)		1.79 (0.59, 5.4)		
	Tertile 3	0.21 (0.07, 0.67)	0.007	0.67 (0.21, 2.1)	0.49	0.67 (0.19, 2.3)	0.48	0.12
DHA	Tertile 1	1		1		1		
	Tertile 2	0.96 (0.39, 2.4)		0.46 (0.14, 1.5)		0.97 (0.32, 2.9)		
	Tertile 3	0.48 (0.16, 1.5)	0.23	0.78 (0.26, 2.3)	0.79	0.50 (0.15, 1.7)	0.28	0.91
Long chain n3 PUFA (EPA+DPA+DHA)	Tertile 1	1		1		1		
	Tertile 2	0.47 (0.19, 1.2)		0.86 (0.28, 2.6)		0.91 (0.3, 2.8)		
	Tertile 3	0.35 (0.12, 1.1)	0.043	0.82 (0.26, 2.6)	0.74	0.48 (0.14, 1.7)	0.27	0.52
Total n3 PUFA	Tertile 1	1		1		1		
	Tertile 2	0.88 (0.35, 2.2)		0.53 (0.17, 1.6)		1.75 (0.57, 5.4)		
	Tertile 3	0.47 (0.16, 1.4)	0.21	0.47 (0.15, 1.5)	0.24	0.28 (0.07, 1.1)	0.08	0.80
LA	Tertile 1	1		1		1		
	Tertile 2	0.64 (0.25, 1.6)		0.93 (0.35, 2.5)		—		
	Tertile 3	0.34 (0.03, 3.6)	0.21	0.25 (0.04, 1.4)	0.14	—	—	0.24
AA	Tertile 1	1		1		1		
	Tertile 2	0.68 (0.27, 1.7)		0.69 (0.24, 2.0)		1.31 (0.42, 4.0)		
	Tertile 3	0.67 (0.26, 1.7)	0.40	0.44 (0.13, 1.5)	0.19	0.72 (0.20, 2.5)	0.57	0.93
Total n6 PUFA	Tertile 1	1		1		1		
	Tertile 2	0.54 (0.20, 1.4)		0.66 (0.25, 1.7)		—		
	Tertile 3	0.33 (0.03, 3.5)	0.14	0.19 (0.04, 1.0)	0.05	—	—	0.13
n3/n6 Ratio	Tertile 1	1		1		1		
	Tertile 2	1.1 (0.37, 3.0)		0.85 (0.28, 2.5)		0.77 (0.27, 2.1)		
	Tertile 3	0.78 (0.28, 2.2)	0.56	0.89 (0.30, 2.7)	0.84	0.24 (0.04, 1.5)	0.12	0.29

AA, Arachidonic acid ; ALA, α-linolenic acid; CI, confidence interval; CRP, C-reactive protein; DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; EPA Eicosapentaenoic acid; LA, Linoleic acid; OR, Odd ratio; PUFA, polyunsaturated fatty acid

Models are adjusted for sex, age, educational level (primary, secondary, superior), physical activity (irregular, <1h equivalent walking/day, ≥1h equivalent walking/day), smoking status (never smoked, former smoker, current smoker), energy intake, alcohol intake, number of dietary records available, baseline BMI, difference between baseline and follow-up BMI, and 'Prudent' dietary pattern.

[1]Median intake (interquartile range) of vitamin E in each tertile, adjusted on energy intake [2]Interaction was computed with tertiles of PUFA intakes and tertiles of vitamin E intakes taken as continuous variables.

Supplemental Table 1 Food groups contributing to intakes of vitamin E and PUFAs [1]

		Vit E, % Intake	ALA, % Intake	EPA, % Intake	DPA, % Intake	DHA, % Intake	LA, % Intake	AA, % Intake
Animal fat other than butter	Animal fat used for food preparation	0.39	1.8	0.22	1.1	0.21	0.88	0.83
Butter	Butter	2.6	8.5	0	0	0	1.6	0
Margarines	All types of margarine	7.0	2.9	0	0	0	3.3	0
Vegetable oil poor in n3 PUFA	Peanut oil, sunflower oil, corn oil, grape seed oil	35.4	3.8	0	0	0	41	0
Vegetable oil rich in n3 PUFA	Walnut oil, rapeseed oil, soybean oil	0.35	3.7	0	0	0	1.1	0
Olive oil	Olive oil	5.7	6.1	0	0	0	4.1	0
Cake, cookies and pastries	cakes, tarts, biscuits, doughnuts, croissants,	5.2	8.2	0.25	1.6	3.1	5.7	7.3
Desserts	Puddings, custards, ice cream	0.96	1.0	0.20	0.86	1.4	0.85	3.5
Cheese	All types of cheese	2.0	11	0	0	0	2.2	2.1
Dairy products	Yogurt, cottage cheese	0.65	1.9	0	0	0	0.45	0
Milk	All types of milk and milk beverages	0.79	2.3	0	0	0	0.61	0
Eggs	Eggs	1.9	1.3	0.67	4.1	7.5	2.3	18
Fruit	Fruit	6.2	5.3	0	0	0	1.4	0
Vegetables	All types of vegetables	6.9	7.2	0	0	0	1.3	0
Soups	All types of soups	3.3	1.8	2.6	1.5	2.6	2.5	0.49
Legumes	Dried peas, lentils, corn	0.23	1.1	0	0	0	0.25	0
Potatoes	Potatoes, sweet potatoes, plantain, igname	1.7	0.01	0	0	0	1.1	0
Fatty fish	Salmon, tuna, trout, sardine, anchovy, mackerel, herring, eel, carp, dogfish	1.3	1.2	38	25	41	0.42	6
Lean fish	catfish, monkfish, pikefish, codfish, plaice, hake, haddock, halibut, saithe, flatfish, whiting, line fish, scorpion fish, turbot fish	0.87	0.1	17	5.5	17	0.02	2.4
Seafood	shellfish, mollusks	1.2	0.09	17	4.8	12	0.02	3.3
Meat	Pork, beef, veal,	1.2	6.1	9.7	20	2.5	2.8	15

		Vit E, % Intake	ALA, % Intake	EPA, % Intake	DPA, % Intake	DHA, % Intake	LA, % Intake	AA, % Intake
	lamb, horse							
Poultry	chicken, turkey, duck	0.47	2.4	2.7	8.7	2.7	2.7	15
Processed meat	ham, pâté, sausage, bacon and other processed meat	0.64	5.3	5.4	18	4.0	6.7	18
Organ meat	liver, heart, brain, tongue, tripe, intestine	0.12	0.17	2.2	5.4	1.8	0.17	4.2
Nuts	Nuts	1.8	4.7	0	0	0	3.5	0
Pizza	Pizza, quiche, pies	2.3	3.0	2.1	1.9	2.0	2.7	2.8
Soft drinks	All type of soda, cola and fruit juices with added sugar	0.05	0.04	0	0	0	0.01	0
Salty snacks	Salted crackers, chips, olives	0.95	0.35	2.1	0.67	1.8	1.3	0.25
Sugar and sweets	Chocolate, sugar, ham, honey, sweet bread spreads	0.82	1.0	0	0	0	1.1	0
Refined grains	Refined-grain bread, rice and pasta	2.2	4.2	0.07	0.03	0.10	5.0	0.73
Whole grains	Whole-grain bread, rice and pasta	1.0	0.83	0	0	0	0.96	0

AA, Arachidonic acid ; ALA, α-linolenic acid; DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; EPA Eicosapentaenoic acid; LA, Linoleic acid

[1]Food groups providing less than 1% of intakes in any of the nutrients presented were deleted from the table.

Dietary patterns and risk of elevated C reactive protein concentrations 12 years later

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Synthèse

Introduction

Les études expérimentales portant sur les relations entre les apports en nutriments et l'inflammation de bas grade ont permis de mettre en évidence des mécanismes par lesquels les nutriments pourraient avoir des propriétés pro- ou anti- inflammatoires. Les acides gras polyinsaturés, les vitamines et nutriments antioxydants ont ainsi démontré leur capacité à interagir avec le système immunitaire et à intervenir dans de nombreuses étapes clés de la réaction inflammatoire.(Calder et al., 2009) Par ailleurs, de nouvelles méthodes de l'épidémiologie nutritionnelle ont permis d'examiner par des méthodes a posteriori des patterns de consommations alimentaires associés à l'inflammation de bas grade.(Hoffmann *et al.*, 2004b, Hu, 2002a) Une méthode en particulier, la régression des rangs réduits (RRR) semble prometteuse dans ce contexte.(Hoffmann et al., 2004b, Hu, 2002a) En effet, elle permet de lier les deux approches, en construisant des patterns alimentaires spécifiquement associés à des variables (soit des nutriments, soit des indicateurs intermédiaires), pour permettre par la suite d'évaluer les associations entre ce pattern et une pathologie.

D'une part, les études appliquant cette méthode dans le cadre de l'étude des relations entre nutrition et inflammation sont peu nombreuses, et d'autre part elles sont toutes transversales. (Centritto et al., 2009, Heidemann *et al.*, 2005, Hoffmann *et al.*, 2004c, Schulze *et al.*, 2001)

Notre objectif était donc de construire un pattern alimentaire spécifiquement associé aux apports en nutriments reconnus comme ayant des propriétés pro- ou anti-inflammatoires, puis d'étudier les relations entre ce pattern et l'inflammation de bas grade (mesurée par la CRP sanguine) dans le long terme.

Matériel et Méthodes

Les sujets éligibles dans cette étude étaient les sujets inclus dans l'étude SU.VI.MAX ayant poursuivi leur suivi par une inclusion dans l'étude SU.VI.MAX 2 (Hercberg et al., 2004, Kesse-Guyot et al., 2011) et pour lesquels les éléments suivants étaient disponibles :

- Mesure d'exposition : présence d'au moins 3 enregistrements de 24 heures dans les deux premières années de suivi (entre 1994 et 1996) de l'étude S.VI.MAX, permettant une mesure précise des apports alimentaires dans les nutriments d'intérêt
- Mesure du critère de jugement : présence d'une mesure de la CRP réalisée lors de l'examen clinique effectué pour l'étude de suivi SU.VI.MAX2 (entre 2007 et 2009)
- Mesure des facteurs d'ajustement : variables socio-démographiques (sexe, âge, niveau d'éducation, statut marital), de mode de vie (statut tabagique, activité physique) et d'anthropométrie (poids et taille mesurés lors d'un examen clinique à un an de l'inclusion dans l'étude SU.VI.MAX 1995-1997 et lors de l'examen clinique de l'étude SU.VI.MAX2 2007-2009, permettant de calculer l'indice de masse corporelle aux deux temps) ainsi que l'affectation initiale dans le groupe placebo ou le groupe de supplémentation de la phase d'essai randomisé de l'étude SU.VI.MAX.

Les apports alimentaires ont été regroupés dans 43 groupes alimentaires spécifiques. Ces groupes alimentaires ont été utilisés en tant que facteurs prédicteurs dans une procédure de RRR.(Hoffmann et al., 2004b) Les nutriments utilisés en tant que facteurs de réponse dans la RRR étaient les suivants :

- β-carotène
- Vitamine C
- Vitamine E
- Acide linoléique (18 :2 n-6 LA)
- Acide α-linolénique (18 :3 n-3 ALA)
- Acide arachidonique (20 :4 n-6 AA)
- N-3 PUFA à longue chaine (EPA+DHA+DPA)

L'ensemble des apports en groupes alimentaires et en nutriments utilisés étaient préalablement ajustés sur à l'apport énergétique.(Willett et al., 1986)

Les patterns alimentaires retenus pour les analyses ultérieures étaient ceux permettant d'expliquer plus de 5% de la variation des nutriments utilisés en tant que facteurs de réponse.

Les associations entre les patterns alimentaires (en tertiles) et l'inflammation de bas grade (évaluée par une CRP>3mg/l(Pearson et al., 2003)) ont été évaluées par régression logistique uni- puis multi-variée en prenant en compte les facteurs de confusion socio-démographiques, de mode de vie et anthropométriques, le groupe de supplémentation et l'apport énergétique total.

Résultats

Parmi les 6850 sujets inclus dans l'étude de suivi SU.VI.MAX2, 3476 avaient effectué une mesure de CRP. Parmi ceux-ci, 2031 ont été inclus dans l'étude.

La procédure de RRR a permis de retenir quatre patterns alimentaires spécifiquement associés aux nutriments réponse, expliquant au total 63,9% de la variation des nutriments retenus en réponse et 13,2% de la variation des apports en groupes alimentaires. Le premier pattern, expliquant 28,1% des apports en nutriments, corrélait positivement avec les apports en nutriments antioxydants (vitamines C et E, β-carotène) et acides gras essentiels (LA et ALA). Les groupes alimentaires ayant les poids positifs les plus importants sur ce pattern étaient les légumes, l'huile d'olive et les autres huiles d'origine végétale et les groupes alimentaires ayant les poids négatifs les plus importants sur ce pattern étaient le beurre, le vin et les produits céréaliers raffinés. Le deuxième pattern était positivement corrélé aux apports en acide arachidonique et en PUFAs n-3 à longue chaine (EPA + DPA + DHA). Les groupes alimentaires ayant un poids positif important sur ce pattern étaient globalement les produits animaux (charcuterie, volaille, poissons gras et produits de la mer, œufs). Le troisième pattern était positivement corrélé aux apports en vitamine C, β-carotène et PUFAs n-3 à longue chaine et négativement corrélés aux apports en LA, AA et vitamine E. Les groupes alimentaires ayant des poids positifs importants sur ce pattern étaient les fruits et jus de fruits et les groupes alimentaires ayant les poids négatifs importants sur ce pattern étaient les huiles végétales pauvres en PUFAs n-3. Le quatrième pattern était positivement corrélé aux apports en AA et en vitamine C et négativement corrélé aux apports en PUFAs n-3 à longue chaine et en vitamine E, reflétant un rapport important entre PUFAs n-6 et n-3. Les groupes alimentaires ayant des poids positifs importants sur ce pattern étaient la charcuterie, les œufs et la volaille, les groupes alimentaires ayant des poids négatifs importants étant les poissons gras.

Le pattern 1 était négativement associé avec une CRP augmentée dans les modèles logistiques uni- et multi-variés. Le pattern 4 était positivement associé à une CRP augmentée dans les modèles logistiques univariés mais la force d'association diminuait lors de l'ajustement.

Discussion

Dans notre étude, un pattern alimentaire riche en légumes et en huiles végétales, conduisant à des apports élevés en acides gras essentiels et en nutriments antioxydants était négativement associé à une CRP augmentée, alors qu'un pattern alimentaire riche en charcuterie et pauvre en poissons gras, conduisant à un rapport entre PUFAs n-6 et n-3 important était positivement associé à une CRP augmentée.

L'association négative entre un pattern alimentaire riche en légumes et en huiles végétales et une CRP augmentée a déjà été observée dans des études construisant des patterns alimentaires selon la procédure classique de l'analyse en composante principale (ACP).(Calder *et al.*, 2011, Centritto et al., 2009, Esmaillzadeh et al., 2007, Lopez-Garcia et al., 2004a, Nanri et al., 2011) Néanmoins, si les patterns construits en ACP partagent certains aspects de notre pattern RRR, ils présentent souvent aussi des apports élevés en fruits et produits céréaliers complets, étant donné que la procédure de construction du pattern implique la maximisation de la variance expliquée au niveau des groupes alimentaires. En effet, il existe une corrélation importante entre les groupes fruits et céréales complètes d'une part et légumes et huiles végétales d'autre part.

L'avantage en cela de la procédure de RRR est qu'elle permet de tester les hypothèses relatives aux nutriments responsables de l'association négative entre ces patterns alimentaires « Prudents » ou « Healthy » et la CRP. Notre étude permet d'une part d'expliquer en partie l'absence d'association entre des patterns alimentaires construits en ACP et la CRP et d'autre part d'avancer l'hypothèse que ce sont les consommations élevées en légumes et en huiles végétales qui pourraient expliquer les associations négatives observées.

Par ailleurs, l'association positive entre un pattern reflétant un fort ratio d'apport entre PUFAs n-6 et n-3 et la CRP permet de renforcer les hypothèses et observations qui ont été faites sur l'importance de la balance entre ces acides gras dans l'inflammation.(Simopoulos, 2008)

Les forces de cette étude résident dans la mesure précise des apports alimentaires par des enregistrements de 24 heures répétés et l'utilisation de procédures statistiques innovantes d'extraction de patterns et permettant de tester des hypothèses liées aux mécanismes sous-jacents impliqués dans les relations à long terme entre apports alimentaires et CRP.

Les limites de cette étude résident dans l'absence de mesure de l'inflammation au début de l'étude, les associations observées pouvant être des relations résiduelles de relations transversales plutôt que de véritables associations prospectives, et l'utilisation d'une mesure de la CRP non sensible, avec une limite de détection à 1mg/l.

Conclusion

La construction de patterns alimentaires spécifiquement associés à des nutriments supposés avoir des propriétés pro- ou anti-inflammatoires permet d'améliorer la connaissance des relations entre alimentation et inflammation dans le long terme. Cette étude aurait besoin d'être répliquée dans d'autres populations pour renforcer les résultats observés.

Manuscrit

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Dietary patterns and risk of elevated C-reactive protein concentrations 12 years later

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Abstract

Inflammation mediates several chronic diseases. Micronutrients can act on inflammation, either through modulating cytokine production or by scavenging by-products of activated white cells. Identifying dietary patterns (DP) reflecting these mechanisms and relating them to inflammation is of interest. The objective of the study was to identify DP specifically associated with intakes of nutrients potentially involved in inflammatory processes in a middle-aged population and investigate long-term associations between these DP and C-reactive protein (CRP) status assessed several years later. Subjects included in the Supplementation in Vitamins and Mineral Antioxidants 2 cohort study, having available data on dietary assessment carried out in 1994–5 and CRP measurement in 2007–9, were included in the analysis. DP were extracted with reduced rank regression (RRR), using antioxidant micronutrients and PUFA as response variables. Associations between CRP measurements >3 mg/l and extracted DP were then examined with logistic regression models providing OR and 95 % CI. A total of 2031 subjects (53·2 % women, mean follow-up duration: 12·5 years) were included in the analyses. Of the four extracted DP, a DP with high loading values of vegetables and vegetable oils, leading to high intakes of antioxidant micronutrients and essential fatty acids, was significantly and negatively associated with risk of elevated CRP (OR 0·88; 95 % CI 0·78, 0·98). Conversely, a DP reflecting a high *n*-6:*n*-3 fatty acid intake ratio was positively and significantly associated with elevated CRP (adjusted OR 1·15; 95 % CI 1·00, 1·32). DP extracted with RRR provide support for further exploration of relationships between dietary behaviour and inflammation.

Key words: **Dietary patterns: C-reactive protein: Reduced rank regression: Inflammation**

Inflammation has been implicated in the pathways of numerous diseases[1]. Besides chronic inflammatory conditions, such as rheumatoid arthritis or inflammatory bowel diseases, where inflammation is the primary mechanism of disease activity, low-grade chronic inflammation could be involved in the risk of obesity[2], cancer[3] and atherosclerosis[4].

Regardless of the origin or the site of chronic inflammation, the response is stereotypical and involves overproduction of cytokines, chemokines, eicosanoids and matrix metalloproteinases[1,5]. IL-6 and TNF-α are released by activated leucocytes at the site of inflammation and this in turn can stimulate systemic cytokine production. For instance, IL-6 induces C-reactive protein (CRP) by the liver[6]. Elevated concentrations of cytokines act to amplify the inflammatory process, leading to tissue damage and clinical symptoms[1]. Besides, activated macrophages, monocytes and granulocytes produce free oxygen radicals[7]. Over time, chronic inflammation results in the accumulation of oxidant species, gradually weakening the antioxidant defence systems[8].

Some micronutrients can act on inflammation, either by modulating primary mechanisms of cytokine production or through secondary processes by scavenging by-products of activated leucocytes. PUFA are implicated in the production of inflammatory mediators, eicosanoids, which include

Abbreviations: CRP, C-reactive protein; DP, dietary pattern; PCA, principal component analysis; RRR, reduced rank regression; SU.VI.MAX2, Supplementation in Vitamins and Mineral Antioxidants 2.

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thromboxane, PG and leukotrienes. The principal source for eicosanoids is arachidonic acid and its precursor, linoleic acid. Diets rich in linoleic or arachidonic acid have the potential to increase inflammatory signals. *n*-3 PUFA can serve as alternate sources for eicosanoids, leading to the production of altered eicosanoids with less inflammatory capacity[9]. Diets rich in *n*-3 PUFA display anti-inflammatory capacities, all the more if *n*-6 PUFA intake is low. Conversely, diets rich in *n*-6 and low in *n*-3 PUFA promote inflammation[10].

The response to oxidant stress, such as the one observed in inflammatory processes, involves micronutrients such as vitamins C and E and carotenoids[11,12]. Vitamin C is involved in scavenging free oxygen radicals and protection against lipid peroxidation due to its high reducing power. Vitamin E is a potent chain-breaking antioxidant interrupting lipid peroxidation and preventing the propagation of free radical-initiated reactions[13]. Carotenoids exhibit immunomodulatory activities, stimulate the phagocytic and bacteria-killing ability of peripheral blood neutrophils and peritoneal macrophages[14].

Traditional analysis in nutritional epidemiology, typically studying relationships between single nutrients and disease, does not allow us to capture the complexity of a subject's diet, as nutrients are not consumed individually, but in combination in the food matrix[15]. Comprehensive approaches involving the assessment of dietary patterns (DP) have, therefore, stirred considerable interest in the scientific community, as they represent meaningful combinations of food consumption in the population[15].

Several studies have investigated the role of DP in inflammation processes[16–30], either using *a priori*-based approaches, through existing dietary score (describing Mediterranean diet) or a 'dietary inflammatory index'[16–18], or exploratory analysis, such as principal components analysis (PCA)[19–22,26,29,31,32]. Besides, some have proposed DP associated with biological markers of inflammation to be intermediate factors of CVD or diabetes[20,23,25,30]. However, to the best of our knowledge, no study has evaluated the long-term associations between DP reflecting pro- or anti-inflammatory nutrient intake and inflammation status assessed by serum CRP concentration.

The objective of the present study was to identify DP explaining variation in intakes of nutrients potentially involved in inflammatory processes in a middle-aged population and investigate the long-term associations between those DP and CRP status assessed several years later.

Methods

Study population

The study population was selected from the participants in the Supplementation in Vitamins and Mineral Antioxidants 2 (SU.VI.MAX2) study. The SU.VI.MAX study is a randomised, double-blind, placebo-controlled, primary prevention trial designed to evaluate the effect of supplementation of antioxidant vitamins and minerals at nutritional doses on the incidence of CVD and cancer[33]. Subjects were enrolled in 1994–5 for a planned 8-year intervention trial. In 2007–9, subjects were invited to enroll into an additional follow-up study defining the SU.VI.MAX2 study[34]. Subjects who accepted underwent a clinical examination and had blood drawn for biological tests. In the SU.VI.MAX2 study, a subsample selected on the basis of geographical criteria for operative and logistical aspects was included in the CRP study.

Both the SU.VI.MAX and the SU.VI.MAX2 studies were approved by the Ethics Committee for Studies with Human Subjects of the Paris-Cochin Hospital (nos. 706 and 2364, respectively) and the Comité National Informatique et Liberté (nos. 334641 and 907094, respectively). All subjects gave a written informed consent to participate in the study. This trial is registered under the trial registration code: clinicaltrials.gov: NCT00272428.

All subjects included in the CRP study were eligible for the present study, irrespective of whether they belonged to the placebo or the supplementation group of the initial SU.VI.MAX trial. Subjects having less than three dietary records in the first 2 years of the SU.VI.MAX study (1994–6) and subjects having missing data on anthropometric measurements (at baseline or at the beginning of the SU.VI.MAX2 study), socio-demographic, physical activity and tobacco use variables were excluded from the analyses.

Data collection

Dietary data assessment. Dietary assessment was carried out via repeated 24 h records (1994–6), collected by computerised questionnaires using the Minitel Telematic Network loaded with study-specific software, as described before[33]. The Minitel was a small terminal widely used in France as an adjunct to the telephone. Days of the week for these records were randomised and fixed for each subject so that each day of the week and all seasons were covered. A validated instruction manual was used for coding food portions, including more than 250 generic items, corresponding to 1000 specific foods. A French food composition table was used to calculate nutrient contents[35]. Foods were classified into forty-three food groups (See Supplemental Table 1, available online).

Inflammation measurement. Blood samples were drawn from the participants in the SU.VI.MAX2 study (2007–9), immediately centrifuged and frozen at −80°C and stored. CRP concentrations were measured using an immunoturbidimetric assay (reagent: Tina quant C-reactive protein (latex) assay), with a detection limit of 1 mg/l for CRP. Intra-assay and inter-assay CV were 0·61 and 2·87 %, respectively.

Covariate assessment. Socio-demographic (marital status (single/cohabiting), educational level (primary, secondary or superior)), physical activity (subjects were asked to report if they regularly practised physical activity (yes or no) and if yes, if they practised the equivalent of ≥1 h walking/d (yes or no), herein coded as: irregular, <1 h equivalent walking/d or ≥1 h equivalent walking/d) and smoking status (never smoked, former smoker or current smoker)) data were obtained through self-administered questionnaire at baseline (1994–6).

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Table 1. Characteristics of the study population

(Mean values and standard deviations; number of subjects and percentages)

	Men		Women		
	Mean	SD	Mean	SD	*P**
Sex					
n	950		1081		
%	46·77		53·23		
Baseline age (years)	51·65	4·67	48·05	6·19	<0·001
Age at SU.VI.MAX2 study (years)	64·11	4·68	60·60	6·18	<0·001
Follow-up duration (years)	12·07	0·35	12·12	0·38	0·003
CRP >3 mg/l					0·656
n	133		144		
%	6·55		7·09		
Supplementation allocation					0·522
Placebo					
n	447		524		
%	47·05		48·47		
Antioxidants					
n	503		557		
%	52·95		51·53		
Martial status					<0·001
Single					
n	86		195		
%	9·05		18·04		
Cohabiting					
n	864		886		
%	90·95		81·96		
Highest achieved diploma					0·013
Primary					
n	201		192		
%	21·16		17·76		
Secondary					
n	331		442		
%	34·84		40·89		
Superior					
n	418		447		
%	44·00		41·35		
Smoking status					<0·001
Never smoked					
n	368		666		
%	38·74		61·61		
Former smoker					
n	474		310		
%	49·89		28·68		
Current smoker					
n	108		105		
%	11·37		9·71		
Physical activity					<0·001
Irregular					
n	217		244		
%	22·84		22·57		
< 1 h/d					
n	228		403		
%	24·00		37·28		
≥ 1 h/d					
n	505		434		
%	53·16		40·15		
Baseline BMI (kg/m^2)	25·21	2·99	23·04	3·49	<0·001
BMI at SU.VI.MAX2 study (kg/m^2)	26·26	3·41	24·48	4·15	<0·001
BMI variation (kg/m^2)	1·05	1·54	1·45	2	<0·001
Dietary intake at baseline					
Long chain *n*-3 fatty acids (g/d)	0·45	0·35	0·35	0·28	<0·001
Linoleic acid (g/d)	13·09	4·53	10·12	3·69	<0·001
α-Linolenic acid (g/d)	0·98	0·32	0·77	0·26	<0·001
Arachidonic acid (g/d)	0·20	0·08	0·15	0·06	<0·001
β-Carotene (μg/d)	4237·25	2537·62	3896·96	2574·48	<0·001
Vitamin C (mg/d)	101·20	48·56	96·67	44·65	<0·001
Vitamin E (mg/d)	14·41	5·04	11·88	4·28	<0·001

SU.VI.MAX2, Supplementation in Vitamins and Mineral Antioxidants 2; CRP, C-reactive protein.

*P obtained from t tests for continuous variables and χ^2 tests for categorical variables.

Anthropometric measurements were taken at a clinical examination 1 year after inclusion into the SU.VI.MAX study (1995–7) and at the SU.VI.MAX2 study (2007–9). The weight was measured in subjects in light clothing and with no shoes to the nearest 0·1 kg, and the height was measured to the nearest cm with a wall-mounted stadiometer in the same conditions.

Statistics

BMI was calculated as the weight (in kg) divided by the square of height (in m) and obesity was defined as BMI $\geq 30\,kg/m^2$.

DP were extracted using reduced rank regression (RRR)[36]. RRR derives patterns from predictors to maximise the explained variation of a pre-defined set of responses. Responses chosen for RRR were nutrients that have been consistently associated with inflammation in the literature, i.e. PUFA (linoleic acid, α-linolenic acid, arachidonic acid, EPA, docosapentanoic acid and DHA) and antioxidant micronutrients (β-carotene, vitamin C and vitamin E). Food group consumption and nutrient intake used as predictors were energy-adjusted using the residual method[37]. Sensitivity analysis using different numbers of response variables (especially different regrouping sets for fatty acids and tests including or excluding dietary fibres) indicated that the greatest explanation of the total variation in foods and in responses was obtained using linoleic acid, α-linolenic acid, arachidonic acid, very-long-chain *n*-3 PUFA (EPA, docosapentanoic acid and DHA combined), β-carotene, vitamin C and vitamin E. Results of sensitivity analyses are available online, in Supplemental Table 2. Factors explaining more than 5% of the total explained variation in responses were retained in the analysis. Extracted DP scores were used as they appeared in the RRR.

CRP was categorised as ≤ 3 and >3 mg/l, according to the evidence in CVD risk[38], and logistic regression models were applied to estimate OR (95% CI) of high levels of inflammation across tertiles of DP scores. *P* for trend across DP was computed using DP scores as continuous variables. We first ran models testing crude associations, then models were adjusted in three ways: (1) sex, baseline age, educational level (primary/secondary/university), marital status (single/cohabiting), baseline smoking status (never smoker/former smoker/current smoker), baseline physical activity (irregular/<1 h equivalent walking/d/≥ 1 h equivalent walking/d), energy intake and number of dietary records available (initial supplementation allocation group had no effect on high levels of CRP, but for consistency, the variable was included in the adjustment procedure); (2) model 1 + BMI at inclusion; (3) model 2 + change in BMI during the follow-up. Interactions were tested between extracted DP and supplementation allocation group (antioxidant *v.* placebo) in the SU.VI.MAX study, obesity status at baseline, obesity status at SU.VI.MAX2 study and variation of obesity status with time.

Sensitivity analyses were performed in order to test the robustness of the present results. Data were re-analysed after excluding subjects with a history of cancer or CVD (myocardial infarction or stroke) during the dietary data collection period and obese subjects at baseline. All analyses were carried out using SAS software (version 9.1; SAS Institute, Inc.)

Results

Of the 6850 subjects enrolled in the SU.VI.MAX2 study, 3476 were included in the CRP study. Of these, 861 were excluded for insufficient number of dietary records, 502 for missing data on anthropometric measurements at baseline, forty-three for missing data on socio-demographic variables and thirty-nine for missing data on tobacco use or physical activity. The final sample, therefore, included 2031 subjects.

Characteristics of included subjects are displayed in Table 1. In particular, 53·2% were women. Mean baseline age was 51·6 (SD 4·7) years for men and 48 (SD 6·2) years for women, with a mean follow-up duration of more than 12 years. In all, 52·2% subjects were allocated to antioxidant supplementation in the initial trial. There were no differences between placebo and antioxidant groups in baseline characteristics (data not tabulated). Compared with those included in the sample, subjects excluded for insufficient dietary records or missing data were more frequently single. Included subjects did not differ otherwise from the excluded ones.

Mean number of dietary records available for each subject was 10.12 (SD 3.0), with a mean of 7.02 (SD 2.7) records on week days and 3.10 (SD 2.0) on week-end days. Seasonality was accounted for at the individual level, as a mean of 4.57 (SD 1.6) records per subject were available for the May–October period, while 5.55 (SD 2.0) records per subject were available for the November–April period.

Table 2. Explained variations of nutrients and food groups by extracted dietary pattern (DP)* (*n* 2031)

	DP1	DP2	DP3	DP4	Total explained variation
Explained variation in food groups	4·6	2·9	3·3	2·4	13·2
Explained variation in nutrients	28·1	16·7	12·1	7·1	63·9
Pearson correlation coefficient					
β-Carotene	0·36	−0·08	0·31	0·07	
Vitamin C	0·38	−0·07	0·70	0·25	
Vitamin E	0·86	−0·12	−0·14	−0·11	
Linoleic acid	0·82	−0·07	−0·38	0·02	
α-Linolenic acid	0·46	−0·10	0·03	0·04	
Arachidonic acid	0·11	0·77	−0·19	0·45	
Very-long-chain *n*-3 PUFA	0·24	0·73	0·25	−0·46	

* DP obtained with reduced rank regression using antioxidant nutrients and PUFA as response variables in the procedure.

Four factors were extracted with RRR, explaining 63·9% of total variation of response variables and 13·2% of variation of food groups. Correlation between extracted DP and nutrients intake and food groups loadings on the DP are presented in Tables 2 and 3. The first pattern (DP1) was positively correlated to intake of antioxidant micronutrients (vitamin C, vitamin E and β-carotene) and essential fatty acids (linoleic and α-linolenic acids). Food groups loading highly positively on this pattern were vegetables, olive oil and other vegetable oils and groups loading highly negatively were butter, wine and refined grains. The second pattern (DP2) was positively correlated to intake of arachidonic acid and very-long-chain *n*-3 PUFA. Food groups loading highly positively on this pattern were fatty fish, seafood, processed meat, organ meat, eggs and poultry. The third pattern (DP3) was positively correlated to intake of vitamin C, β-carotene and very-long-chain *n*-3 PUFA and negatively correlated to intake of linoleic acid, arachidonic acid and vitamin E. Food groups loading highly positively on this pattern were fruits and fruit juices and groups loading highly negatively on this pattern were vegetable oils poor in *n*-3 PUFA. The fourth pattern (DP4) was positively correlated to intake of arachidonic acid and vitamin C and negatively correlated to intake of long-chain *n*-3 PUFA and vitamin E. This pattern reflected a high *n*-6:*n*-3 PUFA intake ratio. Food groups loading highly positively on this pattern were processed meat, eggs and poultry and group loading highly negatively on this pattern was fatty fish (Tables 2 and 3).

Table 3. Loadings of food groups in dietary pattern (DP)* scores (*n* 2031)†

	DP1	DP2	DP3	DP4
Animal fat other than butter				
Beer				
Breakfast cereals			0·137	
Butter	−0·174			
Cake, cookies and pastries				
Cheese				
Coffee				
Condiments	0·182			
Desserts				
Dried fruit				
Eggs		0·286		0·405
Fruits	0·189	−0·151	0·467	
Dairy products			0·206	
Fatty fish	0·155	0·632	0·239	−0·514
Fruit juice			0·463	0·239
Legumes				
Reduced-fat dairy products				
Lean fish	0·104		0·144	−0·216
Margarines	0·181	−0·105		−0·132
Meat			−0·154	0·134
Milk				
Nuts	0·211	−0·141		
Olive oil	0·359			−0·105
Organ meat		0·268		0·279
Pizza				
Potatoes				
Poultry		0·263		0·332
Processed meat		0·280	−0·212	0·325
Refined grains	−0·200	−0·122		−0·158
Soft drinks				
Seafood		0·327		−0·114
Salty snacks				
Soups	0·111	−0·115	0·171	
Spirits			−0·103	
Sugar and sweets				
Tea				
Vegetables	0·430		0·205	0·136
Vegetable oil poor in *n*-3	0·529		−0·340	
Vegetable oil rich in *n*-3	0·185			
Water			0·139	
Whole grains		−0·118	0·156	
Wine	−0·162	0·128	−0·138	

*DP obtained with reduced rank regression using antioxidant nutrients and PUFA as response variables in the procedure; loading values in the range of −0.10 to 0.10 are not presented in the table.
†See Supplementary Table S1 (available online) for food groupings.

Logistic regression showed that high CRP concentrations were negatively associated with DP1 and positively associated with DP4 assessed 13 years before in crude and multivariate models (Table 4). Associations between DP1 and high CRP concentrations were even strengthened after adjusting further on BMI and BMI change (models 2 and 3), whereas associations between DP4 and high CRP were weakened after adjusting for BMI and change in BMI. DP2 and DP3 were not significantly associated with high concentrations of CRP.

Interactions between extracted DP and initial supplementation allocation group, obesity status at baseline, obesity status at SU.VI.MAX2 study or change in obesity status during follow-up were not significant. Exclusion of subjects having developed cancer or CVD during the 2-year period of dietary data collection or obese subjects at baseline did not substantially modify results (data not shown).

Discussion

In the present study, RRR was used to determine meaningful combinations of food intake that would explain as much of the variation of potentially pro- or anti-inflammatory nutrient intake as possible. Moreover, we were able to identify major food consumption patterns associated with inflammation: a DP rich in vegetables and vegetable oil (DP1), leading to a high intake of antioxidant micronutrients and essential fatty acids, showed preventive features against subsequent elevated CRP; conversely, a diet poor in fatty fish and rich in other animal products (DP4), leading to a high *n*-6:*n*-3 PUFA intake ratio, exhibited pro-inflammatory characteristics.

The negative association between a DP rich in vegetables and vegetable oil and inflammation is consistent with results from studies using PCA of food consumption in relation to inflammation[39]. Such studies have observed that DP correlated with high intakes of vegetables, fruits, fish and vegetable oils are negatively associated with CRP concentrations[20,21,26,29,31].

For instance, in the Nurses' Health Study, the 'prudent pattern' that positively correlated with consumption of fruit, vegetables, fish, poultry and whole grains was negatively associated with CRP and IL-6 concentrations in quintiles[26]. In the Multi-Ethnic study for Atherosclerosis, a DP with high loading values of vegetables, fish and soup ('vegetables and fish') was negatively, but not significantly, associated with CRP concentrations and with other inflammatory biomarkers (E-selectin and IL-6)[29]. In a healthy Italian population, Centritto *et al.*[20]

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Table 4. Increased C-reactive protein (CRP) according to quartiles of extracted dietary patterns (DP)* (*n* 2031) (Odd ratios and 95 % confidence intervals)

	Tertile 1	Tertile 2		Tertile 3			Continuous		
	OR	OR	95 % CI	OR	95 % CI	*P* for trend	OR	95 % CI	*P*
DP1									
n	105		83		80				
Univariate	1	0·76	0·56, 1·04	0·73	0·53, 0·99	0·045	0·90	0·80, 1·00	0·055
Model 1†	1	0·77	0·56, 1·05	0·73	0·53, 1·00	0·048	0·90	0·80, 1·00	0·051
Model 2‡	1	0·75	0·55, 1·03	0·69	0·49, 0·95	0·022	0·88	0·78, 0·98	0·025
Model 3§	1	0·76	0·55, 1·05	0·69	0·50, 0·97	0·028	0·89	0·79, 0·99	0·039
DP2									
n	83		97		88				
Univariate	1	1·20	0·87, 1·64	1·07	0·78, 1·47	0·688	1·05	0·93, 1·18	0·46
Model 1†	1	1·22	0·88, 1·67	1·04	0·75, 1·44	0·823	1·04	0·92, 1·18	0·55
Model 2‡	1	1·09	0·79, 1·51	0·93	0·67, 1·29	0·641	0·98	0·87, 1·12	0·79
Model 3§	1	1·01	0·73, 1·41	0·87	0·62, 1·21	0·388	0·96	0·85, 1·09	0·52
DP3									
n	95		81		92				
Univariate	1	0·83	0·61, 1·14	0·96	0·71, 1·31	0·810	1·00	0·88, 1·14	0·99
Model 1†	1	0·83	0·60, 1·15	0·95	0·69, 1·30	0·734	1·00	0·88, 1·14	0·99
Model 2‡	1	0·87	0·63, 1·20	1·04	0·75, 1·44	0·819	1·04	0·91, 1·18	0·59
Model 3§	1	0·90	0·65, 1·26	1·10	0·79, 1·52	0·588	1·06	0·93, 1·21	0·41
DP4									
n	79		88		101				
Univariate	1	1·13	0·82, 1·56	1·33	0·97, 1·82	0·078	1·19	1·04, 1·36	0·012
Model 1†	1	1·14	0·82, 1·59	1·32	0·96, 1·82	0·085	1·20	1·04, 1·37	0·010
Model 2‡	1	1·09	0·78, 1·52	1·21	0·87, 1·67	0·250	1·15	1·00, 1·32	0·046
Model 3§	1	1·10	0·79, 1·55	1·19	0·86, 1·65	0·306	1·13	0·98, 1·30	0·090

*DP obtained with reduced rank regression using antioxidant nutrients and PUFA as response variables in the procedure.
†Model 1 adjusted for sex, baseline age, level of education (primary/secondary/university), marital status (single/cohabiting), smoking status (never smoker/former smoker/current smoker), baseline physical activity (irregular/<1 h equivalent walking/d/≥1 h equivalent walking/d), energy intake, number of dietary records available and supplementation allocation group (antioxidant *v.* placebo).
‡Model 2 adjusted for model 1 variables + baseline BMI.
§Model 3 adjusted for model 2 variables + change in BMI during follow-up.

found that a pattern with high loading values for vegetables, olive oil, legumes, soups, fruit and fish ('olive oil and vegetables') was significantly negatively associated with CRP concentrations, even after adjustment for BMI.

DP described earlier share 'healthy components' with our DP1, such as certain food group loadings (vegetables or vegetable oils, in particular), but the DP extraction procedure is intended to investigate the observed correlated food group consumption, maximising explained variation of food intake[36]. As such, PCA-derived DP reflecting a 'prudent' or 'healthy' diet usually combines high intakes of fruits, vegetables, whole grains and fish, and it is not possible to determine which food groups mostly drive the negative association with inflammation. This might possibly explain why some PCA-derived patterns fail to associate significantly with inflammatory biomarkers in the literature. RRR procedure allows for analysing associations between food group consumption and inflammation beyond simple correlations as would PCA, stressing which component of a 'healthy' diet would directly relate to inflammation. The present results argue that vegetables and vegetable oil are probably responsible for the association between these 'healthy' patterns and inflammation.

The positive association between a diet with a high *n*-6:*n*-3 PUFA intake ratio and inflammation is not unexpected: the balance between *n*-6 and *n*-3 PUFA is of major importance in the production of inflammatory mediators[10]. The fact that the association of DP4 with high CRP is weakened after adjusting for BMI and BMI change, however, suggests that the effect of a high *n*-6:*n*-3 PUFA intake ratio on inflammation is probably mostly mediated by adiposity. Dietary fat composition has direct effects on adiposity and adipocyte metabolism[40]. In rodent models, dietary fat composition alters expression of neuropeptide genes involved in weight regulation[41]; diets rich in *n*-6 PUFA promote adiposity[42], whereas diets rich in *n*-3 PUFA reduce adiposity[43,44]. The *n*-6:*n*-3 PUFA intake ratio in pregnant women has been found to make an impact on the adiposity of the child, and diets rich in *n*-6 PUFA increase adiposity in adults[45,46].

It is interesting to underline the fact that, whereas DP were significantly associated with elevated CRP, initial supplementation in the SU.VI.MAX trial study was not. This finding could be explained by three mechanisms: first, supplementation was conducted at nutritional doses. It is therefore possible that the associations between nutrient intake (taken as supplements) and inflammation would need a higher dosage to be proven. Second, the initial SU.VI.MAX trial ended in 2002, and subjects were not advised to take supplements at the disclosure of the results. It is therefore also possible that the improvement in nutritional status obtained with supplementation did not last up to the end of the follow-up, leaving associations with dietary intake perceptible. Third, it is possible that the global nutritional

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profile of food groups rather than the antioxidant compounds *per se* would be responsible of the observed effect.

To our knowledge, long-term associations between DP extracted using RRR and CRP concentrations examined in a longitudinal design have not been previously investigated. Inflammatory biomarkers are usually considered as intermediary factors between nutrition and CVD or diabetes. Therefore, studies using RRR to extract DP introduce CRP in combination with other risk factors for these diseases as response variables in the procedure, in order to analyse associations with prospective onset of CVD or diabetes[20,23,25,27].

Introducing nutrients as response variables in the RRR procedure allowed us to explore the possible mechanisms through which food group consumption could act on inflammatory processes. RRR identified the components of a potential anti-inflammatory diet, relating them to current knowledge in the field. RRR in this perspective, therefore, contributes to new insights on the anti- or pro-inflammatory potential of the diet.

The present study is, however, subject to limitations. First, CRP was measured with a standard procedure, with a detection limit at <1 mg/l, not allowing us to investigate low-grade inflammation. Second, CRP was not measured at baseline, and therefore subjects with high inflammation pre-existing at baseline could not be excluded. The present results could, therefore, reflect pre-existing associations between DP and CRP. However, sensitivity analysis excluding subjects susceptible to having a high CRP at baseline (subjects having declared cancer or CVD in the initial years of the study or obese subjects at baseline) did not significantly alter the present results.

Strengths of the present study include the use of detailed repeated dietary records (24 h records), allowing for an accurate and reliable analysis of nutrient intake, use of valid and original statistical analyses, investigation of the long-term associations between nutrition and inflammation and test of the robustness of the results in sensitivity analysis.

In conclusion, the present results show that DP specifically explaining variation in intakes of potentially pro- and anti-inflammatory nutrients can accurately predict high levels of inflammatory biomarkers several years later. However, the present results need to be reproduced in other populations in order to confirm our conclusions on the beneficial effects of a diet rich in vegetables and vegetable oils on inflammation.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114512005636

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Supplemental Table 1: Food groups included in the analysis of the dietary patterns

Animal fat other than butter	Animal fat used for food preparation
Beer	Beer and ciders
Breakfast cereals	Breakfast cereals
Butter	Butter
Cake, cookies and pastries	Cakes, tarts, biscuits, doughnuts, croissants,
Cheese	All types of cheese
Coffee	Coffee
Condiments	Vinegar, mustard, soya sauce, spices
Desserts	Puddings, custards, ice cream
Dried fruit	Dried fruit
Eggs	Eggs
Fruit	Fruit
Fruit juice	Fruit juices without added sugar
Dairy products	Yogurt, cottage cheese
Reduced-fat dairy	Low-fat dairy
Fatty fish	Salmon, tuna, trout, sardine, anchovy, mackerel, herring, eel, carp, dogfish
Lean fish	Catfish, monkfish, pikefish, codfish, plaice, hake, haddock, halibut, saithe, flatfish, whiting, line fish, scorpion fish, turbot fish
Legumes	Dried peas, lentils, corn
Margarines	All types of margarine
Meat	Pork, beef, veal, lamb, horse
Milk	All types of milk and milk beverages
Nuts	Nuts
Olive oil	Olive oil
Organ meat	Liver, heart, brain, tongue, tripe, intestine
Vegetables	All types of vegetables
Pizza	Pizza, quiche, pies
Potatoes	Potatoes, sweet potatoes, plantain, igname
Poultry	Chicken, turkey, duck
Processed meat	Ham, pâté, sausage, bacon and other processed meat
Refined grains	Refined-grain bread, rice and pasta
Whole grains	Whole-grain bread, rice and pasta
Salty snacks	Salted crackers, chips, olives
Seafood	Shellfish, mollusks
Soft drinks	All type of soda, cola and fruit juices with added sugar
Soups	All types of soups
Spirits	Aperitifs, spirits, liquors
Sugar and sweets	Chocolate, sugar, ham, honey, sweet bread spreads
Tea	Tea
Vegetable oil poor in n-3 fatty acids	Peanut oil, sunflower oil, corn oil, grape seed oil
Vegetable oil rich in n-3 fatty acids	Walnut oil, rapeseed oil, soybean oil
Water	Water
Wine	Wine

DISCUSSION GENERALE ET PERSPECTIVES

1. Discussion

Résultats principaux

Les résultats présentés dans ce travail de thèse permettent de conforter par des approches d'épidémiologie nutritionnelle complémentaires les hypothèses relatives à l'intérêt des PUFAs et des nutriments antioxydants dans l'inflammation.

Nos résultats ont montré une relation inverse entre les apports alimentaires en PUFAs n-3 et n-6 et une CRP augmentée. Une interaction était observée entre les apports en vitamine E et en PUFAs n-3 dans la relation avec la CRP augmentée, la relation inverse observée n'étant significative que chez les sujets ayant des apports faibles en vitamine E.

Si l'association inverse entre apports en PUFAs n-3 et inflammation a été observée dans d'autres études (He et al., 2009, Lopez-Garcia et al., 2004b, Pischon et al., 2003, Poudel-Tandukar et al., 2009, Yoneyama et al., 2007), la relation entre PUFAs n-6 et inflammation est sujette à controverse.(Fritsche, 2008, Johnson et al., 2012) Nos résultats tendent à conforter l'hypothèse selon laquelle les PUFAs n-6, et l'acide linoléique en particulier, auraient aussi une relation inverse avec l'inflammation.(Poudel-Tandukar et al., 2009) De plus, nous avons montré que la vitamine E pourrait intervenir en tant que facteur modulateur de la relation entre PUFAs et inflammation. L'hypothèse d'une interaction entre PUFAs et vitamine E a été soulevée dans le cadre de l'étude de la cancérogenèse (Cognault et al., 2000, Lhuillery et al., 1997, Thiebaut et al., 2005), mais, à notre connaissance, n'a jamais été étudiée dans le cadre de l'inflammation.

D'autres études nécessitent de confirmer l'existence d'une telle interaction, ainsi que les mécanismes biologiques qu'elle pourrait affecter, afin de mieux comprendre les bénéfices relatifs des apports en PUFAs et vitamine E.

Nous avons montré que les concentrations sanguines en β-carotène étaient inversement associées à une CRP augmentée. La relation inverse entre concentration en β-carotène et CRP augmentée était plus forte chez les femmes, les sujets ayant bénéficié d'une supplémentation en vitamines pendant la phase d'essai de l'étude SU.VIMAX, les non-fumeurs et les sujets ayant un poids normal à l'inclusion dans l'étude. Aucune association n'était observée entre les concentrations sanguines en vitamine E et vitamine C et une CRP augmentée.

Si plusieurs études d'observation ont montré une relation inverse entre statut en β-carotène et biomarqueurs inflammatoires(Beydoun et al., 2011, Ford et al., 2003, Kritchevsky et al., 2000, Morris et al., 2010, Singh et al., 2005), une seule étude observationnelle a permis de montrer que cette relation était présente sur le long terme.(Hozawa et al., 2007) Celle-ci a étudié la relation à long terme entre la concentration en caroténoïdes totaux et plusieurs biomarqueurs

inflammatoires, dont la CRP. Notre étude complète ces résultats, en mettant en évidence la relation entre statut en β-carotène et CRP augmentée sur le long terme, et en l'élargissant à d'autres nutriments antioxydants, la vitamine C et la vitamine E. De plus, l'étude des facteurs modulateurs de cette relation a montré qu'elle était plus forte chez les sujets ayant au départ une concentration en β-carotène plus élevée (femmes, non-fumeurs, etc.).

Ces résultats étaient complétés par l'étude de la relation entre CRP augmentée et des profils alimentaires spécifiquement associés à ces nutriments. Un profil d'apports alimentaires riches en légumes et en huiles végétales, permettant des apports alimentaires importants en acides gras essentiels et en nutriments antioxydants était inversement associé à une CRP augmentée. L'intérêt de l'approche par profils alimentaires dans ce cadre est l'interprétation possible des résultats directement en termes de consommation alimentaire. De plus, la méthode statistique de la RRR nous a permis de tester les hypothèses selon lesquelles les relations entre nutrition et inflammation seraient associées aux apports en PUFA et en antioxydants.

Les études rapportant des associations entre profils alimentaires et inflammation ont pour la plupart porté sur des profils alimentaires construits par analyse en composantes principales, rendant donc compte des associations observées entre les apports en groupes alimentaires (voir Tableau 8, page 76) dans la population d'étude, mais ne permettant pas d'associer spécifiquement ces profils aux apports en nutriments d'intérêt dans l'inflammation.

Nos résultats permettent donc d'une part de compléter l'étude des mécanismes reliant apports alimentaires et inflammation, et d'autre part caractériser des profils alimentaires spécifiquement associés aux nutriments reconnus comme pro- ou anti-inflammatoires.

La plupart des études observationnelles qui ont servi de support bibliographique à ces travaux de recherche étaient de type transversal. La structure de nos recherches est originale car elle apermis d'étudier la relation à long terme entre exposition alimentaire (que ce soient les apports alimentaires en PUFAs, le statut en antioxydants ou les profils alimentaires) et CRP augmentée.

De plus, l'étude de facteurs modulateurs des relations entre exposition nutritionnelle et CRP permettait d'approfondir les résultats observés grâce aux méthodes traditionnelles. Par ailleurs, nous avons utilisé une méthode statistique innovante dans la construction des profils alimentaires.

Enfin, la méthode utilisée pour le recueil des expositions nutritionnelles dans l'étude SU.VI.MAX nous a permis d'étudier les apports alimentaires de façon fine et précise, et de compléter cette étude par l'analyse des biomarqueurs sériques de statut nutritionnel. La répétition des recueils alimentaires permet en effet de tenir compte dans les analyses de la variabilité intra-individuelle

dans les consommations alimentaires, et la qualité des données déclaratives est renforcée par l'utilisation de données biologiques.

Cette complémentarité entre les approches utilisées, aboutissant à des résultats concordants, sont une grande force de nos études.

Les limites communes aux trois études présentées dans ce travail résident en deux points principaux :

1. La CRP n'était pas évaluée à l'inclusion pour l'ensemble de la population. Cela ne permettait pas d'étudier la relation longitudinale prospective entre exposition nutritionnelle et inflammation. De fait, il n'est pas exclu que certaines des relations observées soient liées à des relations transversales et non longitudinales. Néanmoins, la CRP étant disponible pour un faible nombre de sujets à l'inclusion, des études de sensibilité nous ont permis dans un certain nombre de cas d'évaluer la part prospective des associations observées et de consolider nos résultats.
2. La méthode de mesure de la CRP ne correspondait pas à une technique de CRP ultrasensible, la limite de détection de la CRP se situant à 1mg/l (alors que la limite de détection de la CRP ultrasensible est à 0.15mg/l). Il ne nous a donc pas été possible de modéliser les relations entre exposition nutritionnelle et CRP de façon linéaire, ou d'étudier les relations existant à des niveaux très faibles de CRP. Néanmoins, le niveau de détection de la CRP restait suffisamment faible pour permettre une catégorisation des individus selon leur risque cardiovasculaire, selon les seuils internationaux.(Pearson et al., 2003)

Par ailleurs, le niveau d'inflammation n'a pas été évalué par d'autres biomarqueurs, ce qui aurait permis de renforcer les résultats obtenus.

Enfin, dans des publications complémentaires, nous avons pu étudier la relation prospective entre les concentrations circulantes de biomarqueurs de l'inflammation et le développement de maladies (diabète, maladies cardiovasculaires et cancers), montrant que les concentrations plus élevées en biomarqueurs de l'inflammation sont associées à une augmentation du risque de maladies (voir publications annexes). Ces travaux, dans leur ensemble, nous ont donc permis d'étudier l'ensemble de la chaine : en amont les mécanismes associant nutrition et inflammation, et, en aval, la relation entre inflammation et pathologies.

Discussion générale : Un processus ubiquitaire pouvant être la clé de nombreuses maladies

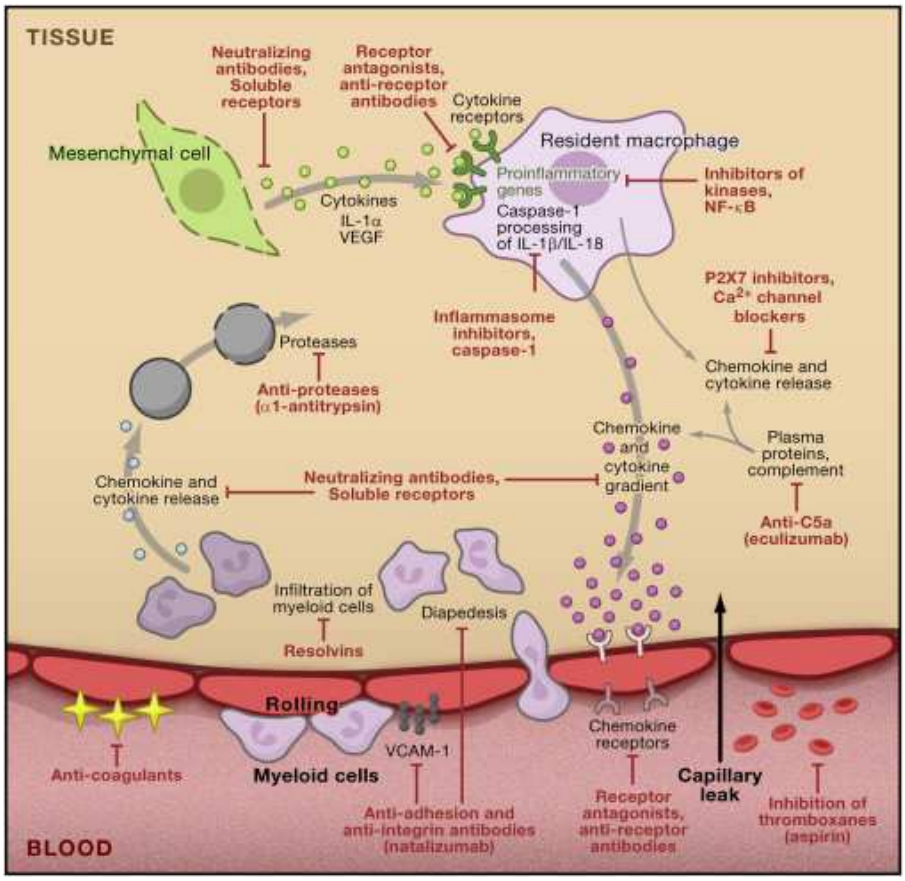
L'inflammation apparaît plus que jamais comme un processus ubiquitaire, faisant intervenir des cellules et des médiateurs spécialisés dont la régulation fine et précise est indispensable au maintien de l'équilibre dans la réponse aux agressions.

En dehors des pathologies présentées plus en détail dans ce travail (maladies cardio-vasculaires, obésité et cancer), d'autres maladies chroniques seraient aussi sous-tendues par des mécanismes inflammatoires. Des découvertes récentes tendent en effet à impliquer des processus systémiques inflammatoires dans des pathologies initialement considérées comme des pathologies mécaniques (ou ayant une part inflammatoire localisée) comme l'arthrose.

Dans ce contexte, l'exploration et l'analyse des multiples interactions participant aux processus inflammatoires sont des enjeux majeurs de la recherche à venir. Certaines cascades de production de médiateurs semblent désormais bien connues (pour les eicosanoïdes par exemple), mais les mécanismes d'entretien et de résolution de l'inflammation sont encore mal connus. Or, la connaissance de ceux-ci est indispensable au développement de stratégies performantes dans la prévention et le traitement des maladies ayant une part inflammatoire.

Par exemple, le développement et l'utilisation d'anti-inflammatoires non stéroïdiens (AINS) spécifiquement ciblés sur l'inhibition de la cyclo-oxygénase 2 (COX-2) ont soulevés des espoirs quant à leur potentiel dans la prévention ou le traitement du cancer colo-rectal.(Oshima *et al.*, 1996, Steinbach *et al.*, 2000, Tsujii *et al.*, 1998) Néanmoins, leurs effets secondaires cardio-vasculaires ont rapidement restreint leurs possibilités d'utilisation.(Bresalier *et al.*, 2005, Mukherjee *et al.*, 2001, Solomon *et al.*, 2005) Cet exemple illustre bien tout d'abord l'ubiquité des processus inflammatoires, mais aussi leurs multiples interactions et la subtile balance existant entre les différentes voies de signalisation. A la lumière de cet exemple, il semble indispensable soit de pouvoir cibler précisément des médiateurs ayant des effets spécifiques, mais qui sont probablement rares, soit de pouvoir agir à un niveau plus fin, sur la régulation même de l'inflammation.

Figure 26 Cibles d'action potentielles des médicaments anti-inflammatoires – issu de Dinarello et al., Cell 2010; 140(6):935-950.(Dinarello, 2010)



Dans le cancer, les cibles thérapeutiques visant les processus inflammatoires spécifiques impliqués dans la cancérogenèse et le développement tumoral sont en cours d'évaluation, et semblent prometteuses pour compléter l'arsenal thérapeutique.(Dinarello, 2010, Hussain *et al.*, 2012)

Dans le cas des maladies cardio-vasculaires, si l'aspirine est avant tout prescrite pour ses propriétés anti-aggrégantes plaquettaires, et à des doses correspondant principalement à cet effet, il n'est pas à négliger qu'une part de ses effets en prévention secondaire soient liés à des effets anti-inflammatoires *a minima*.(Ridker *et al.*, 1997)

En dehors des développements potentiels dans le champ thérapeutique, l'inflammation doit aussi être envisagée comme une cible en prévention.

Si, en effet, il s'agit d'un processus ubiquitaire dont la dérégulation contribue à nombreuses pathologies, prévenir son apparition permettrait d'emblée une avancée considérable dans la prévention des maladies chroniques, en agissant à un niveau très précoce de leur développement.

Comme nous l'avons observé, la nutrition intervient à de multiples niveaux sur la régulation de la balance inflammatoire. Les acides gras sont des précurseurs immédiats de nombreux médiateurs inflammatoires, et les antioxydants participent à la régulation des réactions oxydantes en amont et en aval de la réaction inflammatoire.

Néanmoins, comme nous l'avons vu, si certaines cascades de signalisation sont bien identifiées, les interactions entre les différents mécanismes régulant la balance inflammatoire ne sont pas encore bien définies. Par ailleurs, les nutriments pouvant intervenir dans la réaction inflammatoire sont nombreux, par des mécanismes directs (comme ceux présentés ici), mais aussi indirects qu'il est nécessaire d'identifier et de mesurer.

L'enjeu majeur dans ce cadre est d'évaluer l'intérêt relatif des différents nutriments identifiés dans ces mécanismes, mais surtout d'étudier l'équilibre de leur apport.

2. Perspectives

Exploration de nouvelles expositions

La suite de mes travaux sur les relations entre la nutrition et l'inflammation de bas grade comportera l'exploration de nouvelles expositions nutritionnelles. En effet, les expositions nutritionnelles étudiées jusqu'ici étaient celles pour lesquelles les hypothèses mécanistiques étaient les plus fortes, et qui avaient été identifiées directement dans les processus inflammatoires. Mes travaux de recherche porteront donc sur des nutriments pour lesquels certaines hypothèses mécanistiques d'action dans les maladies chroniques et l'inflammation ont été soulevées, mais pour lesquels le niveau de preuve est encore faible.

Parmi les familles de nutriments qui suscitent un intérêt de recherche croissant et qui pourraient intervenir sur l'inflammation, les polyphénols seront le prochain objet de mes recherches.

Les polyphénols sont une famille complexe de nutriments, comprenant de nombreuses sous-familles pour un total de plusieurs centaines de molécules. Parmi l'ensemble des polyphénols identifiés dans la nature, environ 500 sont retrouvés dans l'alimentation humaine.(Manach *et al.*, 2004)

Les niveaux de consommation des différents polyphénols, ainsi que leurs profils d'apports sont mal connus dans la population. L'objectif de mon travail sera dans un premier temps d'étudier les profils d'apports en polyphénols dans la population, à l'aide de différentes méthodes statistiques : analyse en composantes principales, analyse en correspondances multiples et en clusters. Par ailleurs, un profil alimentaire spécifique de l'apport en polyphénols sera construit

par RRR. La comparaison de ces techniques statistiques permettra de mieux caractériser les profils de consommation dans la population.

La construction de ces scores de consommation ou de ces profils de consommateurs permettra d'une part de caractériser les apports dans la population, mais surtout d'étudier les relations entre apports en polyphénols et des critères de jugement de santé, au-delà des méthodologies classiques évaluant l'association entre une catégorie spécifique de polyphénols et la santé.

Exploration de nouveaux critères de jugement

Comme cela a été décrit précédemment, les mécanismes intervenant dans la régulation de l'inflammation nécessitent d'être précisés, de même que leur relation avec la nutrition. Il apparaît donc indispensable de s'attacher à décrypter les relations entre nutrition et inflammation, en partant d'un modèle de maladie pour laquelle l'inflammation serait l'un des éléments moteur de la pathologie.

La polyarthrite rhumatoïde (PR) est une maladie auto-immune à tropisme articulaire principalement, procédant par poussées. La PR est une pathologie chronique invalidante, touchant principalement les femmes d'âge mûr, et dont la prévalence a été estimée en France en 2001 à 0,31% de la population.(Guillemin *et al.*, 2005) La prévalence dans les études internationales est estimée entre 0,5 et 1,0 % de la population.(Silman et Pearson, 2002, Tobon *et al.*, 2010) L'incidence de la maladie est estimée entre 20 et 50 cas pour 100.000 habitants dans les pays nord-américains et européens.(Scott *et al.*, 2010) Par ailleurs, si certaines études ont montré une relative baisse de l'incidence de la PR entre les années 1950 et 1990, celle-ci serait de nouveau en augmentation depuis 1995.(Doran *et al.*, 2002, Myasoedova *et al.*, 2010)

En dehors du tabac (Sugiyama *et al.*, 2010), les facteurs environnementaux associés au développement de la PR sont peu connus, en particulier les facteurs nutritionnels. Dans l'ensemble, les données de la littérature montrent des lacunes dans l'analyse des comportements alimentaires des sujets atteints de PR, et des associations entre nutrition et activité de la maladie. Enfin, la plupart des études sont de type cas-témoins, avec de faibles effectifs, et très peu d'études observationnelles se sont attachées à explorer de façon dynamique les relations entre comportements alimentaires et activité de la maladie chez les sujets atteints de PR.

Les éléments relatifs à cette maladie en font un bon modèle pour l'étude des relations entre nutrition et inflammation. Son étude permettrait en particulier d'alimenter les hypothèses relatives à la régulation de l'inflammation de bas grade observée dans d'autres pathologies.

L'étude des relations entre nutrition et polyarthrite rhumatoïde sera menée à partir des données issues de la cohorte Nutrinet-Santé. Cette étude d'adultes surveillée pendant une période de 10

ans a pour objectifs de : 1) étudier les relations entre la nutrition (apports en nutriments, aliments, comportements alimentaires, activité physique) et la mortalité globale et spécifique et l'incidence des pathologies chroniques , et 2) étudier les déterminants (sociologiques, économiques, culturels, psychologiques, cognitifs, sensoriels, biologiques, génétiques…) des comportements alimentaires, de l'état nutritionnel et de l'état de santé.(Hercberg *et al.*, 2010) L'ensemble des participants est suivi grâce à un site internet sécurisé et dédié à cet emploi. A l'inclusion, tous les sujets remplissent un dossier de base comprenant différentes parties : questionnaires alimentaires (3 enregistrements alimentaires de 24h sur 3 semaines, dont un pendant un jour de week-end), questionnaire sur l'activité physique, sur les données anthropométriques, sociodémographiques, sur le mode de vie et sur l'état de santé.

Dans le cadre de leur surveillance, les Nutrinautes reçoivent chaque mois un e-mail automatisé les informant sur l'avancement de l'étude et sur les nouveaux questionnaires à remplir pour compléter leur dossier. Des informations sont également collectées régulièrement sur la santé des participants (avec une validation des événements majeurs). La mortalité est suivie de façon exhaustive grâce au registre national de mortalité. Une collecte de données clinico-biologiques est également réalisée sur un sous-échantillon dans le cadre de la Biobanque NutriNet-Santé.

Dans le cadre d'une campagne fixe de questionnaires, les sujets inclus dans l'étude Nutrinet-santé ont reçu un questionnaire spécifique portant sur les pathologies rhumatologiques inflammatoires permettant d'identifier les sujets pour lesquels un diagnostic de PR a été émis.

Les éléments recueillis concernent le diagnostic de maladie inflammatoire chronique (type de diagnostic, date de diagnostic et type de médecin impliqué, hospitalisation), la prise médicamenteuse (thérapeutiques de fonds et traitements symptomatiques) ainsi que les modifications de l'alimentation effectuées par les sujets (régimes sans sel et contrôlés en sucres, régimes d'exclusion spontanés et/ou sur conseil d'un médecin).

A la fin du questionnaire, le sujet est invité à transmettre les éléments en sa possession sur sa maladie (compte-rendus de consultation ou d'hospitalisation), ou de laisser les coordonnées de son médecin, afin de permettre une validation des cas.

La validation des cas est faite à partir des éléments transmis, soit directement par le sujet, soit après contact avec le médecin. L'ensemble des données recueillies permettra non seulement la confirmation du diagnostic de PR, mais aussi la classification des patients en sous-groupes en fonction du type d'articulation touchées, la présence ou non d'auto-anticorps et le niveau initial d'inflammation.

La constitution de ce groupe de cas de PR permettra de développer les recherches autour de la relation entre nutrition et maladie :

- Comparaison des comportements alimentaires entre sujets atteints de la maladie et sujets sains. Le détail obtenu sur les cas permettra d'évaluer ces relations en fonction de certains paramètres relatifs à la maladie : présence d'anti-anticorps spécifiques, ancienneté de la maladie, fréquence des poussées, traitements entrepris.
- Pour les cas diagnostiqués dans les années suivant leur inclusion dans l'étude, étude des déterminants nutritionnels associés à l'apparition de la maladie.

Conclusion générale

Les approches complémentaires (nutriments, aliments, profils alimentaires) en épidémiologie nutritionnelle développées lors de ces travaux ont permis d'étudier finement les relations entre certaines familles de nutriments et l'inflammation. Nous avons pu montrer l'intérêt des PUFAs et des nutriments antioxydants dans l'inflammation de bas grade, au sein d'une cohorte de sujets sains provenant de la population générale.

Ce même type de stratégie sera mise en œuvre pour poursuivre l'étude des relations entre nutrition et inflammation en explorant d'une part de nouvelles expositions nutritionnelles, et d'autres part un nouveau modèle de pathologie inflammatoire.

En effet, il apparaît que les nutriments interviennent à de niveaux divers dans un système intégré complexe, comprenant de nombreuses voies de signalisation parfois redondantes, et impliquant de nombreux médiateurs, qu'est la réponse inflammatoire. Les interactions multiples intervenant au sein de ce système nécessitent des approches multiples et complémentaires, dont des méthodes complémentaires telles que présentées ici en épidémiologie nutritionnelle pour mieux comprendre les mécanismes sous-tendant les relations entre nutrition et maladies chroniques.

Ces travaux permettront de mieux appréhender les relations entre nutrition et inflammation, et de dégager des hypothèses relatives à l'équilibre en apports alimentaires en prévention des pathologies chroniques.

PUBLICATIONS ANNEXE

Nutrition patterns and metabolic syndrom :A need for action in young adults (French Nutrition and Health Survey – ENNS, 2006-2007)

Chantal Julia, Michel Vernay, Benoît Salanave, Valérie Deschamps, Aurélie Malon, Amivi Oleko, Serge Hercberg, Katia Castetbon

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Nutrition patterns and metabolic syndrome: A need for action in young adults (French Nutrition and Health Survey — ENNS, 2006–2007)

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a r t i c l e i n f o

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Abdominal

a b s t r a c t

Objective. The objective of this study was to investigate the relationship between adherence to French diet and physical activity recommendations and metabolic syndrome (MetS) risk.

Methods. 18–74-year-old subjects who underwent dietary assessment and health examination in the 2006–2007 French Nutrition and Health Survey (Etude Nationale Nutrition Santé, ENNS 2006–2007) were included in the analyses (n = 1608). Quintiles of PNNS-GS, the score measuring adherence to French recommendations, were generated. The prevalence of overall MetS risk and separate components across quintiles of PNNS-GS was estimated by adjusted logistic regressions. Interactions were searched for between PNNS-GS and sex, age and currently used medication.

Results. The PNNS-GS was inversely associated with overall MetS risk in subjects not taking antidiabetic, antihypertensive or lipid-lowering medication (12.8% in the lowest quintile vs. 4.6% in the highest PNNS-GS quintile; P b 0.01). This was true in 18–49-year-old subjects (10.0% vs. 1.7%; P b 0.01), but not in 50–74-year-olds (23.8% vs. 11.2%; P = 0.15). In 18–49-year-old adults, including those taking such medication, the HDL component was associated with PNNS-GS (22.1% vs. 7.9%; P b 0.01).

Conclusion. Improvement in diet and physical activity in line with recommendations could be effective in young adults for MetS prevention so as to decrease the risk of cardiovascular disease in France.

Introduction

Metabolic syndrome (MetS) is a high-risk condition associated with overall and cardiovascular mortality (Galassi et al., 2006; Hunt et al., 2004; Lakka et al., 2002). This relationship was first documented in middle-aged adults, but evidence suggests that its impact on health could occur at an earlier age, accounting in part for early cardiovascular disease (Iribarren et al., 2006; Lakka et al., 2002; Milionis et al., 2007). Reducing the prevalence of MetS could therefore lower the incidence of cardiovascular disease and associated mortality.

While the role of specific dietary components and nutrients has been thoroughly investigated (Ness and Powles, 1997; Snijder et al., 2008), the association between overall dietary patterns and health outcomes still needs investigating. Two strategies have been developed to describe dietary patterns and to relate them to health outcomes such as MetS: an "a posteriori" strategy describing dietary patterns using factor or cluster analysis of diet surveys, and an "a priori" strategy using a score based on nutritional recommendations currently known to be associated with various health outcomes (Hu, 2002; Kant, 2004; Waijers et al., 2007).

In 2001 in France, the National Nutrition and Health Program (Programme National Nutrition Santé — PNNS) was implemented in order to improve the health status of the population through nutritional measures (Hercberg et al., 2008). Guidelines with quantitative dietary and physical activity recommendations have since been widely disseminated in the population. Following an "a priori" strategy, the PNNS guideline score (PNNS-GS) was developed to measure adherence to PNNS diet and physical activity recommendations (Estaquio et al., 2009; Malon et al., 2010). The objective of the present study was to examine the relationship between adherence to nutritional recommendations, as assessed using the PNNS-GS, and MetS components and prevalence in a national sample, in order to identify targets for preventive strategies aimed at reducing the risk of MetS.

Methods

Sample design

The design of the French Nutrition and Health Survey (Etude nationale nutrition santé — ENNS, 2006–2007) has been described elsewhere

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(Castetbon et al., 2009). Briefly, the ENNS survey was a multistage stratified descriptive cross-sectional survey, performed on a randomly selected sample of a non-institutionalized population living in mainland France, aged 18 to 74. Sample selection was based on a three-stage design stratified by eight large regions and by the degree of urbanization. The sampling stages involved: 1) selection of 190 geographic zones; 2) random selection of households from phone lists; 3) selection of one subject per household using the date of birth method. The study was approved by the Ethical Committee (Hôpital Cochin no. 2264) and the French Data Protection Authority (authorization no. 905481).

Data collection

Sociodemographic, medical and behavioral data

Socioeconomic, demographic data and smoking status were recorded during face-to-face interviews. Information on current medication and alcohol consumption (usual weekly frequency and amount) was collected through a self-administrated questionnaire.

Dietary data

Dietary data were collected using three 24 h dietary recalls randomly distributed over a two-week period (one of which was a weekend day) performed by telephone by trained dieticians. Subjects were asked to describe in detail their food intake (including composition of homemade recipes) and quantities using a validated photographic manual of food portions or typical measurements (grams, home containers, etc.). As a complement to 24 h dietary recalls, participants were asked about usual weekly frequency of seafood consumption. Seasonality was accounted for at the sample level, as inclusions in the study were performed for over one year (February 2006–March 2007).

Physical activity

Physical activity was assessed using the short form of the International Physical Activity Questionnaire (IPAQ) during a personal interview (Craig et al., 2003). Weekly frequency and duration of physical activity on one of these days, and three levels of intensity (vigorous and moderate physical activity and walking), were reported. Information on daily television viewing time was also collected.

Health examination

Subjects were offered a health examination that took place either at a health examination center of the French National Health Insurance System (CnamTS) or at home. Waist circumference (WC) was measured to the nearest 0.1 cm using a flexible plastic fiber tape placed at the midpoint between the lower rib margin and the iliac crest. Blood pressure (BP) was measured in subjects after a 5 min rest using an automatic validated device, and the mean between the last two of three consecutive measurements taken at 1 min intervals was considered as their actual blood pressure. Blood samples were collected for determination of serum lipids and glucose concentrations after a recommended fasting period of 12 h. Serum cholesterol (total and HDL-cholesterol) and triacylglycerol (TAG) were determined by enzymatic colorimetric methods. Plasma glucose was determined using the hexokinase method and colorimetric measurement.

Data treatment

Dietary data and construction of PNNS-GS

Individual daily mean amounts of food and nutrient intake were calculated by averaging the three 24-h dietary recall intakes and weighing on the type of day (weekday or weekend day). For alcoholic beverages and seafood, a weekly frequency questionnaire was used. Foods and beverages were then classified into groups according to advice and other information (examples, tips, recipes, etc.) provided in PNNS guides and media campaigns. Composite foods could contribute to several groups in amounts proportional to the recipes. The PNNS-GS was first implemented and validated against nutrient intake and biomarkers in the French SUVIMAX cohort study (Estaquio et al., 2009). We adapted it to French ENNS survey data collection methods as previously described (Malon et al., 2010). It is based on quantitative and qualitative recommendations published in PNNS guidelines. It includes 12 components for diet and one for physical activity (Table 1). Food group and physical activity scoring is performed according to quantitative amounts coming from PNNS recommendations with boundaries, and external sources otherwise (Estaquio et al., 2009). Number of servings is calculated by dividing the amount of food eaten (in grams) by the typical portion size, which is specific to each type of food. At least one point is attributed for each component when subject behavior is in accordance with the recommendation. Intermediate points are attributed for subjects who do not attain the recommendation, but who come close. Additional positive points (high intake of fruits and vegetables, low salt intake and high levels of physical activity) or negative points (high intake of sweetened foods and salt) are attributed (Estaquio et al., 2009; Malon et al., 2010). In order to compensate for high scores due to large quantities of food eaten, along with too much energy intake, a penalty is also included in the scoring procedure for individuals with energy intakes greater than their needs (evaluated using the estimated basal metabolic rate (Schofield, 1985) and physical activity level). If energy intake is ≥ 5% of the evaluated energy need, the total score is reduced by the same proportion.

For physical activity, IPAQ group recommendations were applied in order to compute the metabolic equivalent task (MET-min/week) and to group subjects into 3 IPAQ categories (Craig et al., 2003). Intermediate and high IPAQ categories were considered as meeting the PNNS recommendation.

Definition of MetS

According to the International Diabetes Federation (IDF), components of the MetS include: 1) abdominal obesity (waist circumference ≥ 94 cm for men and ≥ 80 cm for women); 2) high TAG (≥ 1.7 mmol/l or under fibrate therapy); 3) low HDL-cholesterol (b1.03 mmol/l for men or b1.29 mmol/l for women); 4) high BP (BP≥ 130/85 mm Hg or antihypertensive medication); and 5) hyperglycemia (glycemia ≥ 5.6 mmol/l or under antidiabetic medication, i.e. oral antidiabetic or insulin therapy). MetS is defined in the presence of abdominal obesity, plus two or more other risk factor components (Alberti et al., 2005).

Statistical analyses

All statistical analyses were carried out using STATA® V.10 software and the "Svyset" procedure to take into account the complex survey design and calibration. Diet-underreporting subjects were identified using the method proposed by Black and were excluded from analyses (Black, 2000). Subjects whose low-energy intake could be explained by acute disease or low-energy diet reported during dietary recall were not considered underreporters. Calibration was made according to French national census data on age, educational diploma, whether the household included or not at least one child, and the period of data collection (Castetbon et al., 2009), in order to take into account potential participation bias in the survey and seasonality of eating habits.

Quintiles of PNNS-GS were generated for the study population, with lower quintiles corresponding to compliance with fewer recommendations. Population characteristics, described across quintiles of PNNS-GS, were analyzed for their relationship with the PNNS-GS (linear regression for continuous variables or logistic regression for dichotomous variables). The P-value for linear trend was determined for PNNS-GS taken as a continuous variable.

Adjusted multivariate logistic regressions were used to assess the association between PNNS-GS and MetS status (overall risk and separate MetS components). Interactions were searched for between PNNS-GS and gender and age and were considered statistically significant if the P-value reached 0.15. Analyses were also performed after excluding subjects taking antidiabetic, antihypertensive or lipid-lowering medication.

Results

Participant characteristics

Among the 3115 subjects having participated in the food consumption survey of the ENNS study (59.7% response rate), 2734 were not considered as diet underreporters. Among these, 2002 were measured for waist circumference and 1849 underwent one blood test. Finally, a total of 1608 subjects (620 men and 988 women) had complete dietary, physical activity, anthropometric and biochemical information. Compared to subjects included in the food consumption survey alone (N= 885), the 1849 subjects who also underwent the blood examination were more likely to be men, older and with a

Table 1
PNNS-GS: components and scores according to PNNS recommendations.

	Recommendation	Scoring criteria[a]	Score
Fruits and vegetables	At least 5/d	[0–3.5[	0
		[3.5–5[	0.5
		[5–7.5[	1
		≥ 7.5	2
Bread, cereals, potatoes and legumes	At each meal according to appetite	[0–1[	0
		[1–3[	0.5
		[3–6[	1
		≥ 6	0.5
Whole grain food	Choose whole grains and whole-grain breads more often	[0–1/3[	0
		[1/3–2/3[	0.5
		≥ 2/3	1
Milk and dairy products	3/d (≥ 55-years old: 3 to 4/d)	[0–1[	0
		[1–2.5[	0.5
		[2.5–3.5] (≥ 55-years old: [2.5–4.5])	1
		N3.5 (≥ 55-years old : N4.5)	0.5
Meat, poultry seafood and eggs	1 to 2/d	0	0
		]0–1[	0.5
		[1–2]	1
		N2	0
Seafood	At least 2/week	b2/week	0
		≥ 2/week	1
Added fat[b]	Limit consumption	Lipids from added fat ≥ 16%EI[c]/d	0
		Lipids from added fat b16%EI[c]/d	1
Vegetable added fat	Favor fat of vegetable origin	No use of vegetable oil or ratio vegetable oil/total added fats ≤ 0.5	0
		No use of added fats or ratio vegetable oil/total added fats N0.5	1
Sweetened foods[b]	Limit consumption	Simple sugars from sweetened foods ≥ 17.5%EI[c]/d	− 0.5
		Simple sugars from sweetened foods [12.5–17.5[%EI[c]/d	0
		Simple sugars from sweetened foods b12.5%EI[c]/d	1
Beverages			
Non-alcoholic beverages	Drink water as desired	b1 l water and N250 ml soda/d	0
	Limit sweetened beverages: no more than 1 glass/d	≥ 1 l water and N250 ml soda/d	0.5
		b1 l water and ≤ 250 ml soda/d	0.75
		≥ 1 l water and ≤ 250 ml soda/d	1
Alcohol	Women advised to drink ≤ 2 glasses of wine/d and ≤ 3 glasses/d for men	Alcohol N20 g/d for women and N30 g/d for men	0
		Alcohol ≤ 20 g/d for women and ≤ 30 g/d for men	0.8
		Abstainer	1
Salt[b]	Limit consumption	N12 g/d	− 0.5
		]10–12] g/d	0
		]8–10] g/d	0.5
		]6–8] g/d	1
		≤ 6 g/d	1.5
Physical activity	At least the equivalent of 30 min/d of brisk walking	[0–30[min/d	0
		[30–60[min/d	1
		≥ 60 min/d	1.5

[a] Servings per day unless otherwise indicated. "[": boundary included; "]": boundary not included.
[b] Established according to French recommended dietary allowances.
[c] EI: energy intake without alcohol.

higher educational level. Calibration according to French national census data on this subsample corrected this participation bias. Subjects included in the present analysis (N = 1608 subjects) did not differ from participants excluded for incomplete data (N = 241). The raw PNNS-GS score ranged from − 2.2 (due to the penalty for inappropriate intake of energy, sugar or salt) to 15 points. Age, gender, education level, lipid-lowering intake, estrogen use, smoking status and energy intake significantly varied according to PNNS-GS values, while antihypertensive and antidiabetic treatments, time spent watching TV and BMI did not (Table 2).

MetS and PNNS-GS

According to the international definition, MetS affected one individual out of five (Table 3). Overall, after adjusting for covariates, PNNS-GS and MetS risks were not significantly associated, nor was a separate MetS component risk associated with PNNS-GS. However, after exclusion of subjects under medication (N = 372), PNNS-GS was inversely associated with MetS prevalence (12.8% in the lowest quintile versus 4.6% in the highest quintile, P b 0.01). The interaction between PNNS-GS and age categorical variable (18–49 years and 50–74 years) was not significant for MetS prevalence, but proved to be statistically significant for the HDL-cholesterol component (P b 0.01). Prevalence of the HDL-cholesterol component and overall MetS risk across quintiles of PNNS-GS was therefore computed separately for each age category (Table 4).

In 18–49-year-old individuals, PNNS-GS was significantly inversely associated with the HDL component risk factor (Table 4), but was not significantly associated with MetS prevalence (P = 0.06). After excluding subjects under medication, PNNS-GS was significantly associated with the HDL component risk factor (P b 0.05) and MetS prevalence (P b 0.01). In 50–74-year-old individuals, no significant association was found between PNNS-GS and MetS components or overall status, even after excluding subjects under medication (Table 4).

Discussion

Our findings are consistent with observations reported from studies which assessed the relationship between dietary patterns and cardiovascular risk factors, including MetS. Most studies, focusing on the relationship between dietary patterns and cardiovascular risk factors taken as continuous variables, revealed an inverse significant association between a healthy diet and a decrease in such markers

Table 2
Participant characteristics across quintiles of PNNS-GS[a] (n = 1608), French Nutrition and Health Survey — ENNS, 2006–2007.

	Total	Quintiles of PNNS-GS					P for trend[b]
		1	2	3	4	5	
Median score (range)	8.3 (− 2.2;15)	5.7 (− 2.2;6.75)	7.3 (6.8;7.8)	8.3 (7.8;8.8)	9.5 (9.0;10.0)	11.0 (10.1;15.0)	
Age (years)	45.3	38.8	43.2	45.6	47.8	51.6	b10− 3
Female (%)	51.7	35.3	44.3	53.6	63.5	63.8	b10− 3
Education level							
Primary school	21.1	14.6	21.2	11.5	12.8	12.1	0.09
Secondary school	40.6	42.3	41.0	41.5	36.5	40.8	0.82
High school	16.7	18.0	17.6	14.0	14.6	12.1	b0.05
University	21.6	14.0	12.2	23.6	27.7	24.3	b10− 3
Treatment (%)	22.5	11.8	11.9	15.1	22.6	12.2	0.37
Antihypertensive	14.9	7.2	8.3	7.9	14.2	6.8	0.67
Antidiabetic	3.1	2.5	2.0	2.1	0.9	2.4	0.97
Lipid-lowering	13.1	4.8	4.0	7.2	11.0	8.2	0.04
Estrogen use (%)	17.9	12.5	8.0	13.0	19.2	19.6	b0.05
Smoking status (%)							
Current smoker	24.7	29.9	25.6	25.3	16.1	15.6	b10− 3
Former smoker	26.3	20.3	24.0	22.2	31.6	26.7	0.07
Sedentary behavior							
Time spent watching TV (min/d)	156.4	169.2	150.1	157.7	152.2	151.4	0.07
Energy intake (EI) (kcal/d)	2 074.3	2 424.5	2 077.1	1 983.0	1 939.3	1935.2	b10− 3
BMI (kg/m²)	25.5	25.0	25.9	25.6	25.7	25.0	0.70

[a] Means and percentages adjusted for sex and age. Age adjusted for sex only and sex adjusted for age only.
[b] Derived from linear regression for continuous variables and logistic regression for dichotomous variables. PNNS-GS taken as a continuous variable.

(Deshmukh-Taskar et al., 2009; Fung et al., 2001; Kant and Graubard, 2005; McNaughton et al., 2007; Nettleton et al., 2008). Studies using MetS components taken as dichotomous variables found the same inverse relationship between a healthy diet and reduction in MetS and MetS component risk whatever the method used to describe the overall dietary pattern. Indeed, in analyses involving cluster analysis for describing dietary behavior, the "Heart Healthier" cluster modeled from the Framingham Offspring Study (female sample) was associated with significantly lower BP and HDL risks than other identified clusters (Sonnenberg et al., 2005); in another study carried out on an Iranian sample of females, the "healthy pattern" arising from factor analysis was also associated with a significantly lower prevalence of all MetS risk components and MetS (Esmaillzadeh et al., 2007). The Dietary Guidelines for Americans Index (DGAI), an "a priori" score derived from MyPyramid recommendations (Fogli-Cawley et al., 2006), was found to be significantly associated with the prevalence of MetS and all MetS risk factors separately except for HDL (Fogli-Cawley et al., 2007). As in our study, an interaction between DGAI and age was found: the association between DGAI and overall MetS prevalence was significant only in adults b55-years old, but was not significant in older persons. However, in contrast with our observations, DGAI was also significantly associated with abdominal obesity and glycemia markers, while PNNS-GS was associated only with the HDL-cholesterol component. Discrepancies between the studies cited here and our results may be explained by construction of nutrition scores and prevalences of risk factors, which differed from ours.

PNNS-GS takes into account a combination of dietary and physical activity factors known to affect HDL-cholesterol levels: carbohydrates, alcohol, type of added fat and physical activity (Ellison et al., 2004; Mora et al., 2006). Among these dietary factors, alcohol and carbohydrates, in particular, are also known to alter TAG concentrations, since HDL and TAG concentrations are interrelated (Grundy and

Table 3
Adjusted[a] prevalence of MetS and MetS components across quintiles of PNNS-GS, French Nutrition and Health Survey — ENNS, 2006–2007.

	Total	Quintiles of PNNS-GS[a]					P[b]
		1	2	3	4	5	
Median score (range)	8.3 (− 2.2;15)	5.7 (− 2.2;6.75)	7.3 (6.8;7.8)	8.3 (7.8;8.8)	9.5 (9.0;10.0)	11.0 (10.1;15.0)	
All subjects (n = 1608)							
Abdominal obesity							
Waist circumference ≥ 94 cm in men Waist circumference ≥ 80 cm in women	43.8	43.3	48.4	45.9	39.5	41.3	0.84
Triacylglycerolemia							
TG ≥ 1.7 mmol/l or fibrate medication	16.8	16.7	19.5	15.4	20.1	13.4	0.70
HDL							
HDL b 1.03 mmol/l in men HDL b 1.29 mmol/l in women	15.9	18.4	16.8	13.9	16.0	15.9	0.38
Blood pressure							
PA ≥ 130/85 mm Hg or antihypertensive medication	37.2	41.3	33.0	40.9	40.7	30.7	0.45
Glycemia							
Glycemia N 5.6 mmol/l or antidiabetic medication	16.0	21.5	12.7	14.8	19.0	13.7	0.48
Metabolic syndrome (IDF)	14.0	15.7	14.5	12.7	15.0	13.1	0.61
Subjects under medication excluded (n = 1236)							
Metabolic syndrome (IDF)	7.0	12.8	8.3	6.7	6.3	4.6	b0.01

[a] Percentages adjusted for sex, age, energy intake (kcal), estrogen use, TV time (min) and smoking status (3 categories: current smoker, former smoker, never smoked) and education level (university, high school, secondary school, primary school).
[b] Derived from logistic regression. PNNS-GS taken as a continuous variable.

Table 4
Adjusted[a] prevalence (%) of each component risk factor in the MetS, and prevalence of MetS according to quintiles of PNNS-GS (IDF criteria), stratified by age, all subjects (n = 1608) and subjects under medication excluded (n = 1236), French Nutrition and Health Survey — ENNS, 2006–2007.

	Quintiles of PNNS-GS					P[b]
	1	2	3	4	5	
All subjects						
Age 18–49-years old (n = 879)						
HDL						
HDL<1.03 mmol/l in men	22.1	18.0	16.7	18.6	7.9	<0.02
HDL<1.29 mmol/l in women						
Metabolic syndrome (IDF)	9.0	8.1	7.2	7.5	2.7	0.06
Age 50–74-years old (n = 729)						
HDL						
HDL<1.03 mmol/l in men	10.9	16.0	9.1	12.9	17.7	0.18
HDL<1.29 mmol/l in women						
Metabolic syndrome (IDF)	28.2	25.2	24.1	29.0	31.4	0.47
Subjects under medication excluded						
Age 18–49-years old (n = 821)						
HDL						
HDL<1.03 mmol/l in men	20.3	16.8	14.5	15.6	8.2	<0.02
HDL<1.29 mmol/l in women						
Metabolic syndrome (IDF)	10.0	6.2	4.8	3.5	1.7	<0.01
Age 50–74-years old (n = 415)						
HDL						
HDL<1.03 mmol/l in men	4.9	14.1	7.6	9.0	11.5	0.57
HDL<1.29 mmol/l in women						
Metabolic syndrome (IDF)	23.8	13.9	12.0	12.3	11.2	0.15

[a] Adjusted for sex, age, energy intake, estrogen use, TV time and smoking status (3 categories: current smoker, former smoker, never smoked) and education level (university, high school, secondary school, primary school).
[b] Based on the logistic regression coefficient of PNNS-GS as a continuous variable.

Denke, 1990). Yet the PNNS-GS was not found to be significantly associated with the TAG risk factor. The absence of an association between PNNS-GS and the TAG risk factor could be explained, in part, by the fact that in our study population, carbohydrate intakes did not vary significantly across quintiles of score, while dietary intake of lipids, leading to alterations in the HDL concentration only, varied significantly (data not shown).

The physical activity component might account for the association between PNNS-GS and the HDL risk factor. However, after removing this component from analyses the association remained significant.

Adults diagnosed as having cardiovascular risk factors probably alter their diet and physical activity according to recommendations, since nutritional modifications are part of medical care (Fung et al., 2001; World Health Organization, 2007). Our results show that the inverse relationship between a favorable nutritional pattern and overall MetS appears only for subjects using no medication, indicating that nutritional advice to this population could constitute effective prevention. In older subjects, associations were probably weakened by factors having contradictory effects: cardiovascular risk is higher in older subjects, thus necessitating more frequent medication, while dietary behavior usually improves with aging.

Our results were limited by the cross-sectional design of our study, which does not allow for causality implications in the described associations, and could account for the lack of associations observed here. Moreover, the rather small sample size and the short period of food consumption collection (i.e. three 24 h recalls over a two-week period) may also have masked any true relationships between PNNS-GS and MetS components. Biases in participation in the health examination might also have lowered the strength of our conclusions. However, calibration carried out according to French national census data limited this bias.

PNNS recommendations (and thus PNNS-GS) are not specifically targeted to MetS prevention, but more generally to all nutrition-related diseases in an overall population perspective. The lack of specificity of the PNNS-GS in populations which include subjects under medication could explain the absence of an association between PNNS-GS and MetS biomarker risk factors. Results concerning the association between PNNS-GS and nutritional status in middle-aged adults tended to support this view: overall, PNNS-GS was associated with several biomarker concentrations (in particular, beta-carotene and vitamin C), but showed few or no significant associations with lipid concentrations (Estaquio et al., 2009).

Conclusion

We found an inverse association between adherence to PNNS recommendations and MetS in young adults not under medication, which probably existed prior to diagnosis of a cardiovascular-disease-related condition. Our results suggest that targeting diet and physical activity changes in this youthful population could be effective in preventing MetS and therefore in lowering cardiovascular-disease risk in the population.

Conflict of interest statement
Nothing declared.

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Relationships between adipokines, biomarkers of endothelial function and inflammation and risk of type 2 diabetes

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Structured abstract

Aims: Identification of novel biomarkers of diabetes risk help understanding mechanisms of pathogeny and improving risk prediction. Our objectives were to examine the relationships between adipokines, biomarkers of inflammation and endothelial function and development of type 2 diabetes; to assess the relevance of including these biomarkers in type 2 diabetes prediction risk models.

Methods: 1345 subjects from the SU.VI.MAX study, who were free of diabetes at baseline and who completed 13 years of follow-up were included in the present analyses. Odds ratios (OR) with 95% confidence intervals (95% CI) of incident type 2 diabetes associated with a 1-SD increase in adiponectin, leptin, C-reactive protein (CRP), soluble intracellular adhesion modecule-1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), E-selectin and monocyte chemoattractant protein-1 (MCP-1) were estimated. Predicitive performances of models including biomarkers were assessed with area under the receiver operating curves (AUC) and integrated discrimination improvement (IDI) statistics.

Results: 82 subjects developed type 2 diabetes during follow-up. The risk of developing type 2 diabetes increased with increasing concentrations of leptin (2.04 (1.28;3.26)), sICAM-1 (1.39 (1.08;1.78)) and sVCAM-1 (1.29 (1.01;1.64)). type 2 diabetes associations with leptin remained significant after adjusting for a combination of biomarkers. Models adjusted for novel biomarkers had improved performance compared to models adjusted for classical risk factors as assessed by IDI, but not by AUC.

Conclusions: Adipokines, biomarkers of inflammation and endothelial function appear as interesting new predictors of incident type 2 diabetes. Their inclusion in predictive scores is however not supported by the present study.

Introduction

Epidemiological research has recently focused on the identification of novel biomarkers implicated in the development of insulin resistance and type 2 diabetes [1], with an emphasis on antioxidants [2;3], adipokines [4-6], inflammatory cytokines [7-9] and endothelial function biomarkers [10]. Adipokines most consistently studied are adiponectin and leptin, which are solely secreted by adipose tissue and would act as hormones with antagonistic effects. While adiponectin is suggested to have anti-inflammatory and insulin-sensitizing properties, leptin is thought to have pro-inflammatory effects and its increase in obese subjects has been related to insulin resistance [11]. The pattern of secretion of these adipokines would reflect adipose tissue dysfunction, associated with endocrinopathy. Inflammation is also involved in the development of insulin resistance: obesity is characterized by a chronic low-grade inflammatory response which inhibits downstream signaling of insulin receptors [12;13]. More recently, endothelial dysfunction [14] has also been linked to the development of insulin resistance, as increased expression of adhesion molecules (soluble intracellular adhesion modecule-1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), E-selectin) has been associated with risk of incident type 2 diabetes [15]. Biological pathways are complex and predominant ones have not been clearly identified. Moreover, if these biomarkers provide new insights on pathogeny, it is not certain that their inclusion in diabetes prediction algorithms would provide information beyond that obtained with well-known risk factors such as anthropometry and classical biological risk factors (glycaemia, lipid concentrations) [1;16]. The objectives of this study were 1) to examine the associations between incident type 2 diabetes and adipokines, biomarkers of inflammation and endothelial function; 2) to evaluate the additonal contribution of new biomarkers to well-known risk factors in the prediction of type 2 diabetes.

Subjects

This study is an ancillary protocol of a nested case-control study. Subjects were selected from the SU.VI.MAX (SUpplémentation en VItamines et Minéraux AntioXydants) study, a double-blind placebo-controlled randomized primary prevention trial designed to assess the effect of a daily antioxidant supplementation on the incidence of cardiovascular disease and cancer [17]. Inclusion of 35-60 years-old women and 45-60 years-old men began in 1994-1995 for a planned follow-up of 8 years. In 2006, participants who had completed the SU.VI.MAX study in 2002 were invited to enroll in a new prospective study, the SU.VI.MAX 2 study, on the impact of nutrition on aging, thus prolonging follow-up duration.[18] Subjects provided written informed consent to participate in the study and both the SU.VI.MAX and the SU.VI.MAX2 studies were approved by the Ethics Committee for Studies with Human Subjects of Paris-Cochin Hospital (no. 706 and no. 2364, respectively) and the Comité National Informatique et Liberté (no. 334641 and no. 907094, respectively). The initial nested case-control study included all cases of cancer and

cardiovascular disease diagnosed during follow-up and controls free these diseases during follow-up (two controls for each case). Cases and controls were matched on sex, age, BMI and initial supplementation group in the trial phase of the SU.VI.MAX study. Subjects with available data on adipokines, biomarkers of endothelial disfunction and inflammation, who were free of diabetes at baseline and with known diabetes status up to 13 years later were eligible for analyses. Further exclusions were made for subjects with missing data on any of the following covariates: sex, age, supplementation allocation in the initial SU.VI.MAX study, family history of diabetes, anthropometric measurements, glycaemia and lipid concentrations.

Material

At inclusion, socio-demographic, health status, medical and family history and anthropometric measurements (weight and height) data were collected. Blood samples were drawn, a part of which were immediately frozen at –80°C and used to determine the following biomarkers' levels for a subsample of the initial SU.VI.MAX cohort: adiponectin, leptin, CRP, sICAM-1, sVCAM-1, E-selectin and MCP-1. Biomarkers' levels were determined with ELISA sandwich technique (R & D laboratory Systems). Intra-assay (IACV) and inter-assay (IRCV) coefficients of variation were all <10%. CRP had the lowest (1.6%) and MCP-1 had the highest (6.2%) IACV, and CRP had the lowest (3.6) and E-selectin had the higest (9.1%) ICRV.

Follow-up included information on current diabetes medication at years 8 and 13. Fasting plasma glucose (FPG), total cholesterol and triglycerides concentrations were obtained at years 3, 5, 6 and 13. Subjects were considered to have incident type 2 diabetes if they had a FPG ≥ 7.0 mmol/l at any follow-up biological test or if they were under antidiabetic medication at any follow-up investigation.

Methods

Body mass index (BMI) was computed as weight (in kg) divided by the square of height (in m). Descriptive statistics were summarized as mean (±SD) for continuous variables or median (25th-75th percentiles) for continuous variables with skewed distributions. The latter variables were log-transformed before analyses. Chi square tests (for categorical variables) and Welch's t-test (for continuous variables) were used to determine differences at baseline between subjects who developed and subjects who did not develop type 2 diabetes after 13 years of follow-up. Associations between biomarkers and incident diabetes were examined with logistic regression models and expressed as odds ratios (OR) with 95% confidence intervals (95% CI). Each biomarker was tested separately in three models: crude model; model 1 adjusted for baseline age, sex, initial supplementation allocation group in the SU.VI.MAX study, family history of diabetes and BMI; model 2 adjusted for model 1 + baseline glycaemia, cholesterol and triglycerides concentrations (referred to as the "full model"). Models were tested using

biomarkers divided into tertiles and as continuous variables. For biomarkers with skewed distributions, the log-transformed variable was centered so that the OR for a 1-SD increase in the corresponding log-transformed biomarker was computed. Logistic regression were applied considering that all participants provided information on diabetes status at the same time, without censoring once diabetes status was confirmed.

Finally, the biomarkers were included together in the full model, according to the degree of significance of their associations in models investigating biomarkers separately and according to the biological pathways they represent. The ability of novel biomarkers to discriminate between subjects who developed diabetes during follow-up and those who did not was assessed using the area under the receiver operating curve (AUC), the integrated discrimination improvement (IDI) and relative integrated discrimination improvement (RIDI) which measure increased discrimination and percentage of increased discrimination when a variable is added to a prediction model [19;20]. Models including biomarkers separately were compared to the full model, then biomarkers were included sequentially according to the level of their IDI when compared to the full model, and again compared to it.

AUC comparison used nonparametric methods implemented with the SAS % ROC macro.[21] Tests of significance for IDI were one-sided, as improvement in model fit was expected. All other statistical tests were two sided, and $P<0.05$ were considered significant. All analyses were performed with SAS software (version 9.1; SAS Institute Inc).

Results

From the 2370 subjects with available data on biomarkers, 127 were excluded for prevalent diabetes and 664 for missing data on diabetes status at follow-up, leaving 1581 eligible subjects for analysis. 64 subjects were excluded for missing data on family history of diabetes, and 172 for missing anthropometric measurements. Further exclusions were made on missing data on any other covariates. Subjects excluded for missing data had lower baseline glycaemia (5.59 mmol/l vs. 5.67 mmol/l, P=0.04) but otherwise did not differ from included subjects.

The initial intervention in the SU.VI.MAX study had no effect on subsequent fasting blood glucose outcome and type 2 diabetes. [2]

Subjects who developed type 2 diabetes during the 13 years of follow-up were more often men (63.4% versus 48.2%, P=0.008), and had higher BMI (28.24 kg/m^2 versus 24.38 kg/m^2, P<0.001), and FPG (6.20 mmol/l versus 5.63 mmol.l, P<0.001) at inclusion. Also, they had significantly higher baseline plasma values of leptin (P<0.001), MCP-1 (P=0.046), E-selectin (P<0.001), sICAM-1 (P=0.002), hsCRP (P<0.001) and lower plasma values of adiponectin (P<0.001).(Table 1)

In crude models, all biomarkers were significantly associated with risk of developing diabetes, both when biomarkers were considered in tertiles and as continuous variables, except for sVCAM-1, for which associations were not significant.(Table 2) After further adjustment on socio-demographic and anthropometric variables, and baseline glycaemia and lipid plasma levels, (Model 2), the above associations were weakened and kept significant only for leptin (OR 2.04 (1.28;3.26)) and sICAM-1 (OR 1.39 (1.08;1.78)). Conversely, sVCAM-1 became significantly associated with risk of developing type 2 diabetes (OR 1.29 (1.01;1.64)) (Table 2). To test the independent associations between statistically significant biomarkers and type 2 diabetes, biomarkers were added simultaneously in model 2. (Table 3) Leptin remained an independent risk factors of type 2 diabetes (OR 2.10 (1.31;3.37)), whereas associations with endothelial function biomarkers were no longer significant when both sICAM-1 and sVCAM-1 were included in the model.

The AUC of model 2 alone was 0.845, and the inclusion of biomarkers increased it to a maximum of 0.859, none of the observed increases in AUC being statistically significant. However, the inclusion novel biomarkers in model 2 significantly improved predictive performance, as assessed by IDI for sICAM-1 (IDI= 0.012, P=0.033) and leptin (IDI=0.011, P=0.033). The simultaneous inclusion of all biomarkers significantly improved prediction, reaching a RIDI of 30%.

Discussion

A strong association between leptin and sICAM-1 concentrations and risk of type 2 diabetes was observed in this study, independently of other classical risk factors of type 2 diabetes. The addition of novel biomarkers to the prediction of the development of type 2 diabetes significantly improved test performance compared to a model including classic risk factors for diabetes. However, even if significant as assessed by IDI, differences in AUC were not significant, and are probably of marginal importance, as initial AUC with classical biomarkers was already very high (Model 2-0.845).

Results of studies on association between leptin concentrations and incident type 2 diabetes are somewhat heterogeneous. While in animal studies evidence supports a protective role of leptin in the development of diabetes, epidemiological studies tend to suggest an adverse association. [22;23] High levels of leptin, as observed in obese subjects, therefore would likely reflect leptin resistance, which would support an adverse relation between its increase and type 2 diabetes [11]. In the MONICA/KORA nested case-cohort study, leptin levels were positively associated with the risk of developing type 2 diabetes (P for trend <0.001). Adjustment for BMI substantially decreased the associations, which became non significant after further adjustment on CRP, sICAM-1 and E-selectin [6].

Consistent with our results, similar associations between biomarkers of endothelial function and risk of type 2 diabetes were observed in the Nurses' Health Study: RR for the highest quintile (vs. the lowest) were 5.43 (3.47;8.50) for E-selectin, 3.56 (92.28;5.58) for sICAM-1 and 1.12 (0.76;1.66) for sVCAM-1 after adjusting for anthropometry. Further adjustment for CRP did not significantly alter these associations.[10]

As to other biomarkers, the lack of association in our study somewhat differs from the current literature: a meta-analysis of 15 prospective studies found an inverse association between plasma adiponectin and incidence of type 2 diabetes, with no substantial difference across studies, or after adjustment for BMI: overall relative risk (RR) of diabetes across studies was 0.72 (0.67;0.78) per 1-log µg/ml increment of adiponectin level [5]; A meta-analysis of the association between levels of CRP and risk of incident type 2 diabetes found a consistent positive association across nine prospective studies. [7] Overall RR after weighing for age and sex was 4.00 (2.83;5.65) for subjects with CRP levels ≥2.6mg/l compared to subjects with levels <0.5mg/l. Differences might be due to differences in study design or setting, as well as lack of power in our single analysis.

Adipokines are thought to participate in the development of type 2 diabetes through effects on insulin resistance and inflammation [1;23]. Endothelial dysfunction may also directly contribute to insulin resistance by reducing blood flow, resulting in impaired delivery of glucose and insulin to metabolic targets [15]. Low-grade inflammation has been found to contribute independently to both insulin resistance and endothelial dysfunction through kinase transcription factors and circulating pro-inflammatory factors. Inflammation, endothelial dysfunction and adipokines tend to have intricated effects with reciprocal relationships, and isolating predominant mechanisms appears challenging. In a study among aboriginal canadian populations, Ley and al. [4] found that adiponectin was the only marker independently associated with risk of type 2 diabetes after adjustment for anthropometry and inflammation biomarkers. Endothelial function markers were not included in the study. Another study focusing on biomarkers of endothelial function proposed a model adjusted for CRP, thus taking into account inflammatory factors: E-selectin and sICAM-1 levels remained significantly associated with risk of incident type 2 diabetes after adjustment for CRP levels (P for trend <0.001 for both biomarkers) [10]. In Pima Indians, among adipokines, inflammatory factors and endothelial function markers, only adiponectin was significantly associated with incident type 2 diabetes, while inflammatory and vascular biomarkers had no significant effect [8]. Finally, in the MONICA/KORA study, risk of incident type 2 diabetes was associated with adiponectin levels (P <0.001), but not with leptin in fully adjusted models [6]. Our results suggest that adipokines and endothelial function biomarkers might act through independent biological pathways, as associations between sICAM-1 or sVCAM-1

concentrations to a model including leptin remained significant. However, including both biomarkers of endothelial function in the model resulted in a loss of significance. This could be due to the fact that being in the same biological pathway inclusion of both biomarkers would lead to overadjustment. Our results also suggest that adipose tissue dysfunctional parameters, as adipokines secretion may be interpreted, can improve individualize risk patterns for type 2 diabetes, more clearly than BMI alone. The associations observed in our study between biomarkers and type 2 diabetes therefore give further insights into the biological pathways responsible for the development of the pathology.

Information conveid by the studied biomarkers significantly improved the prediction of type 2 diabetes in comparison to using only classical risk factors of diabetes. However, even if significant, improvement in prediction would be marginal, considering the high AUC achieved by the model including classic risk factors for diabetes. Our results on this case are consistent with others who adressed the issue: a study by Chao et al. [24] suggested that the inclusion of novel biomarkers (CRP, Tumor necrosis factor receptor 2, interleukin 6, E-selectin, sICAM-1 and sVCAM-1) did not improve the prediction models for type 2 diabetes, as evaluated by AUC and IDI, even if IDI values found by these authors are consistent with ours.

Therefore, even if biomarkers are interesting in giving new insights in type 2 diabetes pathogenesis comprehension, their inclusion in clinical practice for type 2 diabetes prediction would not appear justified. [25]

Strenghs of our study include diagnosis of incident type 2 diabetes based on objective measures of FPG and medication use, follow-up of more than 13 years and test for prediction improvement with novel and stable statistical methods. Furthemore, comparison between excluded and included subjects only showed minor differences.

Limitations need to be adressed : the ancillary nature of our study undermines our conclusions. subjects in our sample were selected based on later incidence of cardiovascular disease and cancer, with matching controls. Therefore our population is older and with higher BMI at baseline than the entire cohort. It is therefore more at risk of diabetes as well. This selection bias undermines our ability to generalize to other populations, but remain of interest as to associations in a at-risk population. Moreover, biomarkers of adiposity, endothelial function and inflammation have been found to be associated to cardiovascular disease and cancer risk.[26-28] Subjects in the cardiovascular risk or cancer group are therefore more likely to have a biomarker profile including lower adiponectin, higher leptin, ICAM-1, VCAM-1, MCP-1 and CRP. Such profiles in subjects free of diabetes would have lead us to underestimate the associations between biomarkers and diabetes risk. Finally, even with 13 years of follow-up, only a small

number of subjects developed diabetes during follow-up and only a subsample of subjects were tested for biomarkers at inclusion, thus limiting the power of our analyses; concluding that associations between biomarkers and risk of incident type 2 diabetes are independent of classic risk factors for diabetes and independent of one another would imply that other confounding sources (dietary and energy intakes) and mediators have been accounted for, which might not be the case.

Our study adds to the current knowledge of predominant biological pathways to development of type 2 diabetes, as adipokines and endothelial function biomarkers seem to have independent activities. The inclusion of novel biomarkers in risk prediction however significantly but marginally improved risk prediction.

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SC and LF designed the study. CJ and LF performed data analyses. CJ wrote the manuscript. NC provided essential material. All the authors consistently contributed to the discussion and edited the manuscript. This work has been supported by a grant from the "Société Francophone du Diabète". The sponsors had no influence on design, analysis or interpretation of results, and took no part in the writing of this paper.

Declaration of Conflicting Interests

None declared

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Table 1 Characteristics of the study population

	Free of diabetes N= 1263		developed type 2 N= 82		P
Sex					
Hommes	609	(48)	52	(63)	0.008
Femmes	654	(52)	30	(37)	
Group allocation					
Placebo	618	(49)	43	(52)	0.538
Antioxidants	645	(51)	39	(48)	
Educational level					
Primary	276	(22)	14	(17)	0.295
Secondary	473	(38)	37	(46)	
Superior	505	(40)	29	(36)	
Physical activity					
Irregular	299	(24)	27	(33)	0.134
<1h/day	355	(28)	21	(26)	
≥1h/day	596	(48)	32	(40)	
Family history of diabetes					
No family history	1014	(80)	54	(66)	0.002
Family history	249	(20)	28	(34)	
Age (y)	51.06	±5.88	5.88	±5.88	0.201
BMI (kg/m^2)	24.38	±3.45	28.24	±4.50	<0.001
Fasting plasma glucose	5.63	±0.54	6.20	±0.54	<0.001
Total cholesterol (mmol/l)	6.08	±1.00	6.23	±1.10	0.238
Triglycerides (mmol/l)	1.06	±0.62	1.47	±1.05	0.001
Adiponectin (µg/ml)	11.18	(6.09;13.76)	7.94	(4.03;10.27)	<.0001
Leptin (µg/ml)	8.97	(3.51;11.49)	16.15	(4.8;20.56)	<.0001
MCP-1 (ng/ml)	261.84	(202;296)	277.41	(222;306)	0.046
E-Selectin (ng/ml)	38.11	(26.52;48.15)	47.15	(28.8;62.49)	<.0001
sICAM-1 (ng/ml)	240.76	(197;275)	269.14	(222;314.33)	0.002
sVCAM-1 (ng/ml)	690.40	(544;801)	715.70	(551.17;829)	0.373
hsCRP (mg/l)	2.30	(0.57;1.87)	3.62	(1.01;3.11)	<.0001

Data are frequencies (percentage) for categorical variables, mean ±SD, or median (25th; 75th percentiles) for non-normally distributed continuous variables. P values obtained with Chi-square tests for categorical variables and Welch's test for continuous variables (for non-normally distributed variables, tests were performed on the log-transformed variable).

Table 2: Odds ratios of incident diabetes according to biomarkers status

	Tertile 1	Tertile 2		Tertile 3			Continuous		
	OR	OR	95% CI	OR	95% CI	P for trend	OR	95% CI	P
Adiponectin									
N	*44*	*24*		*14*					
Crude	**1**	**0.53**	(0.31;0.88)	**0.30**	(0.16;0.56)	0.000	**0.60**	(0.48;0.75)	0.000
Model 1	**1**	**0.57**	(0.32;1.02)	**0.56**	(0.27;1.18)	0.069	**0.73**	(0.55;0.97)	0.029
Model 2	**1**	**0.63**	(0.35;1.14)	**0.71**	(0.33;1.53)	0.239	**0.81**	(0.60;1.09)	0.166
Leptin									
N	*17*	*21*		*44*					
Crude	**1**	**1.28**	(0.67;2.46)	**2.81**	(1.58;5.00)	0.000	**2.02**	(1.57;2.60)	0.000
Model 1	**1**	**1.07**	(0.54;2.12)	**2.21**	(1.02;4.81)	0.048	**2.27**	(1.46;3.54)	0.000
Model 2	**1**	**0.88**	(0.43;1.80)	**1.67**	(0.74;3.76)	0.213	**2.04**	(1.28;3.26)	0.003
MCP-1									
N	*18*	*28*		*36*					
Crude	**1**	**1.59**	(0.87;2.92)	**1.97**	(1.10;3.53)	0.023	**1.23**	(0.99;1.52)	0.058
Model 1	**1**	**1.70**	(0.88;3.27)	**1.58**	(0.82;3.04)	0.218	**1.07**	(0.83;1.37)	0.621
Model 2	**1**	**1.75**	(0.88;3.46)	**1.71**	(0.86;3.39)	0.157	**1.09**	(0.83;1.42)	0.549
E-selectin									
N	*23*	*12*		*47*					
Crude	**1**	**0.52**	(0.25;1.05)	**2.02**	(1.20;3.38)	0.002	**1.73**	(1.34;2.24)	0.000
Model 1	**1**	**0.45**	(0.22;0.94)	**1.18**	(0.67;2.07)	0.338	**1.25**	(0.96;1.62)	0.095
Model 2	**1**	**0.45**	(0.21;0.95)	**1.07**	(0.60;1.93)	0.557	**1.18**	(0.90;1.55)	0.244
sICAM-1									
N	*16*	*26*		*40*					
Crude	**1**	**1.63**	(0.86;3.07)	**2.48**	(1.37;4.50)	0.002	**1.51**	(1.2;1.90)	0.000
Model 1	**1**	**1.70**	(0.87;3.34)	**2.35**	(1.25;4.41)	0.007	**1.45**	(1.14;1.84)	0.003
Model 2	**1**	**1.49**	(0.75;2.99)	**2.02**	(1.06;3.86)	0.031	**1.39**	(1.08;1.78)	0.010
sVCAM-1									
N	*27*	*22*		*33*					
Crude	**1**	**0.80**	(0.45;1.43)	**1.16**	(0.68;1.96)	0.563	**1.11**	(0.89;1.39)	0.342
Model 1	**1**	**1.09**	(0.59;2.02)	**1.63**	(0.92;2.89)	0.087	**1.25**	(0.99;1.57)	0.059
Model 2	**1**	**1.04**	(0.55;1.98)	**1.83**	(1.01;3.31)	0.044	**1.29**	(1.01;1.64)	0.041
hsCPR									
N	*13*	*21*		*48*					
Crude	**1**	**1.62**	(0.80;3.28)	**4.10**	(2.19;7.67)	0.000	**1.60**	(1.32;1.94)	0.000
Model 1	**1**	**1.03**	(0.50;2.13)	**1.94**	(0.98;3.82)	0.024	**1.21**	(0.96;1.54)	0.110
Model 2	**1**	**0.90**	(0.43;1.91)	**1.71**	(0.85;3.45)	0.059	**1.15**	(0.90;1.47)	0.278

Model 1 adjusted for baseline age, sex, supplementation group, family history of diabetes and BMI; Model 2 adjusted for variables in Model 1 + baseline glycaemia, total cholesterol and triglycerides concentrations. Results for continuous variables are odd ratios for a 1-SD increase of the centered log-transformed variable.

Table 3 : Odds ratios of incident diabetes according to biomarkers status – in combination

Sequence	Leptin		sICAM_1		sVCAM_1	
	OR	95% CI	OR	95% CI	OR	95% CI
+ sICAM_1	2.07	(1.29;3.33)	1.39	(1.09;1.79)		
+ sVCAM_1	2.09	(1.31;3.35)			1.32	(1.03;1.69)
+ sICAM_1 + sVCAM_1	2.10	(1.31;3.37)	1.30	(0.99;1.71)	1.18	(0.90;1.55)

Models adjusted for baseline age, sex, family history of diabetes, baseline BMI, glycaemia, total cholesterol and triglycerids concentrations (Model 2). Results are odd ratios for a 1-SD increase of the log-transformed variable.

Table 4: Predictive ability of novel biomarkers in predicting risk of incident diabetes.

	IDI	95% CI	P	RIDI	AUC	Δ AUC	P
Model 2	Comparing model				0.845		
Model 2 + Adiponectin	0.003	(-0.001,0.008)	0.125	2.14	0.844	-0.001	0.80
Model 2 + Leptin	0.012	(0.001,0.022)	0.034	7.84	0.854	0.010	0.10
Model 2 + E-selectin	0.002	(-0.002,0.006)	0.176	1.53	0.846	0.001	0.63
Model 2 + MCP_1	0.001	(-0.0003,0.003)	0.092	0.97	0.844	-0.001	0.57
Model 2 + sICAM_1	0.011	(0.001,0.020)	0.033	7.18	0.850	0.005	0.30
Model 2 + sVCAM_1	0.007	(-0.0003,0.014)	0.059	4.75	0.849	0.004	0.34
Model 2 + hsCRP	0.002	(-0.001,0.006)	0.137	1.63	0.845	0.000	1.00
Model 2 + Leptin + sICAM_1	0.023	(0.009,0.037)	0.003	15.61	0.859	0.014	0.09
Model 2 + Leptin + sICAM_1 + sVCAM_1	0.026	(0.011,0.041)	0.002	17.76	0.859	0.015	0.08
Model 2 + Leptin + sICAM_1 + sVCAM_1 + Adiponectin	0.031	(0.014,0.047)	0.001	20.84	0.858	0.014	0.16
Model 2 + Leptin + sICAM_1 + sVCAM_1 + Adiponectin + E_selectin	0.031	(0.014,0.047)	0.001	20.78	0.858	0.014	0.15
Model 2 + Leptin + sICAM_1 + sVCAM_1 + Adiponectin + E_selectin + hsCRP	0.031	(0.014,0.048)	0.001	20.92	0.859	0.014	0.14
Model 2 + Leptin + sICAM_1 + sVCAM_1 + Adiponectin + E_selectin + hsCRP + MCP_1	0.032	(0.015,0.048)	0.001	21.65	0.858	0.013	0.16

Model 2 adjusted for sex, baseline age, supplementation allocation group, BMI, family history of diabetes, baseline glycaemia, total cholesterol and triglycerides concentrations

IDI: integrated discrimination improvement; RIDI Relative integrated discrimination improvement; AUC : Area under the receiver operating curve; ΔAUC difference in AUC

+ indicates variables included in the model compared to Model 2

Association Between Prediagnostic Biomarkers of Inflammation and Endothelial Function and Cancer Risk: A Nested Case-Control Study

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Experimental and prevalent case-control studies suggest an association between biomarkers of inflammation, endothelial function, and adiposity and cancer risk, but results from prospective studies have been limited. The authors' objective was to prospectively examine the relations between these biomarkers and cancer risk. A nested case-control study was designed within the Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) Study, a nationwide French cohort study, to include all first primary incident cancers diagnosed between 1994 and 2007 (n = 512). Cases were matched with randomly selected controls (n = 1,024) on sex, age (in 2-year strata), body mass index (weight (kg)/height $(m)^2$; <25 vs. ≥25), and SU.VI.MAX intervention group. Conditional logistic regression was used to study the associations between prediagnostic levels of high-sensitivity C-reactive protein (hs-CRP), adiponectin, leptin, soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1, soluble E-selectin, and monocyte chemoattractant protein 1 and cancer risk. All statistical tests were 2-sided. Plasma sICAM-1 level was positively associated with breast cancer risk (for quartile 4 vs. quartile 1, multivariate odds ratio (OR) = 1.86, 95% confidence interval (CI): 1.06, 3.26; $P_{trend} = 0.048$). Plasma hs-CRP level was positively associated with prostate cancer risk (for quartile 4 vs. quartile 1, multivariate OR = 3.04, 95% CI: 1.28, 7.23; $P_{trend} = 0.03$). These results suggest that prediagnostic hs-CRP and sICAM-1 levels are associated with increased prostate and breast cancer risk, respectively.

breast neoplasms; case-control studies; C-reactive protein; intercellular adhesion molecule 1; neoplasms; prostatic neoplasms

Abbreviations: CI, confidence interval; CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein 1; OR, odds ratio; Q, quartile; SD, standard deviation; sICAM-1, soluble intercellular adhesion molecule 1; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; sVCAM-1, soluble vascular cell adhesion molecule 1.

Editor's note: An invited commentary on this article appears on page 14.

The identification of prediagnostic biomarkers associated with subsequent cancer risk is a key challenge. Markers of inflammation, adiposity, and endothelial adhesion may be good candidates (1–4). C-reactive protein (CRP), which is produced in the liver in response to elevated cytokine levels after an inflammatory stimulus, is a widely used systemic biomarker for diagnosing acute and chronic inflammation (5). White adipose tissue plays a critical role in the regulation of inflammatory processes, as an endocrine organ, and produces adipokines (6). Leptin is a proinflammatory adipokine inducing T helper 1 cells. Its serum level strongly correlates with proportion of body fat stores. Conversely, adiponectin production is decreased in obesity and generally acts as an antiinflammatory factor. Adhesion

Table 1. Baseline Characteristics of Cancer Cases and Controls, SU.VI.MAX Cohort, France, 1994–2007

	Breast Cancer Cases (n = 218 cases)			Prostate Cancer Cases (n = 156 cases)			Overall Cancer Cases (n = 512)			Controls (n = 1,024)			P Value[a]
	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	
Age, years			49.2 (6.1)			54.9 (4.8)			51.4 (6.1)			51.5 (6.1)	0.9
Sex													1.0
Male							229	44.7		458	44.7		
Female							283	55.3		566	55.3		
Body mass index[b]													0.3
<25	164	75.2		74	47.4		314	61.3		628	61.3		
25–<30	37	17.0		67	43.0		150	29.3		323	31.5		
≥30	17	7.8		15	9.6		48	9.4		73	7.1		
Height, cm			162.8 (6.2)			173.4 (6.7)			167.6 (8.3)			166.7 (8.3)	0.05
Intervention group													1.0
Yes	109	50.0		73	46.8		258	50.4		516	50.4		
No (placebo)	109	50.0		83	53.2		254	49.6		508	49.6		
Smoking status													0.001
Never smoker	126	57.8		63	40.4		245	47.9		516	50.4		
Former smoker	46	21.1		75	48.1		175	34.2		392	38.3		
Current smoker	46	21.1		18	11.5		92	18.0		116	11.3		
Alcohol intake, g/day			9.2 (11.2)			24.1 (19.9)			16.6 (18.6)			14.9 (16.7)	0.06
Physical activity													0.2
Low	64	29.4		35	22.4		129	25.2		259	25.3		
Moderate	75	34.4		40	25.6		156	30.5		273	26.7		
High	79	36.2		81	51.9		227	44.3		492	48.1		
Educational level, years													0.3
<12	130	59.6		89	57.1		306	59.8		584	57.0		
≥12	88	40.4		67	43.0		206	40.2		440	43.0		
PSA level for men, ng/mL						3.6 (3.6)			2.9 (3.3)			1.3 (1.5)	<0.0001
PSA category for men, ng/mL													<0.0001
<3				96	61.5		164	71.6		425	92.8		
≥3				60	38.5		65	28.4		33	7.2		
Plasma hs-CRP level, mg/L			2.1 (4.3)			2.5 (5.1)			2.5 (5.9)			2.1 (4.5)	0.006
Plasma sICAM-1 level, ng/mL			247.0 (80.0)			245.2 (65.5)			253.2 (80.0)			240.3 (65.4)	0.005
Plasma sVCAM-1 level, ng/mL			689.5 (226.9)			682.5 (194.6)			689.0 (233.9)			683.8 (200.7)	0.8

Table continues

Table 1. Continued

	Breast Cancer Cases (n = 218 cases)			Prostate Cancer Cases (n = 156 cases)			Overall Cancer Cases (n = 512)			Controls (n = 1,024)			P Value[a]
	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	
Plasma soluble E-selectin level, ng/mL			33.8 (14.7)			41.0 (15.3)			38.1 (16.2)			37.9 (15.1)	0.8
Plasma MCP-1 level, pg/mL			248.7 (159.4)			278.8 (85.9)			266.0 (129.2)			257.5 (113.8)	0.1
Plasma leptin level, ng/mL			13.0 (12.0)			5.3 (4.6)			9.6 (9.7)			9.8 (10.3)	0.6
Plasma adiponectin level, μg/mL			13.8 (9.0)			7.1 (3.7)			10.6 (7.7)			11.0 (8.7)	0.4

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein 1; PSA, prostate-specific antigen; sICAM-1, soluble intercellular adhesion molecule 1; SD, standard deviation; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; sVCAM-1, soluble vascular cell adhesion molecule 1.

[a] P value for the comparison between overall cancer cases and controls by Student's t test or χ^2 test where appropriate. Data for biomarker variables were log-transformed to improve normality. All statistical tests were 2-sided.

[b] Weight (kg)/height $(m)^2$.

molecules such as E-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1, and the chemokine monocyte chemoattractant protein 1 (MCP-1) are important in cell–cell and cell–basement membrane interactions. They are also intimately involved in inflammatory reactions (7).

For all of these biomarkers, a role in carcinogenesis has been postulated, notably based on prevalent case-control studies that have reported higher serum/plasma levels of CRP (2), leptin (3), and soluble adhesion molecules (7–9) and lower levels of adiponectin (1) in patients with cancer compared with controls, for various cancer sites. Studies on single nucleotide polymorphisms in the genes of CRP (10, 11), adipokines (12–14), and soluble adhesion molecules (15–17) have also suggested that these markers may affect cancer risk. The prognostic use of these markers has also been demonstrated in many forms of cancer (18–21). However, few prospective studies published so far have provided relevant analyses to investigate the relations between these biomarkers and cancer risk, and where results exist they are conflicting (22–28).

Thus, our objective was to prospectively examine the relations between biomarkers of inflammation, adiposity, and endothelial function and development of cancer.

MATERIALS AND METHODS

Study population

The Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) study was a population-based, double-blind, placebo-controlled, randomized trial initially designed to assess the relation of daily antioxidant supplementation to the incidence of cardiovascular disease and cancer (29). A total of 13,017 subjects were enrolled throughout France in 1994–1995. The intervention study lasted 8 years, and follow-up for health events was maintained until July 2007. Subjects provided written informed consent, and the study was approved by the Ethical Committee for Studies with Human Subjects at the Paris-Cochin Hospital and the Commission nationale de l'informatique et des libertés.

Baseline data collection

At enrollment, all participants underwent a clinical examination and had anthropometric measurements taken by study nurses and physicians. They completed questionnaires on sociodemographic data, smoking, alcohol intake, and physical activity. A 35-mL venous blood sample was collected in Vacutainer tubes (Becton Dickinson, Rungis, France) from participants who had been fasting for 12 hours at the time of the visit. Blood samples were centrifuged immediately after blood draw, and plasma aliquots were then preserved in sodium heparin. Less than 1 hour after blood draw, plasma aliquots were stored at −20°C in dry ice for shipment to the central biobank (maximum 24 hours), where they were stored frozen in liquid nitrogen (−70°C). For male participants, total prostate-specific antigen level was measured by immunometry (Roche

Table 2. Odds Ratios for the Relations Between Biomarkers of Inflammation, Endothelial Function, and Adiposity and Overall Cancer Risk From Conditional Logistic Regression Analyses[a], SU.VI.MAX Cohort, France, 1994–2007

	No. of Cases	No. of Controls	Unadjusted[b] Results		Results From Multivariate Model[c]		Results From Multivariate Model Including All Studied Biomarkers	
			OR	95% CI	OR	95% CI	OR	95% CI
hs-CRP								
Q1	111	272	1	Reference	1	Reference	1	Reference
Q2	124	261	1.17	0.86, 1.60	1.18	0.86, 1.62	1.20	0.87, 1.66
Q3	119	265	1.13	0.82, 1.55	1.08	0.78, 1.49	1.10	0.78, 1.53
Q4	158	226	1.81	1.32, 2.48	1.78	1.28, 2.47	1.78	1.26, 2.52
P for trend			0.0006		0.002		0.004	
sICAM-1								
Q1	113	269	1	Reference	1	Reference	1	Reference
Q2	129	258	1.21	0.89, 1.64	1.23	0.90, 1.67	1.32	0.96, 1.82
Q3	118	265	1.05	0.77, 1.43	1.05	0.77, 1.45	1.09	0.78, 1.53
Q4	152	232	1.56	1.16, 2.11	1.48	1.09, 2.02	1.51	1.06, 2.14
P for trend			0.012		0.035		0.068	
sVCAM-1								
Q1	139	244	1	Reference	1	Reference	1	Reference
Q2	121	265	0.81	0.60, 1.08	0.83	0.62, 1.13	0.80	0.59, 1.10
Q3	116	266	0.76	0.56, 1.04	0.77	0.56, 1.05	0.70	0.50, 0.97
Q4	136	249	0.95	0.71, 1.27	0.99	0.73, 1.34	0.85	0.61, 1.18
P for trend			0.7		0.9		0.3	
Soluble E-selectin								
Q1	126	257	1	Reference	1	Reference	1	Reference
Q2	134	250	1.08	0.81, 1.45	1.09	0.81, 1.47	1.00	0.74, 1.36
Q3	113	272	0.85	0.63, 1.16	0.84	0.62, 1.14	0.79	0.57, 1.09
Q4	139	245	1.17	0.86, 1.58	1.14	0.83, 1.56	1.01	0.72, 1.42
P for trend			0.7		0.9		0.5	

Table continues

Diagnostics, Mannheim, Germany) using a specific antibody with a highly sensitive technique standardized to the reference Stanford material (30).

Cases ascertainment

Confirmed or suspected events were self-declared by subjects during the follow-up process. Investigations were conducted in all cases to obtain medical data from participants, physicians, and/or hospitals. All information was reviewed by an independent expert committee, and cases were validated by pathologic report and classified using the International Classification of Diseases, Tenth Revision, Clinical Modification.

Nested case-control study

Among the 890 first primary invasive incident cancer cases diagnosed between inclusion in the SU.VI.MAX cohort in 1994 and July 2007, 368 had missing data for body mass index (weight (kg)/height $(m)^2$; measured during the clinical examination) or for a prediagnostic blood sample at baseline and were not included in the present study. Ten cases were further excluded because no control was available in the cohort with the required matching criteria. For each cancer case, 2 controls were randomly selected from participants of identical sex, age (in 2-year strata), body mass index (<25 vs. ≥25), and intervention group, with complete follow-up and without cancer diagnosis by the end of follow-up.

Baseline plasma samples of the corresponding subjects were used to determine the levels of high-sensitivity CRP (hs-CRP), leptin, adiponectin, soluble ICAM-1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble E-selectin, and MCP-1. Biomarkers' levels were determined with an enzyme-linked immunosorbent assay sandwich technique (R&D Laboratory Systems, Minneapolis, Minnesota). Three samples of known concentrations were tested in 30 separate assays to assess interassay precision. Three samples of known concentrations were tested 20 times on 1 plate to assess intraassay precision. The intraassay and interassay coefficients of variation were all less

Table 2. Continued

	No. of Cases	No. of Controls	Unadjusted[b] Results		Results From Multivariate Model[c]		Results From Multivariate Model Including All Studied Biomarkers	
			OR	95% CI	OR	95% CI	OR	95% CI
MCP-1								
Q1	119	261	1	Reference	1	Reference	1	Reference
Q2	130	258	1.11	0.82, 1.50	1.07	0.79, 1.46	1.03	0.75, 1.41
Q3	130	252	1.13	0.84, 1.53	1.09	0.80, 1.48	1.04	0.76, 1.42
Q4	133	253	1.16	0.85, 1.58	1.08	0.79, 1.48	1.03	0.74, 1.43
P for trend			0.3		0.6		0.6	
Leptin								
Q1	134	249	1	Reference	1	Reference	1	Reference
Q2	121	264	0.85	0.63, 1.16	0.82	0.60, 1.12	0.77	0.55, 1.06
Q3	126	258	0.91	0.67, 1.23	0.87	0.63, 1.20	0.82	0.58, 1.14
Q4	131	253	0.97	0.68, 1.38	0.84	0.56, 1.25	0.70	0.46, 1.07
P for trend			0.9		0.4		0.2	
Adiponectin								
Q1			1	Reference	1	Reference	1	Reference
Q2	135	248	0.83	0.61, 1.12	0.86	0.63, 1.17	0.87	0.63, 1.19
Q3	119	265	0.98	0.72, 1.33	1.04	0.76, 1.41	1.11	0.80, 1.52
Q4	134	251	0.88	0.65, 1.19	0.92	0.67, 1.26	0.92	0.66, 1.27
P for trend	124	260	0.6		0.9		1.0	

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; OR, odds ratio; Q, quartile; sICAM-1, soluble intercellular adhesion molecule 1; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; sVCAM-1, soluble vascular cell adhesion molecule 1.

[a] Cutoffs for sex-specific quartiles were: hs-CRP—0.6, 1.1, 2.2 in men and 0.5, 0.9, 1.9 in women; sICAM-1—199.0, 243.8, 288.0 in men and 194.0, 232.0, 272.8 in women; sVCAM-1—532.6, 652.0, 786.0 in men and 538.0, 652.0, 800.0 in women; soluble E-selectin—41.5, 30.6, 51.4 in men and 23.6, 33.0, 42.9 in women; MCP-1—225.0, 267.0, 315.0 in men and 183.0, 222.0, 268.0 in women; leptin—2.4, 4.1, 6.6 in men and 5.9, 10.0, 17.0 in women; adiponectin—4.3, 6.4, 9.2 in men and 8.9, 12.0, 16.3 in women. All statistical tests were 2-sided.

[b] Cases and controls were matched by sex, age, body mass index, and SU.VI.MAX intervention group.

[c] Adjusted for sex, age, body mass index, height, SU.VI.MAX intervention group, alcohol intake, physical activity, smoking status, and educational level.

than 10%. Hs-CRP had the lowest intraassay coefficient of variation (1.6%), and MCP-1 had the highest (6.2%). Hs-CRP had the lowest interassay coefficient of variation (3.6%), and soluble E-selectin had the highest (9.1%). Thirty specimens were measured as blinded duplicates on separated plates and showed only small variations for the second decimal digit. Cases and matched controls were measured on the same plate, but the case/control status of each sample within a plate was not known by the investigator (blinded determination). Specimens with values below the detection limit were observed only for leptin, and they represented only 2.5% of the totality of biologic samples. These observations were handled by conferring on them the detection limit value indicated by the manufacturer. For the other analytes, no sample exhibited values below the detection limit.

Statistical analyses

The participants' baseline characteristics were compared between cases and controls using Student's t tests or χ^2 tests. Associations between biomarkers and incident cancer were examined with conditional logistic regression models and expressed as odds ratios with 95% confidence intervals. Associations of biomarkers with overall, breast, and prostate cancer risk were successively tested. Associations between each single biomarker and cancer risk were studied in nonadjusted and multivariate models. Multivariate models that simultaneously included all biomarkers were also fitted. For all models, the odds ratios for sex-specific quartiles and the odds ratios for a 1-standard-deviation (SD) increase in the corresponding biomarker (considered as a continuous variable) were both computed. Multivariate models were adjusted for sex, age, body mass index, height, SU.VI.MAX intervention group, alcohol intake, physical activity, smoking status, educational level, and baseline prostate-specific antigen level (for prostate cancer analyses only). Further adjustment for other site-specific classical risk factors was also tested: family history of breast cancer, number of children, use of hormone replacement therapy for menopause and menopausal status at baseline (in breast cancer analyses), and family history of prostate cancer (in

Table 3. Odds Ratios for the Relations Between Biomarkers of Inflammation, Endothelial Function, and Adiposity and Breast Cancer Risk From Conditional Logistic Regression Analyses[a], SU.VI.MAX Cohort, France, 1994–2007

	No. of Cases	No. of Controls	Unadjusted[b] Results		Results From Multivariate Model[c]		Results From Multivariate Model Including All Studied Biomarkers	
			OR	95% CI	OR	95% CI	OR	95% CI
hs-CRP								
Q1	52	112	1	Reference	1	Reference	1	Reference
Q2	55	109	1.09	0.68, 1.75	1.16	0.71, 1.88	1.30	0.77, 2.19
Q3	53	111	1.05	0.66, 1.67	0.93	0.57, 1.51	1.06	0.63, 1.79
Q4	58	104	1.24	0.75, 2.05	1.25	0.73, 2.14	1.40	0.79, 2.49
P for trend			0.5		0.7		0.4	
sICAM-1								
Q1	48	123	1	Reference	1	Reference	1	Reference
Q2	57	104	1.44	0.90, 2.32	1.47	0.90, 2.41	1.75	1.03, 2.98
Q3	49	116	1.07	0.67, 1.72	1.15	0.70, 1.89	1.43	0.83, 2.47
Q4	64	93	1.77	1.12, 2.81	1.57	0.97, 2.54	1.86	1.06, 3.26
P for trend			0.05		0.1		0.048	
sVCAM-1								
Q1	61	92	1	Reference	1	Reference	1	Reference
Q2	51	114	0.69	0.44, 1.08	0.72	0.46, 1.15	0.72	0.44, 1.17
Q3	50	123	0.61	0.38, 0.98	0.64	0.39, 1.04	0.56	0.33, 0.95
Q4	56	107	0.79	0.50, 1.25	0.84	0.51, 1.36	0.72	0.42, 1.24
P for trend			0.3		0.4		0.1	
Soluble E-selectin								
Q1	56	114	1	Reference	1	Reference	1	Reference
Q2	63	104	1.19	0.77, 1.84	1.25	0.80, 1.95	1.11	0.69, 1.79
Q3	45	116	0.80	0.50, 1.28	0.79	0.48, 1.28	0.72	0.42, 1.23
Q4	54	102	1.08	0.67, 1.74	1.02	0.61, 1.70	0.90	0.51, 1.60
P for trend			0.8		0.5		0.3	

Table continues

prostate cancer analyses). Two-way interactions between each biomarker and smoking status were explored, but no interaction was detected.

All statistical tests were 2-sided, and $P < 0.05$ was considered significant. All analyses were performed with SAS software, version 9.1 (SAS Institute Inc., Cary, North Carolina).

RESULTS

A total of 512 incident cancer cases were diagnosed during follow-up: 218 breast cancers, 156 prostate cancers, and 138 other cancers (50 colorectal cancers, 32 thyroid cancers, 24 lung cancers, 20 skin melanomas, 8 esophagus cancers, and 4 stomach cancers). Thus, a total of 512 sets of 1 case and 2 matched controls were included for the current analyses. Median follow-up time was 6.5 years in cases and 13 years in controls. Characteristics of cancer cases and noncases are described in Table 1. Overall cancer cases were more frequently current smokers and had higher prostate-specific antigen levels at baseline (for men).

In multivariate models, plasma hs-CRP level (for quartile 4 (Q4) vs. quartile 1 (Q1), odds ratio (OR) = 1.78, 95% confidence interval (CI): 1.28, 2.47; P for trend = 0.002) and plasma sICAM-1 level (for Q4 vs. Q1, OR = 1.48, 95% CI: 1.09, 2.02; P for trend = 0.035) were associated with increased overall cancer risk (Table 2). When all biomarkers were entered simultaneously into a multivariate model, the association with hs-CRP remained statistically significant (P for trend = 0.004), but the association with sICAM-1 became borderline nonsignificant (P for trend = 0.068; Table 2).

Regarding findings on site-specific cancers, in the multivariate model including all biomarkers, sICAM-1 was significantly associated with increased breast cancer risk (for Q4 vs. Q1, OR = 1.86 (95% CI: 1.06, 3.26), P for trend = 0.048 (Table 3); for a 1-SD increase, OR = 1.26 (95% CI: 1.03, 1.53), P = 0.02 (data not tabulated)). Hs-CRP was significantly associated with increased prostate cancer risk when data were considered as quartiles (for Q4 vs. Q1, multivariate OR = 3.04, 95% CI: 1.28, 7.23; P for trend = 0.03) (Table 4), though this association was not detected when hs-CRP was coded as a continuous variable

Table 3. Continued

	No. of Cases	No. of Controls	Unadjusted[b] Results		Results From Multivariate Model[c]		Results From Multivariate Model Including All Studied Biomarkers	
			OR	95% CI	OR	95% CI	OR	95% CI
MCP-1								
Q1	52	114	1	Reference	1	Reference	1	Reference
Q2	54	110	1.08	0.68, 1.72	1.10	0.68, 1.79	1.04	0.63, 1.73
Q3	53	100	1.16	0.73, 1.85	1.25	0.77, 2.01	1.22	0.74, 2.00
Q4	59	112	1.17	0.73, 1.86	1.14	0.70, 1.85	1.09	0.66, 1.81
P for trend			0.5		0.5		0.5	
Leptin								
Q1	55	103	1	Reference	1	Reference	1	Reference
Q2	64	102	1.16	0.73, 1.83	1.09	0.67, 1.76	0.99	0.59, 1.65
Q3	51	116	0.80	0.51, 1.26	0.81	0.50, 1.33	0.75	0.44, 1.29
Q4	48	115	0.69	0.39, 1.20	0.64	0.34, 1.20	0.51	0.26, 1.02
P for trend			0.09		0.1		0.08	
Adiponectin								
Q1	58	110	1	Reference	1	Reference	1	Reference
Q2	48	119	0.77	0.49, 1.23	0.80	0.50, 1.30	0.83	0.50, 1.38
Q3	55	102	1.04	0.64, 1.68	1.12	0.68, 1.85	1.29	0.76, 2.21
Q4	57	105	1.04	0.65, 1.67	1.13	0.68, 1.87	1.15	0.67, 1.97
P for trend			0.6		0.4		0.3	

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; OR, odds ratio; Q, quartile; sICAM-1, soluble intercellular adhesion molecule 1; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; sVCAM-1, soluble vascular cell adhesion molecule 1.

[a] Cutoffs for female-specific quartiles were: hs-CRP—0.5, 0.9, 1.9; sICAM-1—194.0, 232.0, 272.8; sVCAM-1—538.0, 652.0, 800.0; soluble E-selectin—23.6, 33.0, 42.9; MCP-1—183.0, 222.0, 268.0; leptin—5.9, 10.0, 17.0; adiponectin—8.9, 12.0, 16.3. All statistical tests were 2-sided.

[b] Cases and controls were matched by sex, age, body mass index, and SU.VI.MAX intervention group.

[c] Adjusted for age, body mass index, height, SU.VI.MAX intervention group, alcohol intake, physical activity, smoking status, and educational level.

(for a 1-SD increase, OR = 1.03, 95% CI: 0.82, 1.30; P = 0.8).

Further adjustment for other site-specific classical risk factors did not modify the findings. Sensitivity analysis excluding cases that were diagnosed during the first 2 years of follow-up (54 cases) did not modify the results, nor did sensitivity analyses excluding subjects with the highest hs-CRP values (>17.25 ng/mL (i.e., mean +3 SDs); 18 subjects) (data not shown).

DISCUSSION

Plasma hs-CRP level was associated with increased overall and prostate cancer risk in this prospective study. A positive association between plasma sICAM-1 level and risk of breast cancer was also observed, independently of other known risk factors. Growing evidence from mechanistic (31–33), genetic (10), and epidemiologic (2) studies points to a role of inflammation in carcinogenesis (34). Serum/plasma CRP level has been found to be elevated in patients with various malignancies (35). Elevated CRP is also a predictor of lower survival rates in patients with cancer after surgical resection (35). It has been suggested that inflammation creates a tissue microenvironment where the reactive oxygen and nitrogen species released by inflammatory cells could cause potentially malignant DNA alterations (31) and that some inflammatory cytokines and proteins in chronic inflammation promote tumor growth (36).

Consistent with our finding, prospective studies have shown a higher overall cancer risk in subjects with elevated prediagnostic serum CRP levels (2, 11, 22, 24, 37, 38). However, uncertainty remains as to whether this association is restricted to certain cancer locations and which cancer sites are of particular concern. Several studies have suggested that increased lung cancer risk is particularly associated with hs-CRP levels and could partly drive observations for overall cancer risk (11, 39). In our study, the number of lung cancer cases was insufficient to perform specific analysis for this location. In contrast, consistent with our findings of a null association between hs-CRP and breast cancer risk, a recent meta-analysis found similar results; those authors reported an odds ratio for breast cancer of 1.10 (95% CI: 0.97, 1.26) for a log unit increase in CRP level (24).

Table 4. Odds Ratios for Each Biomarker and Prostate Cancer Risk From Conditional Logistic Regression Analyses[a], SU.VI.MAX Cohort, France, 1994–2007

	No. of Cases	No. of Controls	Unadjusted[b] Results		Results From Multivariate Model[c]		Results From Multivariate Model Including All Studied Biomarkers	
			OR	95% CI	OR	95% CI	OR	95% CI
hs-CRP								
Q1	28	82	1	Reference	1	Reference	1	Reference
Q2	41	84	1.43	0.80, 2.56	1.76	0.84, 3.69	2.06	0.90, 4.71
Q3	35	83	1.23	0.66, 2.31	1.70	0.77, 3.75	1.83	0.75, 4.48
Q4	52	63	2.56	1.41, 4.65	2.52	1.18, 5.39	3.04	1.28, 7.23
P for trend			0.04		0.03		0.03	
sICAM-1								
Q1	39	82	1	Reference	1	Reference	1	Reference
Q2	43	82	1.10	0.65, 1.85	1.30	0.68, 2.52	1.19	0.56, 2.50
Q3	37	75	1.03	0.59, 1.82	1.33	0.64, 2.76	1.10	0.49, 2.47
Q4	37	73	1.07	0.62, 1.83	1.36	0.69, 2.69	1.00	0.43, 2.34
P for trend			0.9		0.4		0.8	
sVCAM-1								
Q1	35	72	1	Reference	1	Reference	1	Reference
Q2	39	79	1.02	0.58, 1.78	1.06	0.51, 2.20	0.85	0.36, 1.99
Q3	42	83	1.04	0.60, 1.81	1.05	0.50, 2.22	0.86	0.36, 2.07
Q4	40	78	1.05	0.61, 1.81	1.33	0.66, 2.71	1.16	0.50, 2.70
P for trend			0.8		0.4		0.6	
Soluble E-selectin								
Q1	43	72	1	Reference	1	Reference	1	Reference
Q2	37	83	0.77	0.45, 1.29	0.81	0.41, 1.59	0.66	0.31, 1.42
Q3	33	81	0.69	0.40, 1.20	0.80	0.41, 1.56	0.64	0.30, 1.35
Q4	43	76	0.97	0.56, 1.65	1.22	0.61, 2.43	0.84	0.38, 1.88
P for trend			0.8		0.6		0.8	

Table continues

In our prospective study, the positive association with hs-CRP was observed for prostate cancer risk. In contrast with our findings, previous results from prospective studies investigating the relation between CRP and prostate cancer (11, 22, 24, 28, 37, 38, 40–42) have been mostly nonsignificant, as was shown in a recent meta-analysis (24). However, most of these studies included few prostate cancer cases (fewer than 100) (24, 37, 38), did not measure CRP with a high-sensitivity assay (28, 38), or focused only on men aged 65 years and older (41). As for the remaining studies, results are conflicting. Three studies obtained nonsignificant results (11, 22, 40), but two of them were not specifically designed to explore cancer of the prostate (11, 22). In contrast, Stark et al. (42) observed that CRP level was positively associated with increased risk of prostate cancer (all grades) among normal-weight men and with increased risk of high-grade prostate cancer among all subjects. Thus, further large prospective studies are needed to better understand whether CRP levels are associated with incident prostate cancer. In a recent study, Meyer et al. (43) showed that persons who were homozygous for the variant allele of rs12757998 had both an increased risk of prostate cancer and increased CRP levels, suggesting a link between genetic variation in the RNASEL gene (encoding ribonuclease L) and prostate cancer risk, potentially mediated through inflammation. Regarding prognostic studies, CRP has been observed to be an adverse prognostic marker for men with castration-resistant prostate cancer (21). CRP haplotype is also associated with high prostate-specific antigen level as a marker of metastatic prostate cancer (44).

Several prevalent case-control studies have observed higher circulating levels of sICAM-1 in breast cancer cases compared with controls (7–9). Genetic studies showed that some single nucleotide polymorphisms on the ICAM-1 gene were associated with increased breast cancer risk (16, 17, 45), although this point is debated (46). Higher levels of sICAM-1 were also associated with poorer clinicopathologic features (such as number of metastases and response to chemo-endocrine therapy) and poorer overall survival in a prognostic

Table 4. Continued

	No. of Cases	No. of Controls	Unadjusted[b] Results		Results From Multivariate Model[c]		Results From Multivariate Model Including All Studied Biomarkers	
			OR	95% CI	OR	95% CI	OR	95% CI
MCP-1								
Q1	36	73	1	Reference	1	Reference	1	Reference
Q2	49	78	1.27	0.74, 2.19	1.13	0.58, 2.22	1.10	0.51, 2.36
Q3	38	79	0.98	0.55, 1.74	0.87	0.42, 1.78	0.66	0.29, 1.50
Q4	33	82	0.83	0.47, 1.46	0.59	0.29, 1.21	0.52	0.24, 1.14
P for trend			0.3		0.09		0.07	
Leptin								
Q1	46	81	1	Reference	1	Reference	1	Reference
Q2	27	90	0.54	0.30, 0.96	0.47	0.22, 0.97	0.42	0.19, 0.95
Q3	42	74	1.02	0.59, 1.76	0.89	0.44, 1.77	0.88	0.41, 1.91
Q4	41	67	1.19	0.64, 2.22	0.69	0.27, 1.75	0.58	0.20, 1.68
P for trend			0.3		0.9		0.7	
Adiponectin								
Q1	37	74	1	Reference	1	Reference	1	Reference
Q2	39	84	0.92	0.54, 1.58	0.90	0.45, 1.80	0.78	0.37, 1.65
Q3	40	81	0.99	0.57, 1.71	1.38	0.69, 2.76	1.36	0.63, 2.94
Q4	40	73	1.10	0.64, 1.90	1.34	0.68, 2.61	1.18	0.56, 2.48
P for trend			0.7		0.3		0.1	

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; OR, odds ratio; Q, quartile; sICAM-1, soluble intercellular adhesion molecule 1; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; sVCAM-1, soluble vascular cell adhesion molecule 1.

[a] Cutoffs for male-specific quartiles were: hs-CRP—0.6, 1.1, 2.2; sICAM-1—199.0, 243.8, 288.0; sVCAM-1—532.6, 652.0, 786.0; soluble E-selectin—41.5, 30.6, 51.4; MCP-1—225.0, 267.0, 315.0; leptin—2.4, 4.1, 6.6; adiponectin—4.3, 6.4, 9.2. All statistical tests were 2-sided.

[b] Cases and controls were matched by sex, age, body mass index, and SU.VI.MAX intervention group.

[c] Adjusted for age, body mass index, height, SU.VI.MAX intervention group, alcohol intake, physical activity, smoking status, educational level, and baseline prostate-specific antigen level.

study of metastatic breast cancer (20). However, to the best of our knowledge, our study is the first to have investigated the prospective association between prediagnostic level of sICAM-1 and breast cancer risk. The observed positive association is supported by mechanistic plausibility. Indeed, it has been demonstrated experimentally that sICAM-1 stimulates angiogenesis and neovascularization (47, 48), endothelial cell migration and differentiation (48), and tumor growth (49). In the present study, the association between studied biomarkers and breast cancer risk varied slightly between the model with each biomarker included separately and the model with all biomarkers included simultaneously, the association being stronger for sICAM-1 in the latter model. This probably results from the mechanistic interrelations between the studied biomarkers of endothelial adhesion, inflammation and adiposity. Indeed, it is known that leptin and adiponectin generally act as pro- and antiinflammatory factors, respectively (6), and that the synthesis of adhesion molecules (such as sICAM-1) is stimulated both by leptin and by proinflammatory cytokines (49, 50). The mechanistic synergy between all studied biomarkers is better taken into account in the model which included them all simultaneously. In our study, sICAM-1 was moderately associated with E-selectin, sVCAM-1, MCP-1, and hs-CRP, while its associations with leptin and adiponectin were weaker (Pearson correlation coefficients were 0.4, 0.3, 0.2, 0.2, 0.07, and 0.01, respectively; data not tabulated).

Strengths of our study included the use of multiple biomarkers, the nested case-control design, the reasonably large total number of cancers, and the strong priors. Some limitations should be acknowledged. First, a unique measurement of biomarkers at baseline was performed, and no indication was available regarding transient acute infection (cold, throat infection, etc.) concomitant to blood draw. For some biomarkers such as hs-CRP, although the probability of differential bias between cases and controls is low, this limitation could lead to an attenuation of the strengths of observed associations because of intraindividual variation (51). This may have limited our ability to detect an association between hs-CRP and breast cancer, but conversely, this limitation is unlikely to explain the observed relation between hs-CRP and prostate cancer risk, which was statistically significant despite the potential attenuation of odds ratios. Besides, information on intraclass correlation coefficients

over time is available in the literature for each studied biomarker measured in plasma samples. The reported intraclass correlation coefficients over time were relatively high (0.59 for hs-CRP, 0.86 for E-selectin, 0.62 for sVCAM-1, 0.64 for sICAM-1, 0.70–0.75 for MCP-1, 0.74–0.82 for leptin, and 0.81 for adiponectin), demonstrating that a single blood sample can be used in prospective epidemiologic studies for these biomarkers (52, 53).

Second, controls were selected among persons who had complete follow-up without cancer (and were alive) as of the study end date, without matching on follow-up time. Thus, odds ratios should not be directly extrapolated as rate ratios in our study, since the hypothesis of stability of the exposure distribution over time was probably not fully respected (54). In addition, this may have contributed to driving risk estimates away from the null if the studied biomarkers have causal deleterious effects on the risk of mortality. However, among subjects who did not develop cancer during follow-up in our cohort (i.e., potential controls), the mortality rate (1.1%) and the rate of loss to follow-up (5.2%) were relatively low, limiting the potential for bias.

Next, while the number of total cancers was reasonably large, the number of cancers at any given site was relatively small. This represents a limitation because of heterogeneity in associations by cancer site. In addition, there may be heterogeneity of association even within a cancer site (e.g., localized vs. advanced prostate cancer (42)), but the number of cases in the current study was too small to allow for such a stratified analysis.

Lastly, observed relations could be partly affected by unmeasured or residual confounding. However, a broad range of usual risk factors was accounted for, including specific adjustment factors depending on cancer location.

Our study adds to the current knowledge on inflammation-, adiposity-, and endothelial function-related pathways to development of cancer. For the first time, we have shown a prospective positive association between plasma sICAM-1 level and breast cancer risk. In addition, we observed a positive relation between prediagnostic hs-CRP level and prostate cancer risk, which provides new insights in a context of conflicting literature. Large prospective studies are needed to confirm the pertinence of these biomarkers in cancer risk prediction. If these results are confirmed in validation studies, this could lead to better identification of persons at risk of developing cancer and result in more efficiently targeted cancer screening campaigns.

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Pre-diagnostic levels of adiponectin and soluble vascular cell adhesion molecule-1 are associated with colorectal cancer risk

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Abstract

AIM: To examine the relationships between pre-diagnostic biomarkers and colorectal cancer risk and assess their relevance in predictive models.

METHODS: A nested case-control study was designed to include all first primary incident colorectal cancer cases diagnosed between inclusion in the SUpplémentation en VItamines et Minéraux AntioXydants cohort in 1994 and the end of follow-up in 2007. Cases (n = 50) were matched with two randomly selected controls (n = 100). Conditional logistic regression models were used to investigate the associations between pre-diagnostic levels of hs-CRP, adiponectin, leptin, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1, E-selectin, monocyte chemoattractant protein-1 and colorectal cancer risk. Area under the receiver operating curves (AUC) and relative integrated discrimination improvement (RIDI) statistics were used to assess the discriminatory potential of the models.

RESULTS: Plasma adiponectin level was associated with decreased colorectal cancer risk (P for linear trend = 0.03). Quartiles of sVCAM-1 were associated with increased colorectal cancer risk (P for linear trend = 0.02). No association was observed with any of the other biomarkers. Compared to standard models with known risk factors, those including both adiponectin and sVCAM-1 had substantially improved performance for colorectal cancer risk prediction (P for AUC improvement = 0.01, RIDI = 26.5%).

CONCLUSION: These results suggest that pre-diagnostic plasma adiponectin and sVCAM-1 levels are associated with decreased and increased colorectal cancer risk, respectively. These relationships must be conf rmed in large validation studies.

Key words: Colorectal cancer; Adiponectin; Soluble vascular cell adhesion molecule-1; Nested case-control study;

Prospective study

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INTRODUCTION

Colorectal cancer is the third most frequently diagnosed cancer worldwide, accounting for more than one million cases and 600 000 deaths every year[1]. The identification of pre-diagnostic biomarkers associated with subsequent colorectal cancer risk is a key challenge. Markers of adiposity, endothelial adhesion, and inflammation may be suitable candidates[2-5]. Adipose tissue is an endocrine organ that produces adipokines and plays a critical role in the regulation of inflammatory processes[6]. Leptin reflects body fat storage and acts as a pro-inflammatory adipokine. Conversely, adiponectin production is decreased in obesity and generally has anti-inflammatory properties. Adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and the chemokine monocyte chemoattractant protein-1 (MCP-1) are important in cell-cell and cell-basement membrane interactions. They are also intimately involved in inflammatory reactions[7]. C-reactive protein (CRP) is a widely used systemic biomarker for diagnosing acute and chronic inflammation[8].

Previous cross-sectional studies suggest the potential involvement of these biomarkers in colorectal carcinogenesis, with higher blood levels of CRP[9], leptin[10], soluble adhesion molecules[11,12], and lower levels of adiponectin[10,13] observed in patients with colorectal cancer compared to controls. The prognostic value of these markers has also been suggested by research with colorectal cancer patients[10,12]. However, few prospective studies have investigated the association between these biomarkers and colorectal cancer risk, and the current evidence is conflicting[14-19]. In addition, such studies did not evaluate the discriminatory capabilities of these biomarkers regarding colorectal cancer risk by contemporary statistical methods[20,21].

Thus, our objectives were twofold: (1) to prospectively examine the relationships between biomarkers of adiposity, endothelial adhesion, and inflammation and development of colorectal cancer; and (2) to statistically compare the pertinence of models including these biomarkers to standard models with known risk factors of colorectal cancer.

MATERIALS AND METHODS

Study population

The SUpplémentation en VItamines et Minéraux AntioXydants (SU.VI.MAX) study is a population-based, double-blind, placebo-controlled, randomized trial initially designed to assess the effect of a daily antioxidant supplementation on the incidence of cardiovascular disease and cancer[22,23]. A total of 13 017 subjects were enrolled in 1994-1995. The intervention study lasted 8 years, and follow-up of health events was maintained until July 2007. Subjects provided written informed consent and the study was approved by the Ethics Committee for Studies with Human Subjects at the Paris-Cochin Hospital, "Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale", No. 706 and the "Commission Nationale de l'Informatique et des Libertés", No. 334641.

Baseline data collection

At enrolment, all participants underwent a clinical examination and anthropometric measurements carried out by study nurses and physicians. The participants also completed questionnaires on socio-demographic data, smoking, alcohol intake and physical activity. A fasting venous blood sample was obtained. Plasma aliquots were immediately prepared and stored frozen in liquid nitrogen.

Case ascertainment

Confirmed or suspected cancer events were self-reported by subjects during the follow-up process. Investigations were conducted for all such events to obtain medical data from participants, physicians and/or hospitals. All information was reviewed by an independent expert committee and cancer cases were validated by pathological report and classified using the International Chronic Diseases Classification, 10th Revision, Clinical Modification.

Nested case-control study

All first primary incident colorectal cancer cases diagnosed between inclusion in the SU.VI.MAX cohort in 1994 and July 2007 were included in the present study. For each cancer case, two controls were randomly selected among the remaining participants with complete follow-up data and without cancer diagnosis by the end of follow-up. Cases and controls were matched for sex, age (by 2-year strata), body mass index (BMI, $< vs \geqslant 25$ kg/m^2) and intervention group.

Baseline plasma samples of the selected subjects were used to determine the levels of highly-sensitive CRP (hs-CRP), leptin, adiponectin, soluble ICAM-1 (sICAM-1), soluble VCAM-1 (sVCAM-1), soluble E-selectin (sE-selectin) and MCP-1. Biomarker levels were determined with ELISA sandwich technique (R and D Laboratory Systems). Intra-assay (IACV) and inter-assay (IRCV) co-

Table 1 Baseline characteristics of colorectal cancer cases and controls

	Cases (n = 50)		Controls (n = 100)		P value[1]
Age, yr	51.8	± 5.6	52.1	± 5.6	0.8
Gender					1.0
Men	28	56.0%	56	56.0%	
Women	22	44.0%	44	44.0%	
Intervention group					1.0
Yes	27	54.0%	54	54.0%	
No (placebo)	23	46.0%	46	46.0%	
BMI, kg/m²					1.0
< 25	24	48.0%	48	48.0%	
⩾ 25	26	52.0%	52	52.0%	
Waist circumference, cm	88.2	± 12.9	82.2	± 12.1	0.01
Height, cm	169.3	± 7.1	167.8	± 8.5	0.3
Smoking status					0.9
Never smoker	23	46.0%	46	46.0%	
Former smoker	21	42.0%	40	40.0%	
Current smoker	6	12.0%	14	14.0%	
Alcohol intake, g/d	24	± 24.4	15.4	± 16.3	0.01
Physical activity					0.4
Low	10	20.0%	26	26.0%	
Moderate	18	36.0%	25	25.0%	
High	22	44.0%	49	49.0%	
Educational level, yr					0.9
< 12	30	60.0%	61	61.0%	
⩾ 12	20	40.0%	39	39.0%	
Family history of colorectal cancer[2]					
No	45	90.0%	84	84.0%	0.3
Yes	5	10.0%	16	16.0%	
Plasma levels of biomarkers					
Adiponectin, μg/mL	9.0	± 4.7	10.9	± 7.5	0.2
Leptin, ng/mL	8.5	± 5.3	8.6	± 8.7	0.5
sVCAM-1, ng/mL	750.3	± 316.2	677.6	± 215.3	0.2
sICAM-1, ng/mL	249.7	± 80.3	247.8	± 67.3	0.9
sE-selectin, ng/mL	41.1	± 16.9	39.3	± 16.0	0.7
MCP-1, pg/mL	268.2	± 117.4	249	± 78.2	0.3
hs-CRP, mg/L	2.4	± 4.5	2.2	± 4.4	0.3

[1]P value for the comparison of cases and controls by Student t test or χ^2 test, as appropriate. Biomarker variables were log-transformed to improve normality. Values are mean ± SD or n % as appropriate. [2]In first degree relatives. BMI: Body mass index; hs-CRP: Highly sensitive C-reactive protein; sICAM-1: Soluble intercellular adhesion molecule-1; sVCAM-1: Soluble vascular cell adhesion molecule-1; sE-selectin: Soluble E-selectin; MCP-1: Monocyte chemoattractant protein-1.

efficients of variation were all < 10%. hs-CRP had the lowest (1.6%) and MCP-1 had the highest (6.2%) IACV, and hs-CRP had the lowest (3.6) and sE-selectin had the highest (9.1%) IRCV.

Statistical analyses

The participants' baseline characteristics were compared between colorectal cancer cases and controls using Student's t-tests or χ^2 tests. Associations between biomarkers and incident colorectal cancer were examined with conditional logistic regression models and expressed as odds ratios (OR) with 95% confidence intervals (CI). The ORs for sex-specific quartiles and for a 1 standard deviation (SD) increase in the corresponding biomarker were computed in unadjusted and multivariate models. Multivariate models were adjusted for age, sex, BMI, height, intervention group, alcohol intake, physical activity, smoking status, family history of colorectal cancer, waist circumference and educational level.

The improvement in colorectal cancer prediction performance attributed to the biomarkers was assessed with both the area under the receiver operating curves (AUC) and the more recently proposed statistical tool, the Relative Integrated Discrimination Improvement (RIDI)[21]. The latter measures the percentage of increased discrimination upon addition of another variable to the prediction model. The Bootstrap method was used to derive the 95% CI for the RIDI estimates, which were based on 1000 replications. The added prediction performance was determined separately for each biomarker identified as statistically significantly associated with cancer risk (in the logistic regression analyses step), and then for a combination of these biomarkers simultaneously. Tests of significance for AUC improvement were one-sided, as improvement in model fit was expected. All other statistical tests were two-sided, and $P < 0.05$ was considered significant. Analyses were performed with SAS software (v9.1, Cary, NC, United States).

RESULTS

A total of 50 incident colorectal cancer cases were diagnosed during follow-up (30 colon and 20 rectal cancers). Each case was matched with two randomly selected controls; thus, 150 subjects were included in the analyses. Median follow-up was 6.5 years in cases and 13 years in controls. Baseline characteristics of cases and non-cases are presented in Table 1. Compared to controls, cancer cases had a higher waist circumference and a higher alcohol intake.

In multivariate models, a one SD change in plasma adiponectin level was associated with a decreased colorectal cancer risk [OR (95% CI) = 0.45 (0.22-0.91), P = 0.03]. This association was also observed when adiponectin was considered as quartiles (OR for Q4 *vs* Q1 = 0.11 (0.01-0.93), P for linear trend = 0.03) (Table 2).

Quartiles of plasma sVCAM-1 level were positively associated with increased colorectal cancer risk (P for linear trend = 0.02) (Table 2). This association was borderline non-significant when sVCAM-1 was coded as a continuous variable (P = 0.07).

Unadjusted models (matching factors only) showed similar results (data not shown). A sensitivity analysis excluding cases that were diagnosed during the first two years of follow-up (7 cases) did not modify the findings, nor did sensitivity analyses excluding subjects with high hs-CRP values (> 15.5 ng/mL, i.e., mean + 3SD, n = 3 subjects; data not shown).

Indicators of the predictive potential of colorectal cancer risk models (Table 3) showed improvement when adiponectin alone was included in the multivariate model

Table 2 Odds ratios and 95% confidence intervals for quartiles of each biomarker level and colorectal cancer risk from multivariate conditional logistic regression models[1]

	For a change in 1SD	Quartile1	Quartile2	Quartile3	Quartile4
Adiponectin					
OR	0.45	1 (ref)	0.83	0.42	0.11
95% CI	0.22-0.91		0.12-5.65	0.06-2.93	0.01-0.93
P for linear trend	0.03				0.03
Leptin					
OR	0.55	1 (ref)	0.19	2.22	0.29
95% CI	0.21-1.40		0.02-1.9	0.25-20.09	0.02-3.65
P for linear trend	0.2				0.6
sVCAM-1					
OR	1.69	1 (ref)	6.89	11.59	19.11
95% CI	0.96-2.98		0.72-66.4	0.64-209.81	1.4-261.27
P for linear trend	0.07				0.02
sICAM-1					
OR	0.74	1 (ref)	0.38	0.07	0.13
95% CI	0.40-1.40		0.04-3.23	0.01-0.76	0.01-1.93
P for linear trend	0.4				0.08
sE-selectin					
OR	0.95	1 (ref)	1.23	0.9	1.59
95% CI	0.49-1.81		0.13-11.49	0.1-8.14	0.16-15.62
P for linear trend	0.9				0.9
MCP-1					
OR	1.35	1 (ref)	1.67	1.02	2.02
95% CI	0.73-2.49		0.27-10.24	0.17-6.24	0.26-15.97
P for linear trend	0.3				0.4
hs-CRP					
OR	0.8	1 (ref)	0.73	2.22	1.53
95% CI	0.52-1.24		0.06-9.38	0.25-19.9	0.13-17.84
P for linear trend	0.3				0.6

[1]Adjusted for age, sex, body mass index, intervention group, alcohol intake, physical activity, smoking status, family history of colorectal cancer, waist circumference, height and educational level. n = 50 colorectal cancer cases and 100 controls. Cut-offs for sex-specific quartiles were: hs-CRP: 0.6, 1.2, 2.3 in men and 0.5, 0.9, 2.1 in women; sICAM-1: 198.7, 242.0, 287.4 in men and 193.0, 232.5, 286.0 in women; sVCAM-1: 539.0, 653.5, 798.6 in men and 523.0, 651.5, 875.7 in women; sE-selectin: 29.8, 42.5, 51.7 in men and 26.0, 36.3, 44.6 in women; MCP-1: 216.7, 263.5, 316.5 in men and 171.0, 212.0, 238.0 in women; Leptin: 3.1, 5.0, 8.2 in men and 5.4, 9.6, 15.4 in women; Adiponectin: 4.2, 6.6, 10.0 in men and 9.5, 13.9, 16.0 in women. OR: Odds ratio; CI: Confidence interval; hs-CRP: Highly sensitive C-reactive protein; sICAM-1: Soluble intercellular adhesion molecule-1; sVCAM-1: Soluble vascular cell adhesion molecule-1; sE-selectin: Soluble E-selectin; MCP-1: Monocyte chemoattractant protein-1; ref: Reference category.

(*P* for AUC improvement = 0.009). The RIDI statistic indicated a 12.2% (10.9-13.6) improvement. Improvement in the prediction of colorectal cancer risk was limited when sVCAM-1 only was introduced into the multivariate model (*P* for AUC improvement = 0.09), with 9.9% (8.7-11.0) improvement, as indicated by the RIDI statistic. Prediction was substantially improved when adiponectin and sVCAM-1 were simultaneously included in the multivariate model: *P* for AUC improvement was equal to 0.01, and the RIDI reached 26.5% (24.4-28.7).

DISCUSSION

In this prospective study, pre-diagnostic plasma adiponectin level was associated with decreased colorectal cancer risk, independently of other known risk factors. On the contrary, plasma sVCAM-1 level was associated with increased colorectal cancer risk. Models including these two biomarkers showed significantly improved discriminatory capabilities compared to models including only established risk factors.

Lower levels of circulating adiponectin have been observed in prevalent colorectal cancer cases compared to controls[10,13,24-26]. Single nucleotide polymorphism analyses have found that some variants of the adiponectin genes are related to either increased (rs822395, rs1342387) or decreased (rs266729) colorectal cancer risk[27], although no association was detected in a recent study in the United Kingdom[28]. Another study suggested that variants of the adipokine genes may affect colorectal cancer risk in combination with variants in diabetes-related genes[29]. Studies with colorectal cancer patients showed that higher adiponectin levels were associated with a better prognosis[10,13,30]. It has been suggested that adiponectin may be used for estimation of advanced stage of cancer and for estimating risk of cancer recurrence[31]. However, to date, only three nested case-control studies have investigated the prospective association between adiponectin and colorectal cancer risk, showing inconsistent results[16-18]. Two studies did not find any associations; one of them included 381 male colorectal cancer cases[17] and the other included 306 colorectal cancer cases of both genders[16]. Consistent with our findings, the study of Wei *et al*[18], based on 179 male colorectal cancer cases, found an

Table 3 Predictive potential of adiponectin and soluble vascular cell adhesion molecule-1 regarding colorectal cancer risk: Relative integrated discrimination improvement and improvement of area under the curve

	AUC	*P* value for AUC improvement	RIDI (%)	95% CI
Multivariate model[1]	0.89			
+ Adiponectin	0.98	0.009	12.2	10.9-13.6
+ sVCAM-1	0.92	0.09	9.9	8.7-11.0
+ Adiponectin + sVCAM-1	0.98	0.01	26.5	24.4-28.7

[1]Multivariate model was adjusted for age, sex, BMI, intervention group, alcohol intake, physical activity, smoking status, family history of colorectal cancer, waist circumference, height and educational level. Models including adiponectin and/or sVCAM-1 were compared to the multivariate model. n = 50 colorectal cancer cases and 100 controls. BMI: Body mass index; RIDI: Relative integrated discrimination improvement; AUC: Area under the receiver operating curve; sVCAM-1: Soluble vascular cell adhesion molecule-1; CI: Confidence interval.

inverse association between pre-diagnostic adiponectin levels and colorectal cancer risk. Circulating levels of adiponectin in those studies were comparable to the levels found in the present study. However, none of those three studies matched cases and controls on BMI. Adiponectin is strongly related to adiposity, which is, in turn, associated with an adverse effect on colorectal cancer development, especially in stathmin-positive patients, as recently shown by Ogino *et al*[32]. Thus, matching on BMI is crucial and is a strength of our study compared to previous reports in the literature. Several mechanisms support the inverse relationship between adiponectin and colorectal cancer risk[33]. Adiponectin suppresses tumorigenesis in Apc(Min)(/+) mice[34] and also suppresses colonic epithelial proliferation *via* inhibition of the mammalian target of the rapamycin (mTOR) pathway under a high-fat diet[35]. It inhibits colorectal cancer cell growth through the AMP-activated protein kinase/mTOR pathway[36] and possibly the PI3K/Akt signal pathway[37]. Adiponectin also attenuates interleukin-6-induced colon carcinoma cell proliferation *via* STAT-3[38].

Several case-control studies have observed higher circulating levels of sVCAM-1 in colorectal cancer cases compared to controls[11,12,39-41]. In addition, it has been suggested that the serum level of sVCAM-1 may be a valuable prognostic marker in colorectal carcinoma[12,42], reflecting both tumour progression and metastasis[39]. For instance, Mantur *et al*[11] observed a significant correlation of serum levels of sVCAM-1 with tumor, node, metastases (TNM) stage and lymph node involvement in colorectal cancer patients. Yamada *et al*[43] observed a positive association between concentrations of sVCAM-1 and risk of postoperative colorectal cancer recurrence. Consequently, investigations have been conducted to test for the chemopreventive potential of some molecules (e.g., celecoxib) *via* down-regulation of VCAM-1 in the colon cancer cell line HT29[44].

However, to the best of our knowledge, our study is the first to investigate the prospective association between pre-diagnostic levels of sVCAM-1 and colorectal cancer risk. The observed positive association is supported by a mechanistic plausibility. Indeed, it has been demonstrated experimentally that sVCAM-1 stimulates angiogenesis and neovascularization[45,46] and is negatively correlated with the degree of tumour differentiation[41]. Cell adhesion molecule expression has been demonstrated in endothelial cells of small vessels at the invasive margin of tumour cells involved in metastatic spread[47]. The association among immunohistochemical cell adhesion molecule expression, tumour vascularity and leukocyte infiltration suggests an important role for these molecules in host immune response and in tumour progression[48].

Epidemiologic studies usually estimate the strength of the association between a biomarker and disease risk. Assessment of the discriminatory capabilities of a biomarker in predicting risk of the studied pathology is another approach that may lead to slightly different but complementary information[21]. To the best of our knowledge, no study has previously evaluated the discriminatory capabilities of hs-CRP, leptin, adiponectin, sICAM-1, sVCAM-1, sE-selectin and MCP-1 in predicting colorectal cancer risk, using *ad-hoc* statistical methods such as the novel RIDI statistic[21]. Indeed, the use of the traditional AUC method as a comparative measure of prediction between models has certain limitations[49], and the complementary use of the novel RIDI statistic appears to be more sensitive and accurate[21]. Several factors are already known to influence colorectal cancer risk (e.g., age, smoking status, physical activity, *etc.*) and are usually included in predictive models. As shown in Table 3, the RIDI statistic suggests that when quartiles of adiponectin and quartiles of sVCAM-1 plasma levels are added to the model, the ability of the model to predict colorectal cancer risk is improved by 26.5%, compared to a model including only well-established risk factors (age, smoking status, *etc.*). Thus, our results suggest that adiponectin, and possibly sVCAM-1, should not be ignored as predictors of colorectal cancer risk. In addition, the improvement in the predictive potential was substantially increased when both biomarkers were simultaneously added to the model. This might result from the mechanistic interrelations between adiposity and endothelial adhesion, notably though an inflammation pathway[6,50,51]. Large prospective and validation studies are needed to confirm and better quantify the predictive performance of these biomarkers in colorectal carcinogenesis.

Strengths of our study include its prospective design, the simultaneous measurement of seven biomarkers in the same individuals and, to our knowledge, the first assessment of the discriminatory capabilities of these biomarkers for estimating colorectal cancer risk by the novel RIDI statistic.

Some limitations should also be acknowledged. Firstly, the number of cases was limited in this exploratory study. This may explain some of the null results observed; however, it is unlikely to explain the observed relationships between adiponectin, sVCAM-1 and colorectal cancer

risk, which were statistically significant despite the limited statistical power. These associations are consistent with our initial hypothesis and are supported by available mechanistic data. Secondly, a single measurement of biomarker levels (at baseline) was performed and no indication was available regarding transient acute infection (cold, throat infection, *etc.*) concomitant with the blood draws. For some biomarkers such as hs-CRP, although the probability of differential misclassification bias between cases and controls is low, this limitation might have led to an attenuation of the strengths of the observed associations due to intra-individual variation. This may have limited our ability to detect an association between hs-CRP and colorectal cancer. Finally, the observed relationships might have been partly affected by unmeasured or residual confounders, even though such a possibility is limited since a broad range of usual risk factors were accounted for in the statistical analyses.

Our study adds to current knowledge of adiposity- and endothelial adhesion-related pathways in the development of colorectal cancer. For the first time, we have shown a prospective positive association between plasma sVCAM-1 levels and colorectal cancer risk. In addition, we observed an inverse relationship between pre-diagnostic adiponectin levels and colorectal cancer risk, which provides new insights given the conflicting literature. Our results suggest that the inclusion of adiponectin and sVCAM-1 plasma levels in prediction models of colorectal cancer risk may improve their discriminatory capabilities. Large prospective studies are needed to confirm the pertinence of these biomarkers in colorectal cancer risk prediction. If confirmed in validation studies, these results could lead to improved identification of individuals at risk of developing colorectal cancer, which could result in well-targeted cancer screening campaigns.

COMMENTS

Background

Previous studies suggest an association between biomarkers of adiposity, endothelial adhesion and inflammation and colorectal cancer risk, but prospective data are limited and evaluation of predictive performance is lacking.

Research frontiers

Previous cross-sectional and case-control studies have suggested the potential involvement of such biomarkers in colorectal carcinogenesis, with higher blood levels of soluble adhesion molecules and lower levels of adiponectin in patients with colorectal cancer compared to controls. The prognostic value of these markers has also been suggested. Studies on single nucleotide polymorphisms further indicate that these markers may affect cancer risk. However, few prospective studies have investigated the association between these biomarkers and colorectal cancer risk, often providing conflicting evidence.

Innovations and breakthroughs

This work shows a prospective positive association between plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) levels and colorectal cancer risk, which has not been investigated previously. In addition, the authors observed an inverse relationship between pre-diagnostic adiponectin levels and colorectal cancer risk, which provides new insights given the conflicting literature. The inclusion of adiponectin and sVCAM-1 plasma levels in prediction models of colorectal cancer risk improved their discriminatory capabilities.

Applications

This study adds to current knowledge of adiposity- and endothelial adhesion-related pathways in the development of colorectal cancer. If confirmed in large validation studies, these results could lead to improved identification of individuals at risk of developing colorectal cancer, which could result in well-targeted cancer screening campaigns.

Terminology

Leptin reflects body fat storage and acts as a pro-inflammatory adipokine. Conversely, adiponectin production is decreased in obesity and generally has anti-inflammatory properties. Adhesion molecules such as E-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and the chemokine monocyte chemoattractant protein-1 are important in cell-cell and cell-basement membrane interactions. C-reactive protein is a widely used systemic biomarker for diagnosing acute and chronic inflammation.

Peer review

The authors describe the potential role of two biomarkers in the diagnosis of colorectal cancer using a prospective study cohort initiated almost 18 years ago. The availability of this cohort and the derived material is a major strength of the study; even though a limited number of cases developed and were available for analysis. The study design and analytical work is not questionable, and the statistical analysis is "state of the art".

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Prognostic value of multiple emerging biomarkers in cardiovascular risk prediction in patients with stable cardiovascular disease

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Prognostic value of multiple emerging biomarkers in cardiovascular risk prediction in patients with stable cardiovascular disease

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Background: Few studies have examined simultaneously the prognostic value of traditional and emerging biomarkers including atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP), for major cardiovascular disease (CVD) outcomes in patients with stable CVD, and results are equivocal.
Design: and Methods: Mid-regional pro-ANP (MR-proANP) and N-Terminal pro-BNP (NT-proBNP), CRP and homocysteine were measured in stable CVD patients (n ¼ 1456; age: 61.8 y) at inclusion in the SU.FOL.OM3 cohort. Prospective association of biomarkers with risk of heart failure, major cardiovascular (non-fatal myocardial infarction, ischemic stroke or death from CVD) or overall cardiovascular event were examined with Cox proportional-hazards analyses. Increase in prediction risk upon addition of biomarker(s) to the traditional risk model was examined by change in C-statistic, NRI and IDI.
Results: During follow-up (median: 4.7 y), 40 heart failure, 145 major cardiovascular and 493 overall cardiovascular events were diagnosed. In models adjusted for age, sex, smoking, diabetes, serum creatinine and CVD inclusion criteria, NT-proBNP and CRP associated significantly with heart failure. Both natriuretic peptides predicted the risk of major cardiovascular events in adjusted models; Hazard ratio (HR) and 95%CI for each SD increase in MR-proANP and NT-proBNP were 1.24 (1.04e 1.47), and 1.31 (1.09e 1.57), respectively. The addition of NT-proBNP to a traditional risk model increased significantly the area-under-curve for heart failure and overall cardiovascular events (by 6 and 12% respectively); addition of MR-proANP or homocysteine yielded modest (2%) but statistically significant increase for major cardiovascular events.
Conclusion: NT-proBNP consistently predicted CVD outcomes and may be useful singly or in combination with MR-proANP for risk-stratification in high-risk patients.

1. Introduction

New avenues for prevention of cardiovascular disease (CVD) including better diagnosis and risk evaluation are of increasing interest in high-risk groups. In this regard, novel biomarkers such as natriuretic peptides namely atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP) as well as C-reactive protein (CRP) and homocysteine are of interest.

ANP and BNP are vasoactive cardiac peptide hormones with natriuretic, diuretic, and vasodilator activity [1], that could be important diagnostic and prognostic tools for CVD and related mortality in general population and coronary heart disease (CHD) patients [2e8]. Few studies have simultaneously evaluated the prognostic value of both natriuretic peptides (proANP and proBNP) over and above conventional cardiovascular risk factors, beyond the period of hospitalization after an acute CVD event (MI, left ventricular systolic dysfunction and chronic heart failure) [9,10]. In addition, newer assays targeting more stable epitopes of ANP, such as mid-regional pro-ANP (MR-proANP) have become available that could offer more refined risk assessment [11]. In clinical practice established tests such as CRP and homocysteine are often considered in CVD risk assessment. CRP is commonly determined using routinely available assays as a systemic inflammatory marker [12]. Although the evidence linking elevated homocysteine and CVD risk

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is inconsistent [13,14], recent promising findings from NHANES III and Multi-Ethnic Study of Atherosclerosis (MESA) studies showing significant improvement in risk prediction for future CVD and CHD events in intermediate-risk patients upon addition of homocysteine to the Framingham risk model [15], has re-sparked interest in this marker for CVD risk assessment. The current study, thus, evaluated the comparative prognostic value of four biomarkers (natriuretic peptides MR-proANP and NT-proBNP, CRP and homocysteine) alone and in combination, in addition to conventional risk factors, in patients with stable CVD, in whom information on predictive risk has not been extensively evaluated using emerging biomarkers [9,16].

2. Methods

2.1. Study design

The SU.FOL.OM3 trial is a multicenter, double-blind randomized controlled trial (RCT) that evaluated the separate and combined effects of daily supplementation with B-vitamins, and/or n-3 polyunsaturated fatty acid for prevention of CVD [17]. Participants (45e80 y) were recruited via a nationwide network of 417 cardiologists, neurologists or other physicians. Those meeting the CVD inclusion criteria of a history of ischemic stroke or other coronary event i.e. acute coronary syndrome with or without MI with or without ST segment elevation within 1e12 months (mean: 4 months) were assessed at baseline, randomized to receive an active treatment or relevant placebo and followed (median: 4.7 y) [17]. Exclusion criteria included age (< 45 years or > 80 years), ill-defined diagnosis of cardiovascular disease, inability or unwillingness to comply with study treatment, and disease or treatment that might interfere with metabolism of homocysteine or omega 3 fatty acids, in particular methotrexate for treating cancer or rheumatoid arthritis and chronic renal failure (plasma creatinine concentration > 200 mmol/l or creatinine clearance < 40 ml/min).

The protocol was approved by the ethical committee "Comité Consultatif pour la Protection des Personnes se prêtant à la Recherche Biomédicale" (CCPPRB no. 1933) of Paris-Cochin, and the data protection board "Comité National Informatique et Liberté" (CNIL no. 901230). Participants provided written informed consent following protocols approved by these committees. The present trial was registered as ISRCTN41926726 in Current Controlled Trials (http://www.controlled-trials.com/ISRCTN41926726).

2.2. Measurements

In the current study, post-hoc analyses were performed on a subset of 1456 subjects, selected randomly from 2501 participants in the SU.FOL.OM3 study. Blood samples were obtained at baseline assessment, after a 5 to 12-h fast, processed, and plasma stored at À80 °C. All biochemical measurements were analyzed in the same laboratory following standard procedures. Plasma MR-proANP concentrations were determined with a commercial kit using a Kryptor Brahms fluorescence immunoassay (Thermo Fisher Scientific, Clichy, France). NT-proBNP assay was performed by a Roche electrochemiluminescence immunoassay (Modular E, Roche Diagnostics, Meylan, France) using manufacturer's reagents and controls. High-sensitivity CRP assay was performed by an ultrasensitive immune-technique on the Siemens BNII analyser calibrated using reagents and controls provided by the manufacturer (Siemens Healthcare Diagnostics, Saint-Denis, France). Plasma homocysteine was measured by a competitive immunoassay with direct chemiluminescence detection (Siemens Healthcare Diagnostics limited, Camberley, UK). The intra-assay and inter-assay coefficients of variation for all analytes were between 1 and 6%.

Fasting plasma concentrations of glucose, total cholesterol, high density lipoprotein cholesterol (HDL), calculated low density lipoprotein cholesterol (LDL), triglyceride and creatinine concentrations were determined using standard laboratory methods.

Data on age, smoking status (current smokers, former smokers and non smokers), and medication use were collected at inclusion via questionnaires [17].

Body Mass Index (BMI in kg/m^2) was calculated using height and weight measured at baseline by trained staff following standard protocols.

Blood pressure (BP) was recorded at baseline by trained staff in the sitting position, after a 5-min rest, with a semi-automatic device (Digital blood pressure monitor OMROM UA-787) using standardized protocols. Two measurements were taken. In the case of a BP difference greater than 10 mmHg for either systolic BP or diastolic BP, BP was measured again after a 5-min rest. The last two BP values were averaged to interpret hypertension status.

Diabetes mellitus was defined as fasting glucose ! 7 mmol/l and/or use of antidiabetic drugs or insulin.

Finally, our population was 61,8 Æ 8,8 year old, 83% men, medically treated (92%were on antihypertensives, 94%antiplatelet agents, 87% hypolipemic agents and 12% antidiabetic treatment) patients in secondary cardiovascular prevention.

2.3. Outcomes

Three endpoints were examined in the present study: heart failure; major cardiovascular; and overall cardiovascular events. Heart failure events included heart failure and associated death. Major cardiovascular event was defined as a composite of non-fatal MI, ischemic stroke, or death from CVD. Overall cardiovascular events included any cardiovascular event including major cardiovascular events. The assessments of the outcomes are described in details [17]. Briefly, events were reported using questionnaires every six months, as well as information from the general practitioners, cardiologists, or neurologists treating the participants. Hospital discharge summaries and other clinical informations were gathered for all suspected cardiovascular and neurological events; and each event was discussed in a dedicated committee composed of three cardiologists or neurologists. All adjudications were performed by the committees, blinded to the treatment allocation.

2.4. Statistical analysis

Plasma MR-proANP, NT-proBNP, CRP, homocysteine as well as triglyceride were log transformed to achieve distributions consistent with normality. Baseline characteristics were compared between participants with and without cardiovascular endpoints using t-test or c^2 test as appropriate. Cox proportional-hazards regression models were used to study the associations between the biomarkers at baseline (comparing tertiles for each marker) and the risk of specific endpoints examined i.e. heart failure, major cardiovascular events, and overall cardiovascular events. Models were rerun adjusting for significant variables (age, diabetes status, smoking status, and plasma creatinine at baseline) as well as randomization group, sex and CVD inclusion criteria. In addition, Cox proportional-hazards regression models were run considering each biomarker as a continuous variable; hazard ratios (HR) and 95% confidence intervals (CI) were obtained for 1-sd increase. Proportional-hazards assumptions were confirmed by Schoenfeld's tests. Finally, estimates of C-index (with 95% CI) for the Cox proportional-hazards regression models were calculated [18], as well as IDI and NRI [19]. Difference in C-index, indicating improvement in area-under-the ROC curve (AUC), upon addition of biomarker(s), singly or in combination, to a model containing

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traditional CVD risk factors were estimated [20]. All analyses were performed using SAS with P < 0.05 considered statistically significant.

3. Results

The study involved 1456 subjects who were diagnosed with either acute coronary syndrome (31%), or acute MI (49.2%) or stroke (19.5%) at 1e12 months (mean: 4 mo) prior to inclusion in the study. Briefly, subjects were randomly assigned to receive either a placebo (23%) or a supplement (omega-3 fatty acids alone (25%), B-vitamins folic acid and B-12 alone (27%) or with omega-3 fatty acids (25%) [17]. The supplementation had no effect on the occurrence of CVD outcomes [17] nor on specific CVD endpoints examined in this study (heart failure, major or overall cardiovascular event) (Table 1). During the mean follow-up of 4.7 years, 40 cases of heart failure, 145 major events (58 MI, 51 stroke, and 36 deaths from CVD) and 493 cases of overall cardiovascular events (including 36 cases of acute coronary syndrome) were confirmed by study physicians.

Subjects who developed any of these three endpoints over study follow-up were older, more likely to have diabetes, and generally had higher serum total cholesterol and creatinine concentrations (Table 1). In addition, subjects who exhibited a major cardiovascular event were more likely to have had a stroke (at inclusion), and had other CVD risk factors (such as smoking, high systolic BP and high plasma triglycerides) as compared to those who did not develop a major event during follow-up (Table 1). Baseline concentrations of MR-proANP, NT-proBNP, CRP and homocysteine were significantly higher in subjects who developed (versus those who did not develop) heart failure or overall cardiovascular events over the 5 years follow-up. Homocysteine and NT-proBNP were significantly different while MR-proANP approached significance (p ¼ 0.05) in patients who had versus those who did not have major cardiovascular events over the follow-up (Table 1).

Table 1
Baseline characteristics of study participants by cardiovascular outcomes examined (Mean values Æsd or percentages).

Characteristics	Heart failure			Major events[a]			Overall cardiovascular events		
	Yes	No	p	Yes	No	p	Yes	No	p
N	40	1416		145	1311		493	963	
Randomization group (%)			0.75			0.83			0.72
B-vitamins þ omega-3	22.5	24.7		22.8	24.9		24.9	24.5	
Omega-3	27.5	24.9		27.6	24.7		24.5	25.2	
B-vitamins	32.5	27.0		25.5	27.3		25.7	27.8	
Placebo	17.5	23.4		24.1	23.1		24.7	22.4	
Age (years)	66.6 Æ8.3	61.6 Æ8.8	0.0005	63.9 Æ9.3	61.5 Æ8.8	0.002	62.4 Æ9.2	61.4 Æ8.7	0.04
Gender (%men)	85.0	83.2	0.76	81.4	83.4	0.53	83.6	83.1	0.81
Diabetes (%)	45.0	17.6	< 0.0001	26.2	17.5	0.01	23.0	16.0	0.002
Disease at inclusion (%)			0.94			< 0.0001			0.66
Stroke	20.0	19.5		36.5	17.6		18.9	19.8	
Coronary event	80.0	80.5		63.5	82.4		81.1	80.2	
Smoking (%)			0.97			0.02			0.24
Current smoker	10.0	10.5		17.4	9.7		11.8	9.8	
Former smoker	62.5	63.6		59.7	64.0		64.5	63.1	
Non-smoker	27.5	25.9		22.9	26.3		23.7	27.1	
Body mass index (Kg/m^2)	28.8 Æ5.3	27.5 Æ3.9	0.10	27.7 Æ4.3	27.5 Æ3.9	0.50	27.6 Æ4.1	27.4 Æ3.9	0.54
Blood pressure (mm Hg)									
Systolic	140.2 Æ25.0	133.4 Æ21.5	0.10	137.3 Æ23.7	133.2 Æ21.4	0.03	135.0 Æ22.1	132.9 Æ21.4	0.09
Diastolic	82.3 Æ13.3	82.9 Æ12.1	0.80	83.5 Æ12.7	82.8 Æ12.0	0.54	83.0 Æ12.4	82.8 Æ11.9	0.84
Total cholesterol (mmol/l)	4.6 Æ1.3	4.6 Æ1.1	0.82	4.8 Æ1.0	4.6 Æ1.1	0.02	4.7 Æ1.1	4.6 Æ1.1	0.01
LDL cholesterol (mmol/l)	2.8 Æ1.0	2.8 Æ0.8	0.87	2.9 Æ0.8	2.7 Æ0.8	0.06	2.8 Æ0.9	2.7 Æ0.8	0.004
HDL cholesterol (mmol/l)	1.2 Æ0.3	1.2 Æ0.3	0.46	1.2 Æ0.3	1.2 Æ0.3	0.51	1.2 Æ0.3	1.2 Æ0.3	0.84
Triglyceride (mmol/l)[b]	1.3 [1.1e 1.5]	1.3 [1.2e 1.3]	0.97	1.4 [1.3e 1.5]	1.3 [1.2e 1.3]	0.02	1.3 [1.2e 1.4]	1.2 [1.2e 1.3]	0.11
Fasting glucose (mmol/l)	5.9 [5.3e 7.6]	5.5 [5.1e 6.0]	0.001	5.6 [5.2e 6.2]	5.4 [5.0e 6.0]	0.05	5.5 [5.1e 6.2]	5.4 [5.0e 6.0]	0.01
Creatinine (mmol/l)	87.2 Æ21.1	80.1 Æ16.1	0.04	82.7 Æ20.7	80.1 Æ15.7	0.14	81.8 Æ17.4	79.6 Æ15.6	0.01
MR-proANP (pmol/l)[b]	30.0 [14.0e 65.0]	18.0 [10.0e 39.0]	0.02	24.0 [10.0e 61.0]	18.0 [10.0e 37.0]	0.05	20.0 [11.0e 44.0]	17.0 [10.0e 37.0]	0.01
NT-proBNP (ng/l)[b]	676.0 [355.0e 1824.0]	200.0 [94.0e 452.0]	< 0.0001	237.0 [105.0e 710.0]	206.5 [94.5e 458.0]	0.04	247.0 [121.5e 563.0]	194.5 [86.0e 432.0]	< 0.0001
Hs-CRP (mg/l)[b]	3.2 [1.4e 6.5]	1.7 [0.7e 4.0]	0.01	1.7 [0.8e 4.2]	1.7 [0.7e 4.0]	NS	2.1 [0.9e 5.0]	1.6 [0.7e 3.8]	0.002
Homocysteine (mmol/l)[b]	15.9 [13.3e 17.2]	12.9 [10.8e 15.8]	0.008	14.0 [11.8e 16.9]	13.0 [10.8e 15.8]	0.01	13.3 [11.2e 16.4]	12.9 [10.7e 15.6]	0.01

[a] Composite of non-fatal MI, ischemic stroke or death from cardiovascular disease.
[b] Median and 25th e 75th percentile in parenthesis; p value obtained from t-test on log-transformed data.

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Results from Cox proportional-hazards models on these four biomarkers comparing tertiles for the occurrence of the CVD endpoints examined were generally consistent with these findings (Fig. 1). Specifically, all four biomarkers were significantly predictive of risk of heart failure and overall cardiovascular event and homocysteine was predictive of major cardiovascular events. These findings were unchanged in models adjusted for age, sex, randomization group, smoking status, diabetes status, creatinine and CVD inclusion criteria, except that the associations of homocysteine with major and overall cardiovascular events became non-significant (data not shown).

When treated as continuous variables, all four biomarkers predicted significantly heart failure in crude models; in adjusted models, however, MR-proANP was no longer significant (p ¼ 0.07) (Table 2). Regarding major cardiovascular events, only homocysteine was significantly predictive in crude models, and the association for both natriuretic peptides approached significance (p ¼ 0.07 and p ¼ 0.05 for MR-proANP and NT-proBNP, respectively). In adjusted models, both natriuretic peptides emerged significantly associated with risk of major cardiovascular events; in contrast the association of homocysteine was no longer significant (Table 2). As noted for heart failure and major cardiovascular outcomes, the association of homocysteine with overall cardiovascular events did not remain significant after adjusting for CVD risk factors, creatinine and CVD inclusion criteria (Table 2).

In order to examine the incremental risk contribution of the four biomarkers (considered singly and in combination) to the traditional risk model, we also computed C-index [21] that reflects the area-under-the ROC curve (AUC) associated with the Cox models considering the biomarkers as continuous variables (Table 3). We also calculated NRI and IDI [19]. For heart failure, only for NT-proBNP the AUC increased significantly and strongly by 12%as compared to the traditional risk model; C-index and 95%CI were 0.83 (0.77e 0.90) versus 0.71 (0.62e 0.80), respectively. The addition of MR-proANP, CRP, or homocysteine to the traditional risk model did not significantly improve prediction. Similar results were obtained with both NRI and IDI. On the other hand, for major cardiovascular events, addition of MR-proANP, NT-proBNP, or homocysteine to the traditional risk model yielded a modest increase in the C-statistic (p ¼ 0.04, 0.08 and 0.03, respectively). However, NRI was not significant for both MR-proANP and NT-proBNP and IDI was not significant for homocysteine. Finally, adding the four biomarkers singly to the traditional risk model improved the prediction of risk for overall cardiovascular events by 5e 6%that was significant for NT-proBNP and CRP (C-index) and all four biomarkers (NRI and IDI).

Fig. 1. Kaplan Meier plots comparing tertiles of natriuretic peptides, CRP and homocysteine for cardiovascular events in patients with cardiovascular disease (crude analysis). Third tertile is shown in black, second tertile in gray and first tertile in light-gray. Hazard ratios for 3rd versus 1st tertile (up) and second versus first tertile (down) are indicated in the upper left corner of each plot. From top to bottom panels represent the following outcomes: (A) Heart failure; (B) Major cardiovascular events; and (C) Overall cardiovascular events.

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Table 2
Risk of heart failure and other cardiovascular events in relation to natriuretic peptides, CRP and homocysteine: Results from the Cox proportional-hazards models.

	Heart failure			Major cardiovascular events[a]			Overall cardiovascular events		
	Crude model	Adjusted model[b]	Adjusted model[c]	Crude model	Adjusted model	Adjusted model[3]	Crude model	Adjusted model	Adjusted model[c]
MR-proANP	1.43 (1.05e 1.96)[d] p ¼ 0.02	1.34 (0.97e 1.86) p ¼ 0.07	0.91 (0.65e 1.28) p ¼ 0.60	1.17 (0.99e 1.37) p ¼ 0.07	1.23 (1.04e 1.46) p ¼ 0.02	1.16 (0.95e 1.41) p ¼ 0.14	1.12 (1.03e 1.23) p ¼ 0.01	1.11 (1.02e 1.22) p ¼ 0.02	1.04 (0.93e 1.15) p ¼ 0.51
NT-proBNP	3.09 (2.25e 4.25) p < 0.0001	3.28 (2.29e 4.68) p < 0.0001	2.99 (2.00e 4.47) p < 0.0001	1.18 (1.00e 1.39) p ¼ 0.05	1.31 (1.09e 1.57) p ¼ 0.003	1.22 (0.98e 1.52) p ¼ 0.08	1.22 (1.12e 1.34) p < 0.0001	1.22 (1.10e 1.34) p < 0.0001	1.14 (1.02e 1.29) p ¼ 0.03
hs-CRP	1.48 (1.08e 2.03) p ¼ 0.01	1.42 (1.02e 1.98) p ¼ 0.04	1.18 (0.84e 1.68) p ¼ 0.34	1.01 (0.85e 1.19) p ¼ 0.94	0.95 (0.80e 1.13) p ¼ 0.58	0.89 (0.75e 1.06) p ¼ 0.19	1.16 (1.06e 1.28) p ¼ 0.001	1.13 (1.03e 1.24) p ¼ 0.007	1.10 (1.00e 1.21) p ¼ 0.05
Homocysteine	1.39 (1.10e 1.77) p ¼ 0.006	1.34 (0.99e 1.80) p ¼ 0.06	1.32 (0.91e 1.92) p ¼ 0.15	1.25 (1.08e 1.44) p ¼ 0.002	1.13 (0.96e 1.32) p ¼ 0.15	1.17 (0.99e 1.39) p ¼ 0.06	1.11 (1.02e 1.21) p ¼ 0.01	1.08 (0.98e 1.18) p ¼ 0.11	1.09 (0.99e 1.20) p ¼ 0.08

[a] Composite of non-fatal MI, ischemic stroke or death from cardiovascular disease.
[b] Adjusted for age, sex, supplementation group, smoking, diabetes, creatinine level at baseline and CVD inclusion criteria.
[c] Adjusted for age, sex, supplementation group, smoking, diabetes, creatinine level at baseline and CVD inclusion criteria and mutually adjusted for the 4 biomarkers.
[d] Hazards Ratios for 1-sd increase of the log transformed data. SD values were respectively 0.41, 0.51, 0.49 and 0.14 for MR-proANP, NT-proBNP, hs-CRP and homocysteine. Values in parentheses indicate 95% confidence intervals.

When combinations of biomarkers were examined in addition to the traditional risk model, following key findings were noted. Simultaneous addition of ANP and BNP to the traditional risk model yielded a significant increase in the C-index for all three CVD endpoints examined; increase in the AUC was 13% for heart failure; 3% for major cardiovascular events, and 6% for overall cardiovascular events. Addition of CRP and homocysteine (singly or in combination) to the model containing ANP and/or BNP along with the traditional risk model did not add any further improvement in risk prediction (Table 3; and data not shown). Similar results for combinations of biomarkers were obtained using both NRI and IDI.

4. Discussion

The comparative prognostic value of a panel of emerging biomarkers namely MR-proANP, NT-proBNP, CRP, and homocysteine for predicting cardiovascular risk was evaluated in this relatively large prospective study of stable CVD patients, who had a major CVD event on average 4 months prior to baseline assessment. Although studies have examined the improvement in CVD risk prediction using novel and classic biomarkers [5,10,22e25], most focused on individuals in early stages of CVD or following an acute CHD event. In addition, such studies generally assessed at most two of the four biomarkers determined in this study with few exceptions [9,16,24]. To our knowledge, this is the first report of simultaneous examination of both natriuretic peptides (MR-proANP and NT-proBNP), CRP, and homocysteine for prospective risk prediction and incremental risk assessment for these biomarkers (singly or in combination) over traditional risk factors, in patients with stable CVD who were followed over a fairly long period (median: 4.7 years).

The early phases of evaluation of novel markers in CVD risk [26] were assessed for four biomarkers: proof of concept that the biomarker levels differ between patients who develop or not develop outcome; prospective validation that the marker is predictive of outcome; and incremental value over clinical traditional risk variables. In the current study, generally, all four biomarkers were significantly higher in patients who developed (versus those who did not develop) the CVD outcomes. Furthermore, all four tests were associated with the prospective risk of one or more of the CVD endpoints examined. The measurement of NT-proBNP, however, emerged consistently associated with all three CVD endpoints namely heart failure, composite of major cardiovascular events (MI, stroke, or CVD-related death), or a large composite index of cardiovascular events even after adjustment for traditional risk factors. Overall, for every SD increase in NT-proBNP, there was 3-fold increase in risk of heart failure and 1.31- and 1.22-fold increase in the risk of major cardiovascular and overall cardiovascular events in this study cohort. This is consistent with previous reports in patients in early and stable phases of CVD [5,24,27e30]. The increase in risk estimates noted in the current study are in the same order as those reported for different CVD outcomes in a recent meta-analysis involving studies in general population and those with increased vascular risk and patients with stable CVD [7]. In addition, as compared to CRP, NT-proBNP was significantly and strongly associated with risk of heart failure and MR-proANP with risk of major events; these two natriuretic peptides offered significant incremental risk prediction (increase in the AUC for these endpoints by 12 and 2% respectively) compared to the traditional risk model. Limited predictive value of CRP in CVD patients noted in the current study is consistent with findings from previous reports [5,24,27,31].

The literature on ANP in secondary risk prediction for CVD is relatively limited (as compared to other markers) particularly in patients with stable CVD [9,10,16]. In a report concerning the

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Table 3
. Overall C index (95%CI), NRI (sd) and IDI (sd) for Cox proportional-hazards regression models (top-down).

Model	Heart failure	p[a]	Major cardiovascular events	p	Overall cardiovascular events	p
Model 1 (age, sex, supplementation group, smoking, diabetes, creatinine and CVD inclusion criteria)	0.71 (0.62e 0.80)[b]	Ref	0.64 (0.59e 0.69)	Ref	0.54 (0.51e 0.57)	Ref
Model 1. þ MR-proANP	0.73 (0.65e 0.82)	0.13	0.66 (0.61e 0.71)	0.04	0.55 (0.52e 0.58)	0.09
	0.02 (0.07)[c]	0.79	0.04 (0.04)	0.33	0.05 (0.02)	0.01
	0.004 (0.004)[d]	0.30	0.006 (0.003)	0.04	0.004 (0.002)	0.03
Model 1 þ NT-proBNP	0.83 (0.77e 0.90)	0.0008	0.66 (0.61e 0.71)	0.08	0.56 (0.53e 0.59)	0.01
	0.36 (0.11)	0.001	0.04 (0.04)	0.33	0.06 (0.02)	0.02
	0.05 (0.01)	0.0004	0.008 (0.003)	0.01	0.007 (0.002)	0.002
Model 1 þ CRP	0.73 (0.64e 0.81)	0.33	0.64 (0.59e 0.69)	0.16	0.56 (0.53e 0.58)	0.003
	0.07 (0.08)	0.37	À0.02 (0.01)	0.09	0.09 (0.02)	0.0005
	0.006 (0.003)	0.05	0.0001 (0.0005)	0.81	0.005 (0.002)	0.01
Model 1 þ homocysteine	0.72 (0.63e 0.81)	0.17	0.66 (0.61e 0.71)	0.03	0.55 (0.52e 0.58)	0.08
	0.02 (0.06)	0.78	0.07 (0.04)	0.04	0.05 (0.02)	0.01
	0.003 (0.002)	0.12	0.003 (0.002)	0.27	0.004 (0.002)	0.04
Model 1 þ MR-proANP þ NT-proBNP	0.83 (0.77e 0.90)	0.0009	0.67 (0.62e 0.71)	0.02	0.56 (0.53e 0.59)	0.01
	0.36 (0.11)	0.001	0.11 (0.04)	0.01	0.07 (0.03)	0.007
	0.06 (0.02)	0.0006	0.009 (0.003)	0.009	0.008 (0.003)	0.001
Model 1 þ MR-proANP þ NT-proBNP þ CRP þ homocysteine	0.84 (0.77e 0.90)	0.0006	0.68 (0.63e 0.73)	0.002	0.58 (0.55e 0.60)	0.0007
	0.34 (0.11)	0.003	0.13 (0.05)	0.005	0.14 (0.03)	< 0.0001
	0.06 (0.02)	0.0002	0.01 (0.004)	0.005	0.014 (0.003)	< 0.0001

[a] One-tailed p value.
[b] C index and 95%confidence interval in parenthesis.
[c] NRI for the added biomarker and corresponding standard deviation in parenthesis. Cut-off were 0.03 and 0.1 for heart failure, 0.07 and 0.2 for major events and 0.3 and 0.5 for overall cardiovascular events.
[d] IDI for the added biomarker and corresponding standard deviation in parenthesis.

OPTIMAAL substudy, on a sample of 236 patients with acute MI, no predictive association between NT-proANP and subsequent adverse outcomes (reinfarction or death) were noted [10] in contrast to the report of a strong prognostic value of MR-proANP for death in patients with chronic heart failure [9]. Although, NT- and MR-proANP represent derivatives from different regions of the prohormone, MR-proANP is a biologically more stable form of the prohormone and therefore may offer greater precision in risk assessment [11,16]. Indeed, MR-proANP was shown to be a strong and independent marker of mortality in patients with acute ischemic stroke [6] and in a recent study significantly improved discrimination in patients with stable coronary disease when added to a clinical model; C-statistic improved from 0.768 (clinical model) to 0.804 (clinical model with MR-proANP) [16], as was noted in the current study. The latter study deserves further mention given it is one of the few studies in stable coronary artery disease patients where both MR-proANP and NT-proBNP were measured simultaneously. In contrast to our findings, however, although NT-proBNP was correlated with CVD death or heart failure in models adjusted for clinical covariates, the magnitude of risk (HR) per SD was smaller than that for MR-proANP (1.97 versus 1.73, respectively). The differences in study population characteristics in terms of CVD patients in more advanced stage of disease in the current study versus coronary artery disease patients from the PEACE trial in the study by Sabatine et al. [16] may partly explain these differential findings. The increase in AUC of the order of 2 or 2.5% noted for ANP in the current study and in the study by Sabatine et al. [16] may appear small, yet it is typical of models that already contain a combination of strong predictors (traditional risk factors) [15] and from a public health perspective changes of this magnitude may be meaningful and therefore interest-worthy upon considering cost-considerations [32].

In terms of prognosis of overall cardiovascular events, all four biomarkers examined including both natriuretic peptides, CRP and homocysteine offered similar incremental risk prediction as compared to the traditional risk model. Limited prognostic value was noted for homocysteine in CVD risk prediction among patients with stable CVD in the current study. Our findings do not support the recent report of improved risk classification using homocysteine in subjects without overt CVD [15] and are consistent with previous studies in individuals with CVD [14,33e36].

The current study has certain limitations. The study cohort included a fairly large number of patients however fatal CVD events were rare. This is expected as the case fatality is highest before hospitalization and during early hospital-stay related to primary CHD event even though we were able to follow successfully 95.5% of the patients. Due to the small fatal CVD events, we were not able to analyze this outcome separately, and it was examined as part of the composite endpoint major cardiovascular events. Most previous studies have also reported on composite end-points; larger studies are necessary to evaluate the predictive risk for specific coronary or CVD endpoints. In addition, we measured these biomarkers at baseline only (on average w 4 months after the diagnosis of primary CVD). The duration between the diagnosis of CVD and time to inclusion into the cohort being potentially linked to the biomarker levels; thus it was also included as a confounder in our multivariate models to test whether it was significantly associated with the outcomes. However, when this variable was considered in the statistical models, the results were unchanged (data not shown). It is also important to note that conventional cardiovascular risk factors were not all predictive of adverse prognosis in our study population. Indeed, smoking, aging or diabetes were related to cardiovascular events occurrence, but BMI, blood pressure or LDL cholesterol were not. In such cohorts in secondary cardiovascular prevention, partly because of drug treatment focused on those risk factors, the absence of such relation is not unusual. Therefore, our results cannot be applicable to a population in primary cardiovascular prevention. Although our study included a relatively large number of patients, our findings would have to be confirmed by other studies. The number of events being limited, especially for heart failure, we cannot exclude a possible overestimation of the predictive value of the biomarkers. However, the association between NT-proBNP and heart failure seemed strong.

In conclusion, our findings support a significant and incremental prognostic value for both natriuretic peptides in CVD risk assessment among patients with stable CVD. Specifically, NT-proBNP

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appeared to be the strongest biomarker in terms of its consistent and robust associations with risk of various CVD endpoints as well as incremental risk prediction over the traditional risk model for heart failure and overall CVD event. MR-proANP measurement alone had modest incremental value (over traditional risk factors) particularly for prediction of major CVD events. However, the assessment of NT-proBNP and MR-proANP together seemed to offer complementary information to assess risk beyond other established CVD risk factors for all three CVD endpoints examined. Homocysteine and CRP, on the other hand did not offer additional value for risk prediction after adjusting for traditional CVD risk factors. Our findings suggest that NT-proBNP singly, or in combination with MR-ANP, may offer additional tools for cardiovascular risk stratification not only for heart failure but also other major and overall CVD events in high-risk patients.

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Declaration of conflicting interests

The Authors declare that there is no conflict of interest.

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ANNEXES

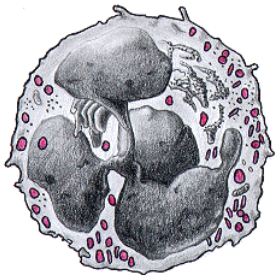
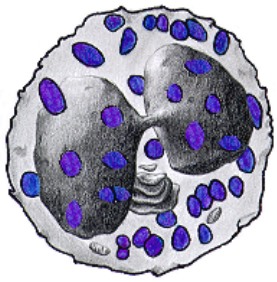
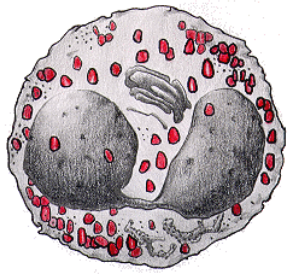
Abréviations

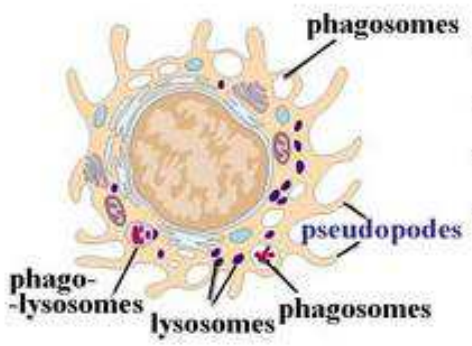
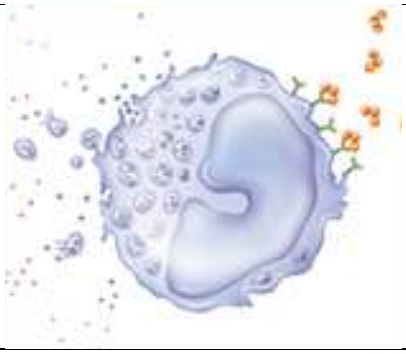
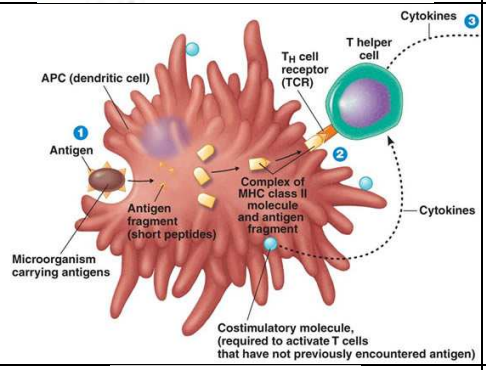
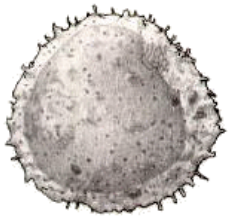
AA	Acide Arachidonique (20:4n-6)
AG	Acide Gras
ALA	Acide α-Linolénique (18:3n-3)
COX	Cyclo-oxygénase
CRP	Protéine C Réactive (C Reactive Protein)
DGAI	Dietary Guidelines for Americans Index
DHA	Acide Docosahexaenoïque (22:6n-3)
DPA	Acide Docosapentaenoïque (22:5n-3)
EPA	Acide Eicosapentaenoïque (20:5n-3)
HEI	Healthy Eating Index
ICAM	Molécule d'adhésion intra-cellulaire (IntraCellular Adhesion Modecule)
IL	Interleukine
INF	Interféron
LA	Acide Linoléique (18:2n-6)
LDL	Low Density Lipoprotein
LOX	Lipoxygénase
LT	Leukotriène
MCP	Protéine chimioattractante des monocytes (Monocyte Chemoattractant Protein)
MICI	Maladies Inflammatoires Chroniques de l'Intestin
NFκB	Nuclear Factor κB
NS	Non Significatif
PG	Prostaglandine
PNNS-GS	Programma National Nutrition Santé - Guideline Score
PUFA	Acide gras poly-insaturé (Poly Unsaturated Fatty Acid)
RRR	Régression des Rangs Réduits (Reduced Rank Regression)
Rv	Résolvine
S	Significatif
SR	Récepteur Scavenger
TBX	Thromboxane
TNFα	Tumor Necrosis Factor α
VCAM	Molécule d'adhésion vasculaire (Vacular Cell Adhesion Molecule)
VEGF	Facteur de croissance vasculaire endothélial (Vascular Endothelial Growth Factor)

Glossaire

Nom	Définition
Dégranulation	Libération des médiateurs chimiques présents dans les granulations intracytoplasmiques des cellules immunitaires (mastocytes en particulier) par un mécanisme d'exocytose
Détersion	Elimination des éléments étrangers, exogènes ou endogènes, et des structures cellulaires ettissulaires nécrosées, présentes dans un foyer inflammatoire
Diapédèse leucocytaire	Passage actif de leucocytes à travers les parois vasculaires, précédant leur migration vers un foyer inflammatoire
Internalisation - endocytose	Mécanisme de transport membranaire permettant de faire pénétrer une particule de petit volume à l'intérieur d'une cellule. L'endocytose a lieu lorsqu'une partie de la membrane entoure entièrement la particule.
Lysosome	Organite cellulaire intracytoplasmique vésiculaire contenant des enzymes de digestion : lipase, oxydase, protéase
Margination	Adhésion des leucocytes à la paroi vasculaire
Opsonine	Molécule ayant la capacité de se lier spécifiquement aux antigènes, permettant leur phagocytose
Phagolysosome	Organite cellulaire intracytoplasmique vésiculaire provenant de la fusion des lysosomes avec une vacuole d'endocytose lors de la phagocytose
Recrutement	Phénomène d'attraction des leucocytes sanguins circulants vers le foyer inflammatoire
Tuméfaction	Terme clinique désignant une augmentation de volume localisée d’un organe
Phagocytose	Ensemble des étapes par lesquelles un phagocyte englobe dans une vacuole lysosomale une structure figurée telle qu’un micro-organisme, un corps étranger ou une autre cellule

Cellules immunitaires – Glossaire spécifique

Nom		Localisation	Fonction
Polynucléaires (=granulocytes)		Sang. Capables de migrer au niveau tissulaire.	Réponse immunitaire innée
Neutrophiles			*Phagocytose* *Dégranulation*
Basophiles			*Réaction d'hypersensibilité immédiate (allergie)*
Eosinophiles			*Réponse immunitaire allergique* *Rôle antiparasitaire*
Monocytes		Sang Capables de migrer au niveau tissulaire	Différentiation en macrophage Phagocytose

Nom		Localisation	Fonction
Macrophages		Tissus	Phagocytose Présentation des antigènes
Mastocytes		Tissus	Granulations intracytoplasmiques contenant de nombreux médiateurs chimiques (dont l'histamine). Degranulation par exocytose lors de l'activation de la cellule
Cellule dendritique		Tissu principalement	Cellule présentatrice d'antigènes
Lymphocyte		Sang Tissus	Cellules de l'immunité acquise spécifiques Plusieurs lignées : B, T, NK

Abstract

Inflammation appears as an ubiquitous mechanism involved in numerous chronic diseases, including metabolic diseases, cardiovascular diseases and cancer. Nutrients are involved at multiple levels and in multiple pathways of inflammation regulation. Polyunsaturated fatty acids are precursors for a range of inflammatory mediators: eicosanoids, resolvins and protectins. Antioxidant nutrients (vitamin C, E and β-carotene) prevent and regulate oxidant reactions, preventing in particular lipid peroxidation in cell membranes, which participate in inflammatory reactions and cellular damages. Epidemiological approaches, particularly through cohort studies allow for a better understanding of the relationships between nutrition and health in the population.

The study of the long-term relationships between antioxidant status (circulating concentrations of vitamin C, E and β-carotene) and C reactive protein (CRP) allowed us to show that β-carotene circulating concentrations were negatively associated to elevated CRP.

The study of relationships between dietary intakes of n-3 and n-6 PUFA and elevated CRP allowed us to show a negative association between n-3 PUFA intakes (particularly docosapentaenoic acid DPA) and elevated CRP and between n-6 PUFA intakes (particularly linoleic acid LA) and elevated CRP

The construction of dietary patterns specifically associated to nutrients having pro- or anti-inflammatory properties (PUFA and antioxidant nutrients' intakes) allowed us to show that a dietary pattern rich in essential fatty acids and antioxidant nutrients was negatively associated to elevated CRP in the long-term.

These consistent results corroborate the importance of adequate intakes of PUFA and antioxidant nutrients in inflammatory mechanisms. However, dietary balance between these nutrients needs to be carefully considered, given the multiple interactions existing in the mechanisms they are involved in.

Keywords

Inflammation, Polyunsaturated fatty acids, Antioxidant nutrients, Dietary patterns, Nutritional epidemiology

Résumé
L'inflammation apparaît comme un mécanisme ubiquitaire sous-tendant de nombreuses pathologies chroniques. Les nutriments participent à de multiples niveaux, de façon directe ou indirecte à la régulation de la réaction inflammatoire. Les acides gras polyinsaturés (PUFAs) sont les précurseurs de nombreux médiateurs de l'inflammation. Les nutriments antioxydants (vitamine C, E et caroténoïdes) interviennent dans la prévention et la régulation des réactions oxydantes participant à la réaction inflammatoire et aux dommages cellulaires l'accompagnant. L'approche épidémiologique, notamment au travers des études de cohortes, contribue à une meilleure compréhension des relations entre nutrition et santé dans la population.
L'étude des relations entre statut en antioxydants (concentrations sanguines en vitamine C, E et β-carotène) et CRP augmentée (Protéine C réactive) à long terme nous ont permis de montrer que le statut en β-carotène était négativement associé à une CRP augmentée.
L'étude des relations entre apports en PUFAs n-3 et n-6 et CRP augmentée nous ont permis de montrer que les apports en PUFAs n-3 ainsi que les apports en PUFAs n-6 étaient négativement associés à une CRP augmentée. Les apports en vitamine E étaient modulateurs de cette relation, qui n'apparaissait significative que pour les sujets avec des apports faibles en vitamine E.
Enfin, la construction de profils alimentaires spécifiquement associés aux nutriments connus pour avoir des propriétés anti ou pro-inflammatoires (PUFAs, nutriments antioxydants) nous a permis de montrer qu'un profil alimentaire riche en acides gras essentiels et en nutriments antioxydants était négativement associé à une CRP augmentée à long terme.
Ces résultat concordants montrent bien l'intérêt d'apports adéquats en PUFAs et en nutriments antioxydants dans les mécanismes inflammatoires. Néanmoins, la balance entre ces différents nutriments doit être prise en compte, en particulier du fait d'interactions multiples entre leurs effets.

Titre en Anglais

Epidemiological aspects of the relationships between nutrition and inflammation

Discipline
Santé – Santé Publique

Mots-clés
Inflammation, Acides gras polyinsaturés, Nutriments antioxydants, Profils alimentaires, Epidémiologie nutritionnelle

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