Approche épidémiologique du rôle des acides gras sur le vieillissement cutané dans le cadre de l'étude SU.VI.MAX

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A mes amis, à ma famille,

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Résumé

De nombreuses études ont été menées ces dernières années cherchant à mettre en évidence l'éventuel effet protecteur de différents micronutriments issus de l'alimentation sur le vieillissement cutané, et plus particulièrement leur effet photoprotecteur. Mais, bien que la peau soit chez l'homme un des principaux lieux de stockage de graisses, jusqu'à présent peu d'attention a été portée au rôle possible des lipides.

L'objectif de cette thèse était d'étudier le lien entre l'apport alimentaire en acides gras et le photo-vieillissement cutané au niveau du visage, chez une population d'hommes et de femmes de la cohorte SU.VI.MAX, âgés entre 45 et 60 ans à l'inclusion dans la cohorte. La sévérité du photo-vieillissement a été appréciée au cours de l'examen clinique d'inclusion à l'aide d'une échelle photographique validée à 6 niveaux. L'apport alimentaire en acides gras a été, quant à lui, estimé à partir de 10 questionnaires récupérant les données alimentaires sur une période de 24 heures (enregistrements 24 heures) qui ont été renseignés au cours des 2 ans et demi qui ont suivi l'inclusion.

Le lien entre la sévérité du photo-vieillissement et les apports en acides gras mono-insaturés (AGMI) a été tout d'abord étudié, et a fait l'objet d'une première publication dans la revue PLoS ONE. Puis, le lien entre la sévérité du photo-vieillissement et les acides gras polyinsaturés (AGPI) n-3 a été étudié, et ce travail est actuellement soumis pour publication dans le Journal of Dermatological Science.

Les analyses ont été réalisées globalement et par aliments sources pour chacun des sexes. Chez les hommes et les femmes, une association inverse a été trouvée entre les apports en AGMI provenant des huiles végétales, en particulier l'huile d'olive, et la sévérité du photo-vieillissement. Par contre, aucun lien n'a été mis en évidence entre le photo-vieillissement et les apports provenant des sources animales (produits laitiers, viandes ou charcuteries).

Lors de l'étude des AGPI n-3, une association inverse a été mise en évidence chez les hommes entre la sévérité du photo-vieillissement et l'apport en acide α-linolénique (ALA, C18:3 n-3). Chez les femmes, une association inverse a été trouvée avec l'apport en acide eicosapentaénoïque (EPA, C20:5 n-3). L'analyse, réalisée selon les sources d'ALA, a permis également de montrer chez les hommes une association inverse entre la sévérité du photo-vieillissement et les apports en ALA des huiles végétales et des fruits et légumes. Chez les

femmes, une tendance inverse a été uniquement mise en évidence avec les ALA des huiles végétales. Par contre, aucun lien n'a été trouvé avec les apports en ALA des produits laitiers.

Ces résultats mettent en avant le rôle potentiel photoprotecteur de l'huile d'olive et des AGPI n-3 sur le vieillissement cutané. Ils contribuent à soutenir les recommandations en faveur d'un régime riche en huile d'olive et en AGPI n-3, comme le régime méditerranéen. Des travaux de recherche complémentaires devraient maintenant être menés afin d'étudier le rôle éventuel des composants mineurs de l'huile d'olive, tels que les polyphénols, sur le vieillissement cutané.

Mots-clés : Epidémiologie, photo-vieillissement, acides gras, nutrition.

Résumé en anglais

Over the last years, number of studies were conducted to highlight the possible protection of micronutrients from diet on skin aging, and in particular photoprotection. Skin is a major fat storage organ in humans, however the role of lipids intakes has been very few investigated.

The aim of this thesis was to investigate the association between the risk of facial severe photoaging and the intake of fatty acids in a population of men and women from the SU.VI.MAX cohort, aged between 45 and 60 years old at inclusion. Severity of facial skin photoaging was graded during a clinical examination at baseline using a 6-grade validated scale illustrated by photographs. Dietary fatty acids intakes were estimated through ten questionnaires on diet over the past 24-hour period (24-h diet records) completed during the first 2.5 years of the follow-up period.

The association between the risk of severe photoaging and monounsaturated fatty acids (MUFA) intakes has been first investigated and is already published in PLoS ONE journal. Then, the association between the risk of severe photoaging and n-3 polyunsaturated fatty acids (n-3 PUFAs) has been studied and this work is currently submitted for publication in the Journal of Dermatological Science.

The analyses have been performed globally and by food sources for each sexe. In both genders, an inverse association was found between intakes of MUFA from vegetable oil, in particular olive oil, and the severity of photoaging. Whereas no association was found with intake of monounsaturated fatty acids from animal sources, whether from dairy products, meat or processed meat.

Concerning n-3 PUFAs, severe photoaging was inversely associated with higher intake of α-linolenic acid (ALA, C18:3 n-3) in men and higher intake of eicosapentaenoic acid (EPA, C20:5 n-3) in women. When considering the different food sources of ALA, an inverse association was revealed in men between severe photoaging and ALA from vegetable oil, as well as with ALA from fruits and vegetable, whereas no association was highlighted with ALA from dairy products. In women, ALA from vegetable oil tended also to be inversely linked to photoaging severity.

These findings support the possible photoprotection effect of olive oil and n-3 PUFAs. They help to support recommendations for a diet rich in olive oil and n-3 PUFAs such as the Mediterranean diet. Further research should be now conducted to investigate the possible role of minor components of olive oil, such as polyphenols, on skin aging.

Key-words: Epidemiology, photoaging, fatty acids, diet

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Liste des abréviations et symboles

$^1O_2$: oxygène singulet

µm : micromètre

AA : acide arachidonique (C20:4 n-6)

acétyl-CoA : acétyl-coenzyme A

ACM : analyse des correspondances multiples

ACP : analyse en composantes principales

AG : acides gras

AGI : acides gras insaturés

AGMI : acide gras monoinsaturés

AGPI : acide gras polyinsaturés

AGPI-LC : acide gras polyinsaturés à longues chaînes

AGS : acide gras saturés

ALA : acide α-linolénique (C18:3 n-3)

AFSSA : agence française de sécurité sanitaire

ADN : acide désoxyribonucléique

ANC : apports nutritionnels conseillés

AO : acide oléique

AP_{-1} : activator protein 1

CCPPRB : comité consultatif de protection des personnes dans la recherche biomédicale

CH_3 : groupement méthyle

CNIL : commission nationale de l'informatique et des libertés

COOH : groupement carboxyle

DHA : acide docosahexaénoïque (C22:6 n-3)

DMLA : dégénérescence maculaire liée à l'âge

DMO : densité minérale osseuse

DPA : acide docosapentaénoïque (C22:5 n-3)

EPA : acide eicosapentaénoïque (C20:5 n-3)

ERO : espèces réactives de l'oxygène

GWAS : genome-wide association study

GEE : generalized estimating equation

HR : hazard Ratio

IMC : indice de masse corporelle

IUNS : international union of nutritional sciences

kcal : kilocalorie

kg : kilogramme

LA : acide linoléique (18:2 n-6)

m : mètre

MC1R : melanocortin 1 receptor

MG : matière grasse

MG/MS : matière grasse / matière sèche

MMP : métalloprotéinase

NADPH : nicotinamide adénine dinucléotide phosphate hydrogéné

NHANES I : first national health and nutrition examination survey

NRJ : énergie totale

OA : acide oléique (C18:1 n-9)

$O_2^{\bullet-}$: anion superoxyde

OR : odds ratio

pH : potentiel hydrogène

Q1 : 1er quartile

Q4 : 4ème quartile

QFA : questionnaire de fréquence alimentaire

SU.VI.MAX. : supplémentation en vitamines et mineraux antioxydants

UV : ultraviolets

UHT : ultra haute température

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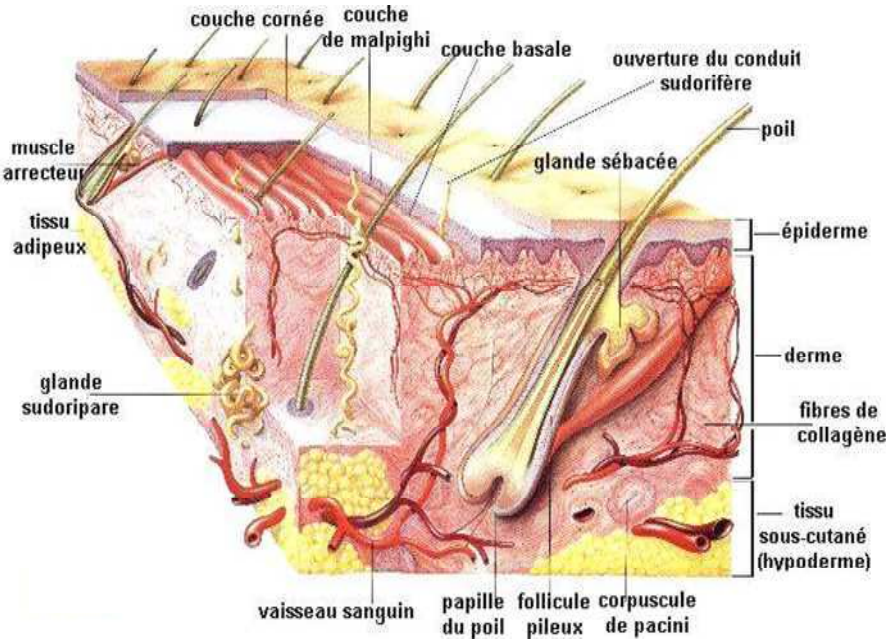
1. Introduction générale et objectifs

1.1 Peau et vieillissement cutané

1.1.1 Structure et fonction de la peau

La peau est l'organe qui est le plus lourd et le plus étendu de l'organisme avec une surface de ± 2 m² et un poids variant entre 4,5 et 5 kg. C'est une enveloppe qui protège l'individu et se compose de trois tissus superposés : l'épiderme (tissu supérieur), le derme (tissu intermédiaire) et l'hypoderme (tissu le plus profond) (Figure 1) (Dubus et Vergier, 2000 ; Melissopoulos et Levacher, 1998). La peau contient également différentes annexes qui participent à l'homéostasie, dont les poils, les glandes sébacées et sudoripares.

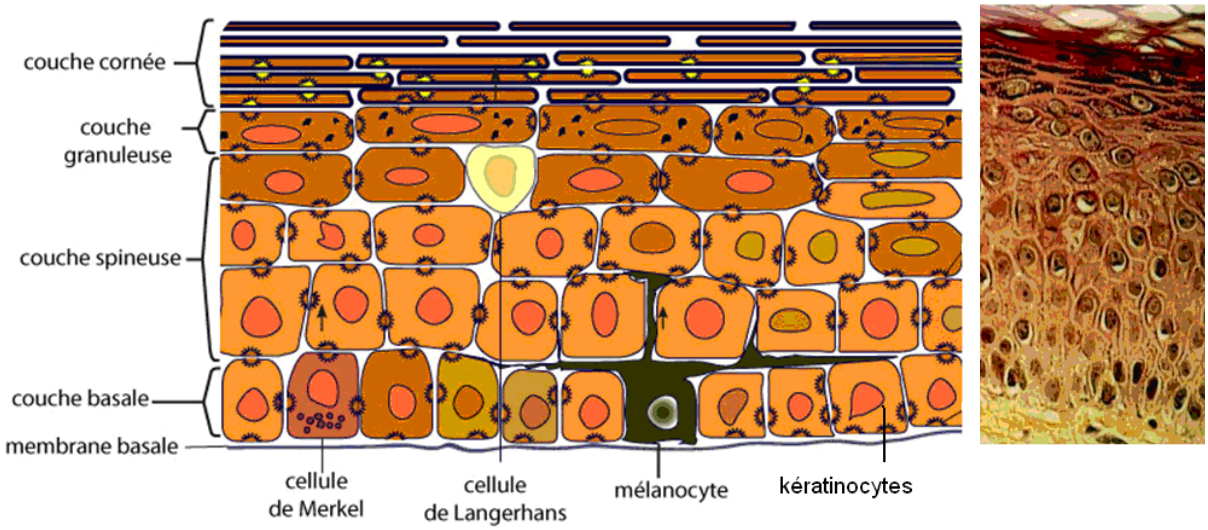
Figure 1. Représentation schématique d'une coupe de peau (tirée de http://www.esthetique.qc.ca/services_fr/peau/schema_peau.html)



1.1.1.1 Epiderme

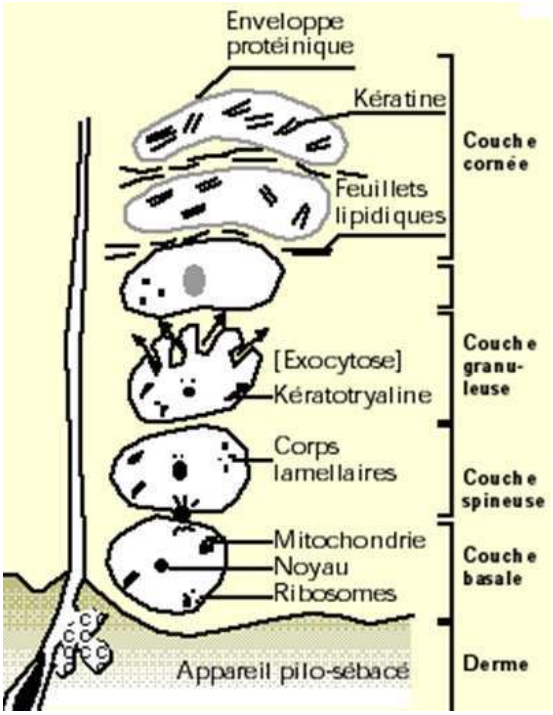
L'épiderme est constitué de 4 ou 5 couches superposées selon la localisation qui vont de la couche cornée (la couche supérieure) à la couche basale (la couche la plus profonde en contact avec la jonction dermo-épidermique). Quatre types de cellules se trouvent dans l'épiderme : les kératinocytes, les mélanocytes, les cellules de Langerhans et les cellules de Merkel (Figure 2).

Figure 2. Epiderme (tirée de http://biologiedelapeau.fr)



Les kératinocytes, qui représentent 90% des cellules de l'épiderme, sont présents dans toutes les couches : de la couche basale, zone de multiplication de ces cellules, aux couches les plus superficielles. Les kératinocytes migrent progressivement vers la surface, ce phénomène étant accompagné d'une modification de leur composition pour aboutir à la couche cornée (Figure 3). En surface, les cellules de la couche cornée perdent leur adhérence puis tombent (phénomène de desquamation).

Figure 3. Différentiation progressive des kératinocytes (tirée de Mazière, 1997)



Les mélanocytes qui représentent environ 1% des cellules de l'épiderme sont uniquement situés dans la couche basale. Ces cellules de grande taille présentent de nombreux prolongements (dendrites) qui sont en contact avec les kératinocytes de leur voisinage. Elles fabriquent de la mélanine sous la forme de petits grains (les mélanosomes) qui migrent dans les dendrites et pénètrent dans les kératinocytes. La qualité et la quantité de mélanine produite dépendent de facteurs génétiques individuels. Il y a deux types de mélanine, la phaeomélanine et l'eumélanine et c'est le ratio entre ces deux types de mélanines qui détermine la couleur de la peau. L'eumélanine est le dispositif le plus efficace pour protéger du rayonnement solaire. En effet, les rayons ultraviolets entraînent une augmentation de la production de l'eumélanine qui ira jouer un rôle de parasol en protégeant les noyaux des kératinocytes (phénomène du bronzage) (Rees, 2003).

Les cellules de Langerhans, qui constituent 2 à 7% de la population cellulaire épidermique, sont présentes dans toutes les couches de l'épiderme et forment un réseau de cellules étoilées reliées entre elles et ayant la capacité de capter toute substance étrangère. Elles jouent un rôle dans l'inflammation cutanée et ont un rôle primordial dans l'immunité cutanée.

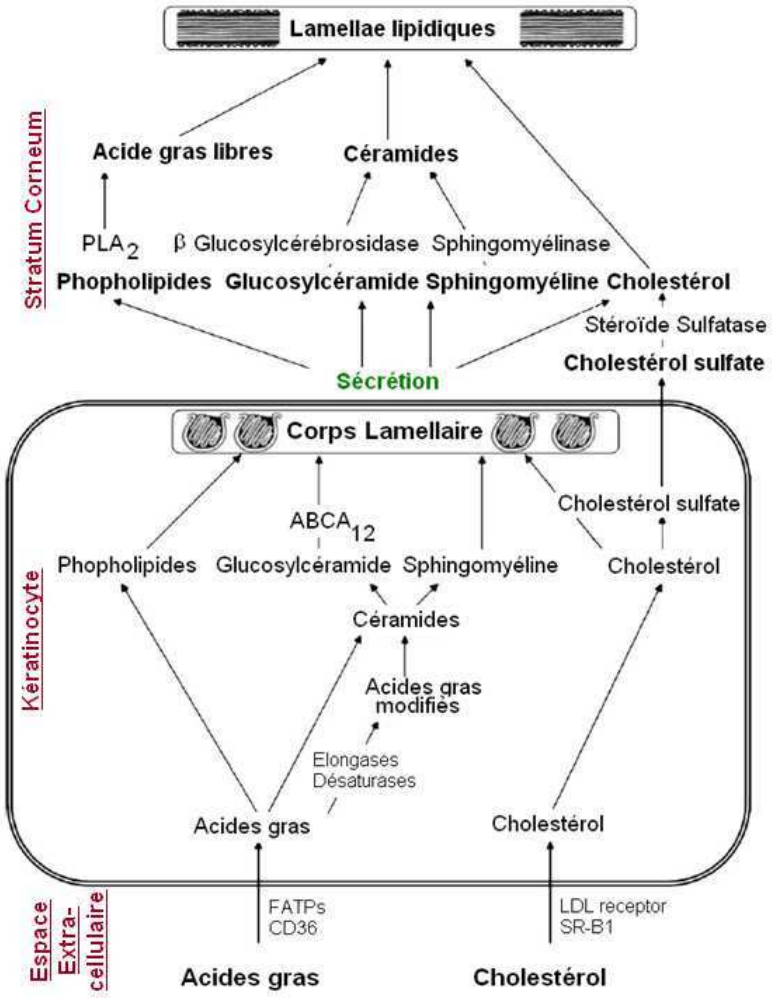
Enfin, les cellules de Merkel, ont une fonction neuro-endocrine. Elles exercent un rôle mécano-récepteur et sont localisées au niveau de la couche basale au contact des terminaisons nerveuses.

Outre les cellules, l'épiderme est composé également de lipides, à la fois des lipides de surface qui forment le film hydrolipidique et des lipides dits intra-épidermiques. Les glandes sébacées synthétisent les lipides de surface qui sont ensuite excrétés dans le sébum. Ces lipides se composent essentiellement de squalène (15%), de cire (25%) et de triglycérides (60%). Les lipides intra-épidermiques sont synthétisés par les kératinocytes. Ils sont composés de céramides (50%), de cholestérol (25%) et d'acides gras libres (25%). La synthèse de ces lipides a lieu au moment de la formation de la couche cornée. Dans les couches épineuses et granuleuses, les corps lamellaires (ou corps d'Odland), petits organites sécrétoires (0,2 à 0,3 µm) issus de l'appareil de Golgi apparaissent (Figures 3 et 4). Ils contiennent à la fois les précurseurs lipidiques des lipides de la couche cornée et les enzymes utiles à leur conversion. Lors du passage de la couche granuleuse à la couche cornée, les corps lamellaires migrent jusqu'à la membrane plasmique des kératinoyctes, où ils fusionnent avec la membrane et déversent leur contenu dans l'espace intercellulaire. Après sécrétion, les précurseurs lipidiques sont modifiés et s'organisent en *lamellae* qui remplissent les espaces

inter-cornéocytaires. La membrane lamellaire intercellulaire ainsi constituée est, contrairement à la plupart des membranes biologiques, dépourvues de phospholipides. Au cours de la différentiation des kératinocytes, de la couche la plus profonde (couche basale) vers la couche supérieure (couche cornée), la composition lipidique change. En effet, la couche basale est riche en phospholipides et pauvre en céramide alors que la couche cornée est pauvre en phospholipides et riche en céramides, acides gras libres et stérols (Elias, 1983).

L'épiderme a essentiellement une fonction de protection de l'organisme : protections mécanique, antimicrobienne, fonction immunitaire, protection contre les rayonnements ultraviolets (UV) (photoprotection) et protection thermique. Il a également une fonction métabolique et endocrine. En effet, la peau sous l'action des ultraviolets B (UVB) synthétise la vitamine D_3 dans la partie profonde de l'épiderme.

Figure 4. Mécanisme de formation des lamelles lipidiques extra-cornéocytaires (tirée de Feingold, 2007)



1.1.1.2 Derme

Le derme est un tissu conjonctif de soutien. Il est divisé en deux parties, le derme papillaire ou superficiel, qui représente un cinquième du derme, et le derme réticulaire ou profond qui représente les quatre cinquièmes du derme.

Le derme est composé de fibroblastes, cellules qui ont pour rôle de synthétiser le collagène, l'élastine, la substance fondamentale et les glycoprotéines. L'ensemble va former la matrice extracellulaire du derme qui permettra d'absorber les forces de tensions. Le derme possède également des cellules migratrices qui participent à la défense de l'organisme dont les leucocytes, les mastocytes et les macrophages. De plus, on retrouve dans le derme des vaisseaux de petit diamètre, des nerfs « libres » et des nerfs reliés à différents corpuscules sensoriels. Il contient également les annexes : poils, glandes sébacées et sudoripares.

Le derme a une fonction de soutien, il supporte l'épiderme. Il a également une fonction métabolique (et nutritionnelle) grâce à son réseau vasculaire et lymphatique. Le réseau sanguin lui permet également de réguler la température corporelle. Le derme est comme l'épiderme impliqué dans les mécanismes de défense (leucocytes, mastocytes et macrophages) et de réparation. Enfin, il assure une fonction sensorielle grâce à ses fibres nerveuses et ses récepteurs sensoriels.

1.1.1.3 Hypoderme

L'hypoderme, ou tissu adipeux blanc, est rattaché à la partie profonde du derme par des expansions de fibres de collagène et d'élastine qui forment des cloisons entre les lobules adipeux. Ces cloisons se fixent en profondeur aux membranes fibreuses qui entourent les muscles (aponévrose) et les os (périoste). Ces cloisons autorisent le passage des fibres nerveuses et des vaisseaux sanguins. Les lobules sont remplis d'adipocytes, des cellules adipeuses dérivées des fibroblastes. Ces cellules sont spécialisées dans l'accumulation et le stockage des graisses. Le tissu adipeux cutané correspond environ à 85% de notre graisse corporelle totale. La distribution du tissu adipeux est clairement différente selon les sexes. Chez les hommes, le tissu adipeux a tendance à s'accumuler dans la partie haute du corps, au dessus de la ceinture, au niveau du ventre et des épaules (répartition androïde). Chez les femmes, le tissu adipeux a tendance à s'accumuler dans la partie inférieure, sous ombilicale, au niveau des cuisses, des hanches et des fesses (répartition gynoïde).

L’hypoderme a une fonction mécanique, il amortit les chocs et permet également de lutter contre le froid. Enfin, l’hypoderme joue le rôle de réserve énergétique. Les graisses contenues dans les adipocytes, peuvent être remises en circulation, via la voie veineuse, lors d'un effort intense ou lors d'une déficience en apport énergétique.

1.1.2 Vieillissement de la peau

Le vieillissement cutané est un processus complexe multifactoriel qui résulte principalement de deux phénomènes : le vieillissement intrinsèque qui concerne l’ensemble du tégument et le vieillissement extrinsèque dû aux facteurs environnementaux et comportementaux (Boisnic et Branchet, 2000 ; Fischer et *al.*, 2002 ; Yaar et Gilchrest, 2007).

1.1.2.1 Vieillissement intrinsèque

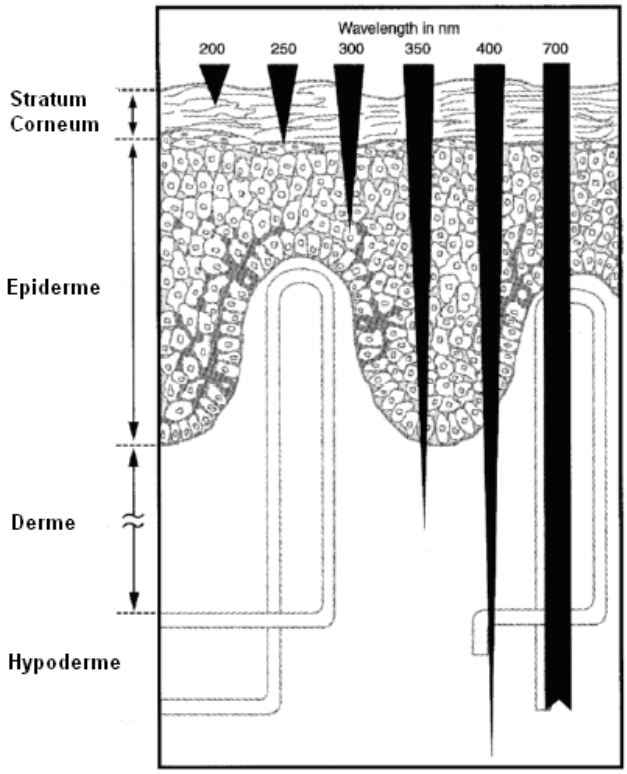
Le vieillissement intrinsèque ou chronologique est inéluctable et peut être considéré comme génétiquement déterminé. Il résulte d’un déséquilibre entre les dommages cellulaires et les mécanismes de défense cellulaire, et aboutit à des modifications de la structure et de la fonction de la peau (Boisnic et Branchet, 2000 ; Passeron et Ortonne, 2003). Ainsi, au cours du vieillissement une diminution du nombre de mélanocytes fonctionnels et de cellules de Langerhans dans l’épiderme est observée. La jonction dermo-épidermique est également modifiée. Les crêtes épidermiques s’aplatissent diminuant ainsi les zones de contact entre l’épiderme et le derme. Les échanges de nutriments et de métabolites entre les deux couches sont ainsi réduits. Le derme s’atrophie également, le nombre de fibres de collagène s’amoindrit et les fibres deviennent plus épaisses et désorganisées. Les fibres élastiques sont également altérées dans le derme papillaire, l’épaisseur des vaisseaux est réduite et la vascularisation du derme papillaire diminuée. De nombreuses modifications visuelles de la peau sont ainsi engendrées, telles que l’apparition de rides, de relâchement cutané, de sécheresse cutanée, de pâleur de la peau, l’apparition de kératose séborrhéique ou encore de taches rubis.

1.1.2.2 Photo-vieillissement cutané

Parmi les différents facteurs responsables du vieillissement cutané extrinsèque, l’exposition aux radiations UV en est le principal, on parle alors de photo-vieillissement cutané. Les

radiations UV vont être à l'origine d'une forte production d'espèces réactives de l'oxygène (ERO) qui mettra à mal les défenses antioxydantes de la peau (Rabe et *al.*, 2006 ; Yaar et Gilchrest, 2007 ; Sage et *al.*, 2012). En effet, le rayonnement UVB, très énergétique pénètre au niveau de l'épiderme où il induit directement des dommages ADN (dimères cyclobutane et les photoproduits 6-4) dans les kératinocytes et les mélanocytes. Le rayonnement UVA, bien que moins énergétique, est reconnu pour être également fortement impliqué dans le photo-vieillissement. Il pénètre plus profondément que les UVB dans la peau et peut atteindre le derme profond et le tissu sous cutané (Figure 5).

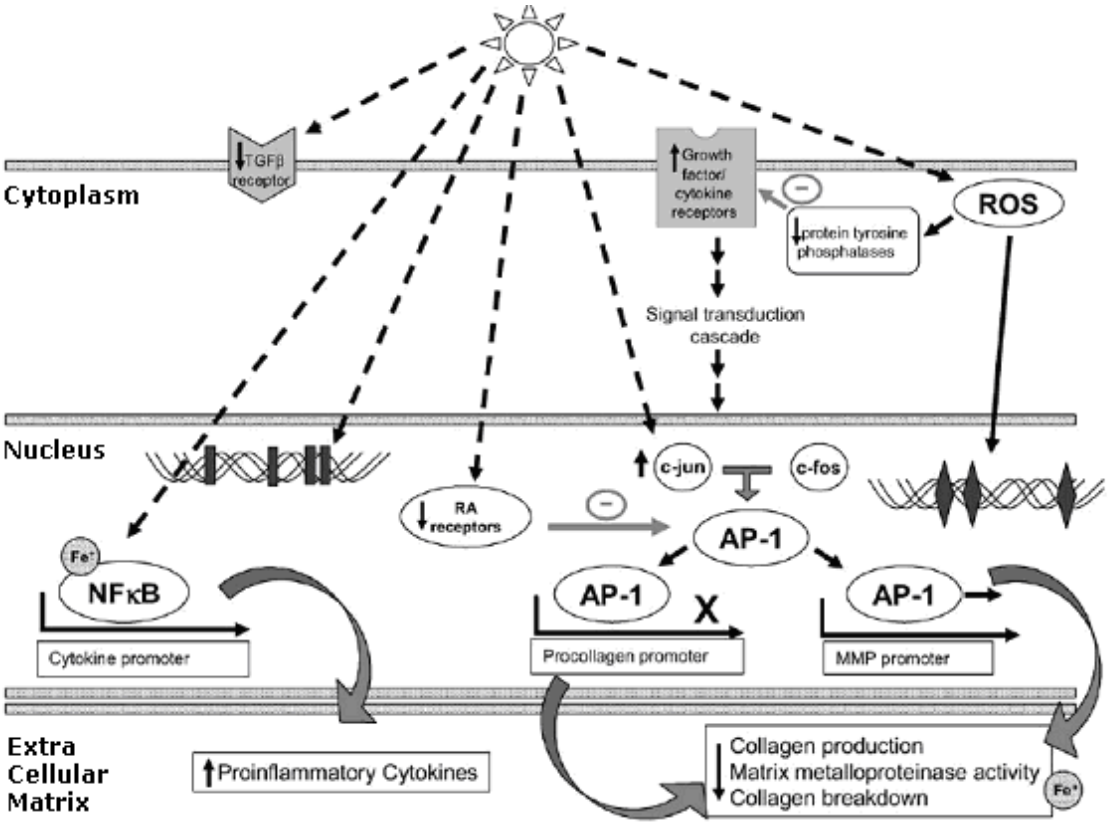
Figure 5. Couches de la peau atteintes par les UV en fonction de leur longueur d'onde (tirée de Goralczyk et Wertz, 2009).



Les UVA sont très faiblement absorbés par l'ADN mais excitent d'autres chromophores endogènes (NADP/NADPH, coenzymes flaviniques, noyau porphyrinique) jouant un rôle de photosensibilisateurs et générant ainsi la production d'ERO (principalement l'oxygène singulet $^1O_2$) qui endommage l'ADN (dimères cyclobutane, 8-oxo-déoxyguanosine), les lipides et les protéines (Sage et *al.*, 2012). Les radiations UV (A et B) sont donc à l'origine d'une surproduction d'ERO qui provoque également une réponse inflammatoire de la peau et une immunosuppression. Ces différentes manifestations augmentent l'expression de

métalloprotéinases (MMP) qui dégradent les macromolécules de la matrice extra-cellulaire (collagène, élastine…) via l'activation des facteurs de transcription AP_{-1} et NF-κB et l'inactivation de récepteurs aux rétinoïdes (Figure 6). Ces différentes altérations sont à l'origine du photo-vieillissement.

Figure 6. Effets des UV sur les kératinocytes et les fibroblastes (tirée de Rabe et *al.*, 2006). Les UV induisent des espèces réactives de l'oxygène (ROS) qui peuvent endommager l'ADN (♦) ou inhiber la tyrosine phosphatase entraînant une cascade intracellulaire qui active le complexe de transcription Activator protein 1 (AP_{-1}). Les UV peuvent également directement activer la protéine c-Jun, et peuvent induire une diminution des récepteurs nucléaires aux rétinoïdes et donc de l'acide rétinoïque (RA) qui agit de façon antagoniste sur le facteur de transcription AP_{-1}. Les UV peuvent également produire directement des mutations de l'ADN (▌), une activation du facteur de transcription NF-κB et une diminution de l'activité des récepteurs TGF-β qui influent sur la production de cytokine inflammatoire et sur la formation du collagène. Abréviation : métalloprotéinases (MMP).



Le photo-vieillissement se superpose alors au vieillissement intrinsèque et engendre lui aussi des modifications de l'épiderme et du derme et accélére le vieillissement. L'épiderme devient irrégulier, parfois atrophié, parfois hyperplasique. Le nombre de cellules de Langerhans diminue tandis que le nombre de mélanocytes hyperplasiques augmente. Le tissu conjonctif

dermique est altéré. La microvascularisation est détériorée : perte des plexus papillaires avec aplatissement des crêtes papillaires, et également dilation et élargissement des vaisseaux dans le derme papillaire et le derme moyen. Le collagène diminue et le tissu élastique dystrophique s'accumule. Le photo-vieillissement cutané se caractérise alors par une peau plus épaisse (élastose solaire), rugueuse, jaunâtre et hyperlaxe. Des ridules, puis des rides profondes apparaissent, des télangiectasies (reflet des altérations vasculaires au niveau du derme), des taches pigmentaires encore appelées lentigines (témoins des altérations des mélanocytes), une hyperplasie sébacée constituée de multiples papules jaunes, molles, ombiliquées en leur centre, ainsi que de kératoses actiniques, considérées comme des lésions précancéreuses.

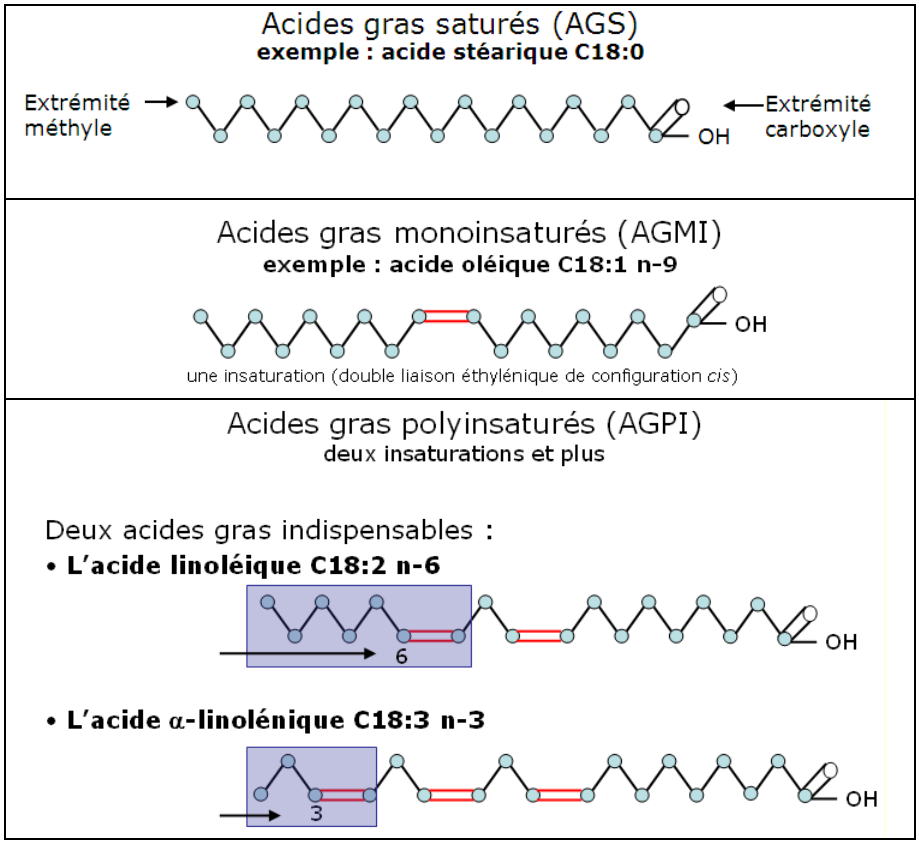
1.2 Acides gras : sources et métabolisme

1.2.1 Nomenclature des acides gras

Les acides gras font partie de la grande famille des lipides (Fahy *et al.*, 2005), ils sont formés d'une chaîne hydrocarbonée avec un groupement carboxyle (COOH) à une extrémité et un groupement méthyle (CH_3) à l'autre extrémité (Guesnet et *al.*, 2005) (Figure 7). Ils sont insaturés ou saturés selon qu'ils contiennent (AGI) ou pas (AGS) des doubles liaisons. Ils peuvent également, lorsqu'ils possèdent des doubles liaisons, présenter des configurations en cis ou trans. A l'état naturel, la majorité des acides gras ont la configuration cis. Différentes nomenclatures existent, les nutritionnistes utilisent la nomenclature qui indique la longueur de leur chaîne carbonée, l'absence ou la présence de doubles liaisons qui reflète la réactivité biochimique, et l'appartenance à la famille (position de la première double liaison par rapport au groupement méthyle terminal). Parmi les AGI, on trouve les acides gras monoinsaturés (AGMI) qui ne présentent qu'une seule double liaison et les acides gras polyinsaturés (AGPI) qui présentent plusieurs doubles liaisons. Parmi les AGPI, il existe deux familles d'AG nommées famille des oméga-3 ou n-3 et famille des oméga-6 ou n-6. Les précurseurs de la famille des oméga-3 et oméga-6 sont respectivement l'acide α-linolénique (ALA, C18:3 n-3) et l'acide linoléique (LA, C18:2 n-6). Ces précurseurs peuvent être allongés et désaturés en leurs dérivés à longue chaîne en particulier : l'acide arachidonique (AA, C20:4 n-6), l'acide

eicosapentaénoïque (EPA, C20:5 n-3), l'acide docosapentaénoïque (DPA C22:5, n-3) et l'acide docosahexaénoïque (DHA, C22:6 n-3).

Figure 7. Structure et nomenclature des principales familles des acides gras (adaptée de Guesnet et *al.*, 2005). La chaîne carbonée est représentée par la succession de sphères (atomes de carbone) reliées entre elles par des liaisons covalentes.



Les acides gras proviennent d'une part de l'alimentation et peuvent être également synthétisés par l'organisme.

1.2.2 Acides gras alimentaires

Les acides gras fournis par l'alimentation se présentent essentiellement estérifiés sous forme de triglycérides qui représentent 95 % des lipides alimentaires (AFSSA, 2010, saisine n°2006-SA-0359). Les AG se présentent également estérifiés sous forme de phospholipides qui constituent 1 à 5% des lipides alimentaires, et également sous forme d'esters de cholestérol qui correspondent eux-mêmes à moins de 1% des lipides alimentaires.

Les acides gras saturés les plus fréquemment rencontrés sont l'acide palmitique (C16:0), l'acide stéarique (C18:0) (Figure 7), l'acide laurique (C12:0) et l'acide myristique (C14:0) que l'on retrouve dans les graisses animales (beurre, fromage, saindoux, lard, suif, huile de poisson…) et également dans certaines matières grasses végétales (huile de noix de coco, beurre de cacao, huile de palme) (Arnault et *al*., 2006).

L'acide oléique (C18:1 n-9) est l'acide gras monoinsaturé le plus largement représenté (Figure 7). Des quantités importantes d'acide oléique sont présentes dans les huiles végétales notamment l'huile d'olive, huile d'arachide et l'huile de sésame, les oléagineux (noix de macadamia, noisette, noix de cajou, amande), dans les fruits (avocat, olive) et également dans les graisses animales comme la graisse de canard et le saindoux. L'acide palmitoléique (C16:1 n-7), qui est représenté en quantité moindre que l'acide oléique, est présent de façon importante dans les huiles de poisson.

Parmi les AGPI, l'acide linoléique (LA, C18:2 n-6) est le plus abondant dans l'alimentation (Figure 7). Les sources importantes sont les huiles végétales, notamment l'huile de tournesol et l'huile de maïs. L'acide α-linolénique (ALA, C18:3 n-3) est également largement rencontré dans la nature. Les aliments riches en acide ALA sont les huiles végétales hautement insaturées, dont l'huile de colza et l'huile de soja, et les noix. Les produits laitiers sont également des sources significatives d'ALA ainsi que les légumes verts malgré leur faible contenu en lipides.

Les principales sources d'acide arachidonique (AA, C20:4 n-6) sont les œufs, les viandes et la charcuterie. Enfin, les acides gras à longue chaîne de la famille des omégas 3 sont présents en quantité importante dans les poissons, crustacés et produits animaux autres que les produits laitiers. Les poissons et les crustacés sont les sources principales de l'acide eicosapentaénoïque (EPA, C20:5 n-3) et de l'acide docosahexaénoïque (DHA, C22:6 n-3). L'acide docosapentaénoïque (DPA, C22:5 n-3) est également trouvé en quantité importante dans la viande et la charcuterie.

1.2.3 Biodisponibilité des acides gras alimentaires

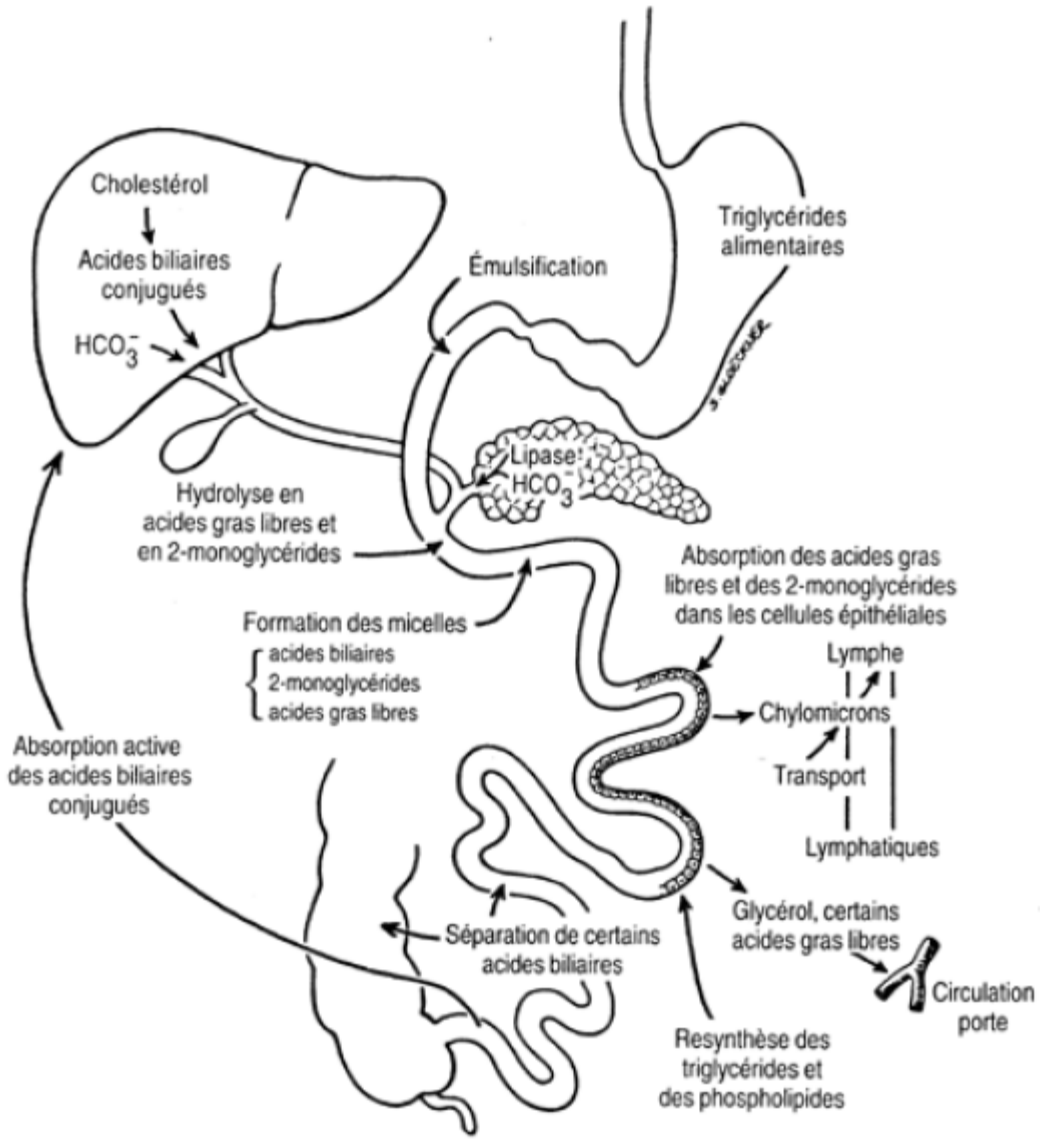
Les mécanismes de digestion, absorption et transport des lipides sont adaptés à leurs caractéristiques hydrophobes, apolaires et très peu solubles dans l'eau (Lecleire, 2008).

La dégradation des lipides commence dans la cavité buccale sous l'action d'une enzyme salivaire : la lipase salivaire. Puis, ils subissent dans l'estomac un brassage en présence d'acide chlorhydrique. Les lipides sont alors mélangés à l'eau en une émulsion avant d'être évacués vers le duodénum où la bile sécrétée par le foie et constituée par ses sels biliaires va permettre de fractionner les grosses gouttelettes en petites gouttelettes entourées de bile (Figure 8). Dans les intestins, les triglycérides sont ensuite hydrolysés par la lipase et la colipase du pancréas qui libèrent des monoglycérides et des acides gras libres absorbables. Les phopholipides sont hydrolysés par la phospholipase A2 qui libère les acides gras et les lysophospholipides. Enfin le cholestérol estérifié sera hydrolysé par la cholestérol-estérase. Les monoglycérides, les acides gras libres, les lysophospholipides et le cholestérol libre participent à la formation des micelles mixtes qui permettent le passage en milieu hydrophile de substances lipophiles vers la bordure en brosse par endocytose. Les micelles sont endocytées dans le jéjunum. Les acides gras à chaine courte et moyenne (C <= 10) sont directement absorbés par diffusion passive et transportés rapidement par l'albumine via la veine porte jusqu'au foie où ils sont métabolisés.

Les monoglycérides, les acides gras libres, les lysophospholipides et le cholestérol libre quittent les micelles pour entrer dans les entérocytes. Les acides gras à longue chaine (C > 10) sont trop lipophiles pour être directement sécrétés dans la circulation. Ils seront resynthétisés en triglycérides, phospholipides et cholestérols estérifiés dans le reticulum endoplasmique de la cellule, puis combinés à une protéine (béta-lipoproteine) pour former les chylomicrons pour être transportable dans le sang. Les chylomicrons quittent l'entérocyte par exocytose, pénètrent dans les chylifères des villosités intestinales et empruntent le système lymphatique pour gagner la circulation sanguine au niveau de la veine sous-clavière gauche. Lorsque les chylomicrons atteignent les capillaires des tissus périphériques, les triglycérides sont hydrolysés en glycérol et en acides gras non estérifiés puis stockés dans le tissu adipeux, ou utilisés comme substrat énergétique dans les cellules musculaires. Les triglycérides et les esters de cholestérol résiduels qui n'ont pas été utilisés forment les lipoprotéines rémanentes qui sont captées par le foie.

Dans des circonstances normales, 95% des lipides alimentaires peuvent être digérés et absorbés par notre système digestif.

Figure 8. Digestion, absorption et transport des lipides (tiré de http://www.biodeug.com/master-1-physio-animale-chapitre-2-fonction-digestive/)



1.2.4 Synthèse des acides gras

1.2.4.1 Synthèse des acides gras saturés

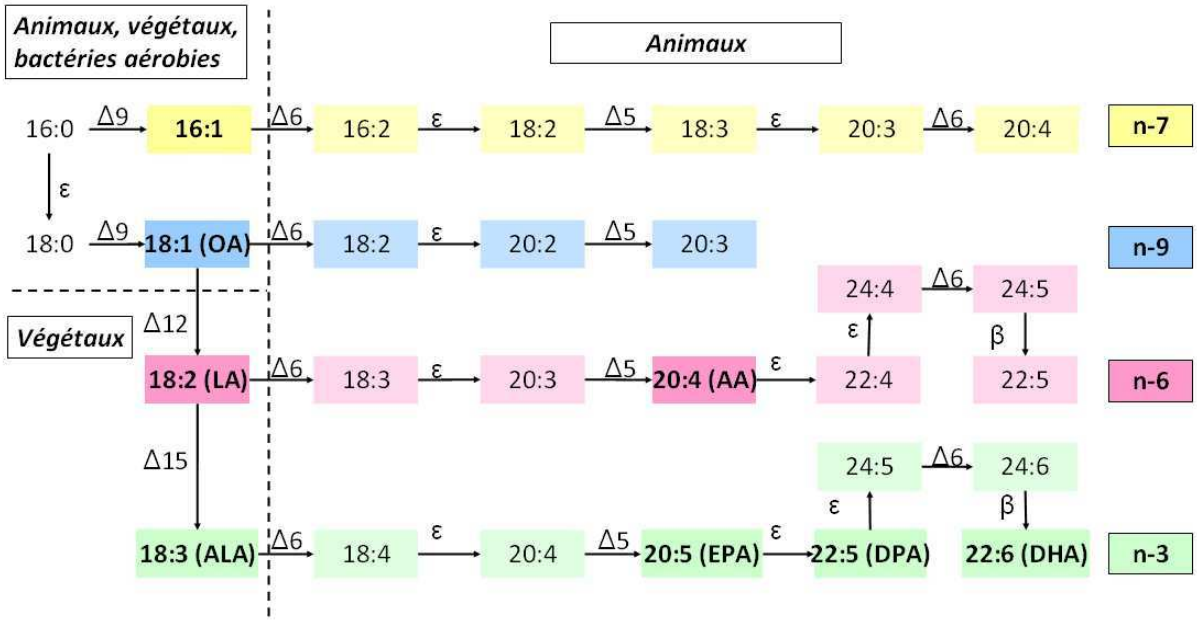
Les acides gras saturés peuvent également être synthétisés de novo par le foie, le cerveau, le tissu adipeux, et la glande mammaire au cours de la lactation (Legrand, 2007). L'importance de cette synthèse va dépendre de l'apport alimentaire. Ainsi, elle augmente lorsque l'apport calorique dépasse les besoins énergétiques ou lors d'une alimentation riche en hydrates de carbone. La synthèse de novo des acides gras ou lipogenèse a lieu dans le cytosol des cellules

des différents tissus. Les acides gras sont formés à partir de l'acétyl-coenzyme A (acétyl-CoA) qui provient de l'oxydation du pyruvate produit lors de la dégradation du glucose, de la dégradation des acides aminés, de l'oxydation de l'alcool et de la fermentation des fibres alimentaires.

1.2.4.2 Synthèse des acides gras monoinsaturés

Chez l'homme, une partie importante des acides gras saturés sont convertis en acides gras monoinsaturés. Une enzyme, la Δ9 désaturase, introduit une double liaison sur l'acide palmitique (C16:0) et sur l'acide stéarique (C18:0) pour former respectivement l'acide palmitoléique (C16:1 n-7) et l'acide oléique (C18:1 n-9) qui sont les deux principaux AGMI (Figure 9). L'acide palmitoléique est faiblement synthétisé par rapport à l'acide oléique qui est lui activement synthétisé par les cellules.

Figure 9. Biosynthèse des acides gras insaturés (adaptée d'après Legrand, 2007)



Le symbole Δ indique une désaturation, avec la place de la double liaison introduite repérée à partir du groupe carboxyle COOH, ε indique une élongation et β une β-oxydation.
Les familles n-7 et n-9 ne sont pas essentielles car leur précurseur est synthétisable par l'homme.
Les familles n-6 et n-3 constituent les acides gras essentiels.

1.2.4.3 Synthèse des acides gras polyinsaturés

Les principaux acides gras polyinsaturés proviennent de l'acide oléique (OA, 18:1 n-9) après une suite d'élongation et de désaturation (Figure 9). L'acide linoléique (LA, 18:2 n-6) et l'acide α-linolénique (ALA, C18:3 n-3) qui ne peuvent pas être synthétisés par l'homme, sont dits acides gras « indispensables », et doivent être apportés par l'alimentation. Il n'existe pas d'interconversion entre les deux familles. Elles partagent par ailleurs les mêmes enzymes pour métaboliser leurs acides gras à longue chaîne et entrent donc en compétition. Leurs dérivés, les acides gras à longue chaîne, sont considérés comme des acides gras « conditionnellement indispensables » puisque l'homme peut les synthétiser s'il dispose des acides gras précurseurs indispensables. Néanmoins, la conversion de LA et ALA en dérivés est peu efficace chez l'homme adulte (Astorg et *al.*, 2007). Des apports adéquats en AGPI à longue chaîne semblent donc importants pour maintenir un fonctionnement optimal des cellules.

1.2.5 Apports nutritionnels conseillés pour les acides gras

L'Agence française de sécurité sanitaire des aliments (AFSSA) s'est autosaisie en 2006 pour réactualiser les apports nutritionnels conseillés (ANC) pour les acides gras et a publié un avis en mars 2010 (AFSSA, 2010, saisine n° 2006-SA-0359). Les nouveaux ANC ont été définis en considérant les besoins physiologiques minimaux et les besoins physiologiques optimaux afin de retarder l'apparition des pathologies (prévention primaire) (Tableau 1). Les ANC sont établis pour le sujet adulte (homme ou femme) pour un apport énergétique de 2000 kcal. Les valeurs sont exprimées en pourcentage de l'apport énergétique sans alcool, excepté pour l'acide eicosapentaénoïque (EPA) et l'acide docosahexaénoïque (DHA) où elles sont exprimées en milligrammes.

Tableau 1. Apports nutritionnels conseillés pour les acides gras (AFSSA, 2010, saisine n°2006-SA-0359)

		Besoin physio-logique minimal*	Prévention du risque					ANC 2010
			Syndrome métabolique diabète-obésité	Pathologies cardio-vasculaires	Cancers : sein et côlon**	Pathologies neuro-psychiatriques	Autres pathologies : DMLA***	
Lipides totaux [a]		30 [b]	30-40	35-40 [c]	35-40	35-40 [d]	< 40	35-40 [c]
Acides Gras indispensables	C18:2 n-6	2	2 [e]	5	2 [e]	2 [e]	≤ 4 [f]	4 [g]
	C18:3 n-3	0,8	0,8 [e]	1 [h]	0,8 [e]	0,8 [e]	0,8 [e]	1 [h]
	C22:6 n-3	250 mg	500 mg	500-750 mg [i]	500 mg	≥ 200-300 mg	500 mg	250 mg
Acides Gras non indispensable	C20:5 n-3	-						250 mg [j]
	C12:0 + C14:0 + C16:0	-	-	≤ 8 [h]	-	-	-	≤ 8
	Acides Gras Saturés totaux	-	- [k]	≤ 12	≤ 12 [l]	-	-	≤ 12
	C18:1 n-9	-	-	≤ 20 [m]	-	-	-	15-20

Acide linoléique : C18:2 n-6, Acide α-linolénique : C18:3 n-3, Acide docosahexaénoïque : C22:6 n-3, Acide eicosapentaénoïque : C20:5 n-3, Acide laurique : C12:0, Acide myristique : C14:0, Acide palmitique : C16:0, Acide oléique : C18:1 n-9.

*correspond pour les acides gras à un apport nécessaire pour éviter tout syndrome de déficit alimentaire en acides gras indispensables. Ces recommandations assurent un bon fonctionnement de l'ensemble de l'organisme et notamment le développement et le fonctionnement cérébral. ** parmi les cancers étudiés, seules les études relatives aux cancers du sein et du côlon permettent d'établir des recommandations. *** parmi les maladies étudiées, seules les études relatives à la dégénérescence maculaire liée à l'âge (DMLA) permettent d'établir des recommandations. «-» absence de données bibliographiques permettant de conclure.

[a] Les valeurs ne s'appliquent que pour un apport énergétique proche de 2000 kcal et une balance énergétique équilibrée. [b] Un besoin minimum de 30 % paraît souhaitable pour assurer l'apport minimum en AGPI indispensables. De plus, il n'y a aucun bénéfice à descendre en deçà de 30 %. [c] Pour des apports de moins de 35 %, il n'y a pas de bénéfice établi pour la santé cardiovasculaire. [d] Les valeurs proposées pour la prévention des risques de maladies cardiovasculaires et de syndrome métabolique peuvent s'appliquer en l'absence de données spécifiques étant donnée la possibilité d'un lien pathogénique. [e] En l'absence de données spécifiques, le besoin physiologique s'applique. [f] Sur la base d'études d'observation qui montrent que des apports excessifs en acide linoléique, supérieurs à 2,5 % ou à 5,5 %, selon les études, sont associés à une disparition de l'effet bénéfique des AGPI n-3 LC. La valeur de 4 % a donc été prudemment choisie. [g] La valeur de l'ANC tient compte du fait qu'un certain nombre de données suggère une limite maximale d'apport en acide linoléique. [h] Cette donnée est déduite d'études épidémiologiques d'observation et non d'études d'intervention formelles. [i] Besoins en EPA+DHA pouvant atteindre 750 mg pour les sujets à haut risque cardiovasculaire (prévention secondaire). [j] Les données regroupant souvent les effets EPA + DHA, la valeur de 250 mg est donc obtenue par soustraction. [k] Absences de données cliniques cohérentes. [l] Données restreintes au cancer du sein. [m] Sur la base de la conjonction d'études épidémiologiques et de données cliniques suggérant une valeur limite d'apport.

1.3. Objectifs de la thèse

La peau est un des principaux lieux de stockage des graisses. Les AGMI et les AGPI n-3, de part leurs rôles supposés sur l'inflammation, pourraient être des candidats susceptibles de protéger la peau des effets néfastes liés aux radiations UV. Lorsque ce travail de thèse a débuté, deux études épidémiologiques avaient déjà été réalisées sur le lien entre AGMI et expression du vieillissement cutané (Purba et *al.*, 2001 ; Cosgrove et *al.*, 2007), et une étude sur les liens avec l'état cutané (Boelsma et *al.*, 2003). Depuis, en 2010, une nouvelle étude épidémiologique a été publiée où les liens entre AGMI et AGPI n-3 (EPA+DHA) et expression du vieillissement cutané ont été étudiés (Nagata et *al.*, 2010).

L'étude de Purba et co-auteurs (2001) conduite sur 453 hommes et femmes âgés de plus de 70 ans vivant en Australie et en Europe (Grèce et Suède) a mis en évidence un lien entre les apports en AGMI et le vieillissement actinique du dos de la main. Les apports en AGMI étaient corrélés négativement avec le vieillissement cutané. Dans cette même étude, un lien inverse a également été mis en évidence entre les apports en huile d'olive et le vieillissement cutané. L'étude de Cosgrove et co-auteurs (2007) réalisée sur 4025 femmes âgées de 40 à 74 ans vivant aux Etats-Unis a montré une association positive entre l'acide oléique et la sécheresse sénile. Dans l'étude de Nagata et co-auteurs (2010), 716 femmes âgées de 20 à 74 ans vivant au Japon ont été incluses. Un lien significatif entre apports en AGMI et élasticité de la peau a été mis en évidence et aucun lien n'a été mis en évidence avec les apports en AGPI n-3 (EPA+DHA). Enfin, l'étude de Boelsma et co-auteurs (2003) conduite sur 302 hommes et femmes âgés de 18 à 75 ans vivant au Pays-Bas a mis en évidence une association négative entre les apports en AGMI et l'hydratation de la peau, et a trouvé une association positive avec le pH cutané. Cependant, compte tenu du peu de données disponibles, des différents critères d'intérêt retenus pour apprécier le vieillissement cutané, les différentes zones de mesures et la variété des habitudes alimentaires des populations étudiées, la réalisation de nouvelles études est nécessaire afin de fournir des résultats supplémentaires et aider à statuer sur le rôle éventuel des AGMI et des AGPI-3 sur le vieillissement cutané.

Dans ce contexte, les objectifs de ce travail de thèse a donc été dans un premier temps d'étudier chez une large population d'hommes et de femmes de la cohorte SU.VI.MAX, d'âge

moyen, âgés entre 45 et 60 ans, le lien entre la sévérité du photo-vieillissement cutané au niveau du visage et les apports alimentaires en AGMI (globalement, puis selon les sources alimentaires). Le deuxième objectif a été d'étudier sur le même échantillon le lien entre le photovieillissement cutané et les apports en AGPI n-3 : acide α-linolénique (C18:3 n-3), acide eicosapentaénoïque (C20:5 n-3), acide docosapentaénoïque (C22:5 n-3), et acide docosahexaénoïque (C22:6 n-3).

2. Matériel et méthodes

2.1 Présentation de l’étude SU.VI.MAX

2.1.1 Objectifs de l’étude

L'étude SU.VI.MAX (acronyme pour « SUpplémentation en VItamines et Minéraux Anti-oXydants ») est une étude épidémiologique d’intervention nutritionnelle qui s’est intéressée aux grandes pathologies chroniques caractéristiques des pays industrialisés, réalisée à l’échelon national (Hercberg et *al.*, 1998a ; Hercberg et *al.*, 1998b).

L’objectif principal de l’étude SU.VI.MAX (1994-2002) était de mesurer l’impact d’une supplémentation en vitamines et minéraux anti-oxydants (6 mg de bêta-carotène, 30 mg de vitamine E, 120 mg de vitamine C, 100 µm de sélénium et 20 mg de zinc) sur l’incidence des cancers et des maladies cardio-vasculaires. L’étude réalisée était randomisée en double aveugle, testant l’effet d’une supplémentation journalière en minéraux et vitamines antioxydants à des doses nutritionnelles – 1 à 3 fois les apports nutritionnels recommandés versus placebo. L’attribution dans les groupes de traitement était stratifiée sur le sexe, l’âge, le tabagisme et le lieu de résidence. Le nombre de sujets nécessaire était estimé entre 12500 et 15000 selon les différentes hypothèses envisagées. Les trois principaux critères de jugement étaient la mortalité générale, l’incidence de cancers tous sites confondus et l’incidence des maladies cardiovasculaires ischémiques. L’objectif de cette étude était également de préciser les relations entre alimentation et santé.

2.1.2 Population

L’échantillon de la cohorte a été constitué selon un sexe ratio ♂/♀ de 0,6. Les hommes étaient âgés entre 45 et 60 ans et les femmes entre 35 et 60 ans en raison de l’âge relativement précoce auquel l’incidence des cancers fréquents chez la femme (sein et utérus) commence à s’élever. Le choix des tranches d’âge était fait pour d’une part, éviter les problèmes nutritionnels particuliers qui peuvent être liés au troisième âge, d’autre part, parce que le critère *Mortalité* est, chez des sujets relativement jeunes, d’interprétation simple et enfin, pour obtenir des sujets de classes d’âge où l’incidence des pathologies (critères de jugement) est suffisamment élevée.

2.1.3 Recrutement et inclusion

L'inclusion des sujets adultes volontaires dans l'étude a commencé en octobre 1994. 79976 candidats se sont portés volontaire suite à une campagne multimédia menée de mars à juin 1994. Un mailing contenant une information détaillé sur l'étude SU.VI.MAX, 15 capsules du traitement, un questionnaire et une demande de consentement avisé a été envoyé à chacun des candidats. Seulement 1/4 des dossiers (n=21481) ont été correctement renseignés et renvoyés. Pour être définitivement éligibles, les sujets devaient être dans la tranche d'âge définie, se déclarer ne pas être atteint de pathologie sévère qui puisse restreindre la participation pendant huit ans, ne prendre aucun supplément contenant des vitamines ou minéraux étudiés, ne manifester aucune inquiétude ou réticence à se conformer aux contraintes du protocole, en particulier à recevoir un placebo et n'exprimer aucune motivation ambiguë ou comportement obsessionnel concernant l'alimentation et la santé. Après vérification des critères d'inclusion 14412 ont été retenus. En réalité, seuls 13017 sujets ont été inclus entre octobre 1994 et avril 1995. Parmi eux, 270 sujets (2% ; dont 115 dans le groupe antioxydant et 155 dans le groupe placebo) ont rompu leur consentement le jour de l'enrôlement au sein de la cohorte ou dans les 3 jours qui ont suivi.

L'étude SU.VI.MAX. a été approuvée par le comité d'éthique pour les études sur l'homme (CCPPRB n°706) de Paris-Cochin, et le "Comité National Informatique et Liberté" (CNIL n°334641) qui implique que toutes les informations médicales sont confidentielles et anonymes.

2.1.4 Suivi des participants

Tout au long de l'étude des données cliniques et biologiques ont été collectées afin de pouvoir vérifier la compliance des sujets à la supplémentation et d'évaluer leur état de santé.

Une fois par an, un examen clinique ou biologique (en alternance) des participants a été réalisé par les équipes médicales de SU.VI.MAX. Chaque participant devait choisir parmi une des 65 principales villes Françaises pour réaliser sa visite annuelle. En fonction de la ville, la visite avait lieu soit dans un centre de médecine préventive soit dans une des deux unités mobiles SU.VI.MAX qui sillonnaient la France. Lors du bilan clinique, un certain nombre de tests de dépistage des cancers étaient réalisés (recherche de sang dans les selles, mammographie pour les femmes de plus de 50 ans, frottis cervical pour les femmes n'en ayant

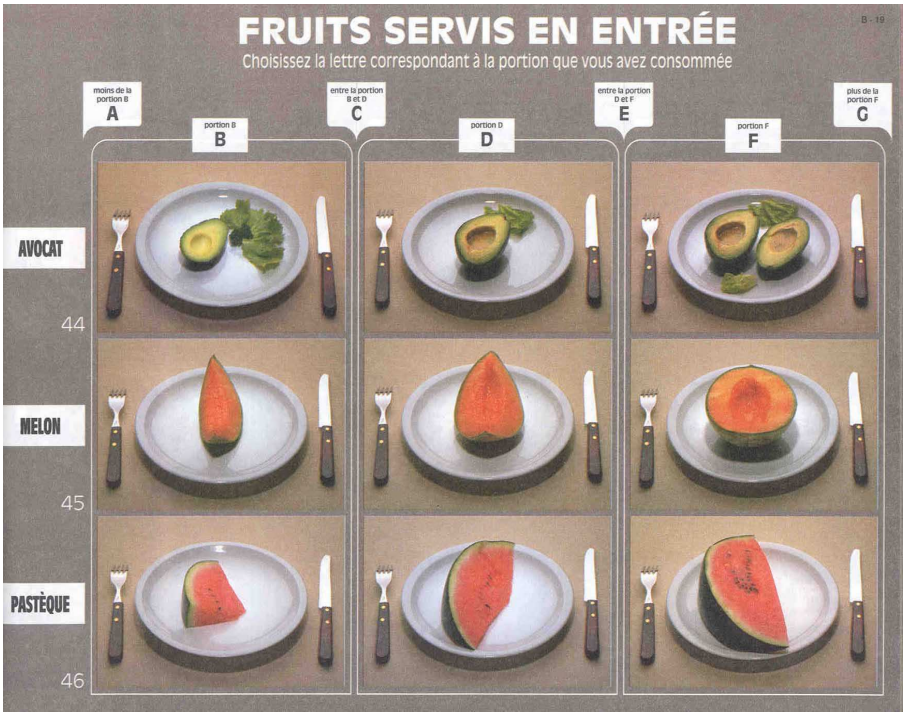
pas eu dans l'année…), ainsi qu'un électrocardiogramme, une mesure de la pression artérielle, un examen clinique et des mesures anthropométriques. En cas d'anomalie aux tests de dépistage, un contact se faisait avec les structures de soins en charge des sujets afin d'assurer le suivi des investigations complémentaires et documenter les diagnostics. Lors du bilan biologique, un prélèvement le matin à jeun de 35 ml de sang était réalisé afin d'effectuer le dosage de différents paramètres biologiques (bêta-carotène, rétinol, vitamine C, vitamine E, zinc et sélénium sériques, hémoglobine, glycémie, iodurie, cholestérol total, triglycérides, apolipoprotéines A1 et B). De plus, tous les événements liés à la santé étaient recueillis chaque mois. Toutes les consultations et hospitalisations étaient ainsi analysées et ont fait l'objet d'investigations détaillées par les médecins de l'équipe SU.VI.MAX, ce qui a permis de documenter les événements médicaux.

2.2 Données alimentaires

2.2.1 Enregistrement alimentaire de 24 heures

Les volontaires de la cohorte devaient transmettre tous les deux mois par minitel (6 fois par an), les données d'un questionnaire sur leurs apports alimentaires sur une période de 24 heures (« enregistrement alimentaire de 24 heures »). Les jours étaient répartis sur la semaine (deux jours pendant le week-end et quatre jours en dehors du week-end) et sur l'année (tous les deux mois) afin de tenir compte des variations de l'alimentation avec les jours de la semaine et avec les saisons et d'être ainsi le plus représentatif possible des apports alimentaires. Chaque questionnaire alimentaire comportait près de 900 items relatifs aux boissons et aliments consommés aux différents moments de la journée (y compris les collations). Les volontaires devaient indiquer les quantités consommées en se référant à un manuel d'instruction des données alimentaires spécialement développé pour l'étude SU.VI.MAX et validé lors d'un pré-test (Le Moullec et *al.*, 1996). Le manuel contient des photos représentant plus de 250 aliments représentés en trois portions. Les volontaires pouvaient faire des choix intermédiaires ou en dehors des deux portions extrêmes pour un total de 7 portions différentes (Figure 10).

Figure 10. Exemple du manuel d'instruction pour les données alimentaires



L'enregistrement alimentaire de 24 heures incluait également des questions sur le type d'huile ou de graisse utilisé pour l'assaisonnement et la cuisson. Les volontaires devaient également renseigner l'origine des aliments (surgelés, conserve…), les lieux de prise alimentaire (domicile, restaurant, cantine…) et les modes de préparation.

2.2.2 Table de composition

La teneur de l'alimentation des participants en énergie, en AGMI, en AGPI n-3 (ALA, EPA, DPA et DHA) et en vitamines E et C a été déterminée à l'aide d'une table de composition alimentaire qui a été développée pour l'analyse des données collectées dans l'étude SU.VI.MAX (Arnault et *al.*, 2006). Cette table a été compilée à partir de données de tables déjà existantes : la table de composition des aliments française (Favier et *al.*, 1995) et sa mise à jour (Ireland et *al.*, 2002), la table américaine US Department of Agriculture National Nutrient Database (US department of agriculture, 2003), la table britannique McCance & Widdowson's Food Composition Table (Ministry of Agriculture Fisheries and Food, 1998), sur des données fournies par l'Iterg et sur la base de publications originales. Cette table permet de disposer de la composition nutritionnelle en acide gras de 689 aliments simples et

301 recettes. La composition nutritionnelle est donnée pour 100 grammes d'aliments ou de recettes.

Puis, l'apport en AGMI et en AGPI n-3 a été déterminé en tenant compte de différentes sources alimentaires. Tout d'abord, une table de recettes développées par des diététiciennes a permis de décomposer les recettes en aliments simples. Chaque recette peut être décomposée jusqu'en 23 aliments simples et à chaque aliment simple correspond un pourcentage. Par exemple, un gâteau en chocolat est décomposé de : 27,47 % d'œuf dur, de 19,08 % de sucre blanc en poudre, de 19,08 % de beurre doux, de 19,08 % de chocolat noir, de 11,45 % de farine blanche, de 3,82 % de fécule de maïs et en 0,02 % de sel. Puis les aliments simples ont été regroupés dans les différentes catégories de sources alimentaires (annexe 1).

2.3 Evaluation du photo-vieillissement cutané

L'évaluation du photo-vieillissement au niveau du visage, a été réalisée par des médecins formés par un dermatologue, à l'occasion de la première campagne de recueil de données cliniques (1995-1996). Pour des raisons techniques, ce recueil a commencé trois mois après le début de la campagne. De ce fait, le photo-vieillissement a été évalué sur un sous-ensemble de la cohorte (3582 hommes et 4983 femmes). L'intensité du photo-vieillissement a été appréciée à l'aide d'une échelle photographique (Larnier et *al.*, 1994). La reproductibilité inter et intra-investigateur de cette échelle a été validée par les auteurs sur un groupe de femmes Caucasiennes (Larnier et *al.*, 1994).

Le photo-vieillissement (Figure 11) est décrit par six niveaux, chaque niveau étant illustré globalement par trois photographies présentant différents aspects du photo-vieillissement (rides, relâchement et lentigines). Comme les grades 1 et 6 étaient peu présentés dans notre population âgée de 45 à 60 ans, ces grades ont été regroupés avec les grades 2 et 5 (1-2: Léger ou Léger/Modéré, 3: Modéré, 4: Modéré/Important et 5-6: Important/Très important), respectivement.

Figure 11. Echelle photographique de photo-vieillissement à 6 niveaux (adaptée de Larnier et al., 1994)

2.4 Facteurs de confusion potentiels

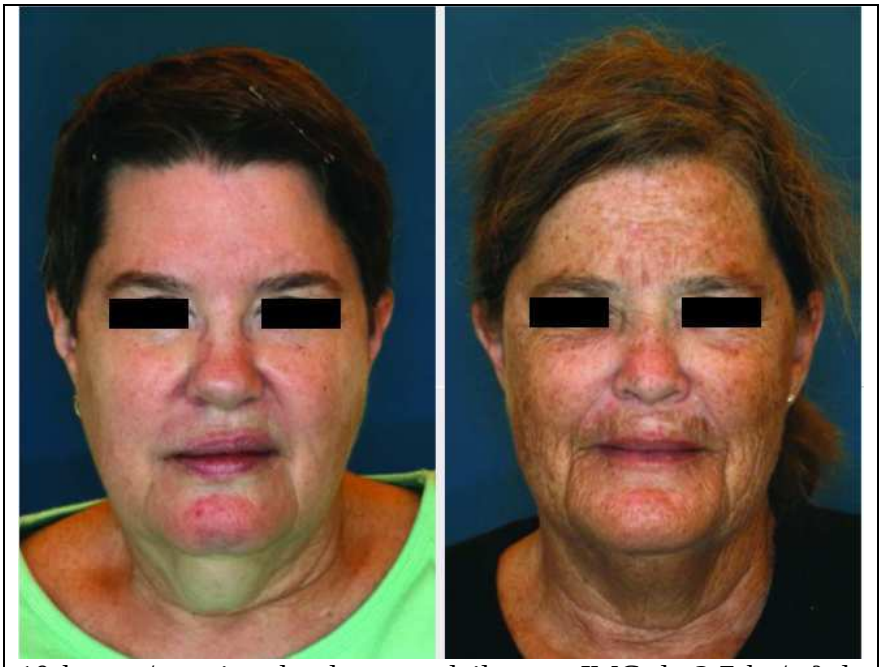
L'exposition au soleil, le phototype, et le tabagisme sont des facteurs de risque connus du photo-vieillissement cutané (Daniell, 1971 ; Kadune et *al.*, 1991 ; Ernster et *al.*, 1995 ; Malvy et *al.*, 2000 ; Rabe et *al.*, 2006 ; Yaar et Gilchrest, 2007). De même, la baisse de la production des hormones sexuelles avec la ménopause est rapportée accentuer le vieillissement cutané (Maheux et *al.*, 1994 ; Callens et *al.*, 1996 ; Dunn et *al.*, 1997). Un indice de masse corporelle (IMC) élevé a été également décrit comme diminuant le risque de vieillissement cutané (Purba et *al.*, 2001 ; *Guinot et al., 2002a*[1]). Une étude réalisée en 2006-2007, aux USA, lors du Twins festival de Twinsburg dans l'Ohio, sur 186 paires de vrais jumeaux a fourni une illustration spectaculaire des effets de ces différents facteurs à patrimoine génétique équivalent (Guyuron et *al.*, 2009). Des photographies ont été prises et un questionnaire sur le

[1] Voir liste des publications

mode de vie a été renseigné puis l'âge apparent a été apprécié à l'aide d'un panel de juge et l'impact du mode de vie sur l'âge apparent étudié.

La figure 12 illustre l'impact de l'exposition au soleil chez deux sœurs jumelles de 61 ans qui ont eu un comportement d'exposition différent. La sœur de droite a déclaré avoir eu en moyenne une durée d'exposition au soleil de 10 heures de plus par semaine que celle de sa sœur. Elle présentait également un IMC plus faible de 2,7 kg/m². Un écart d'âge moyen de 11 ans a été donné entre les deux soeurs.

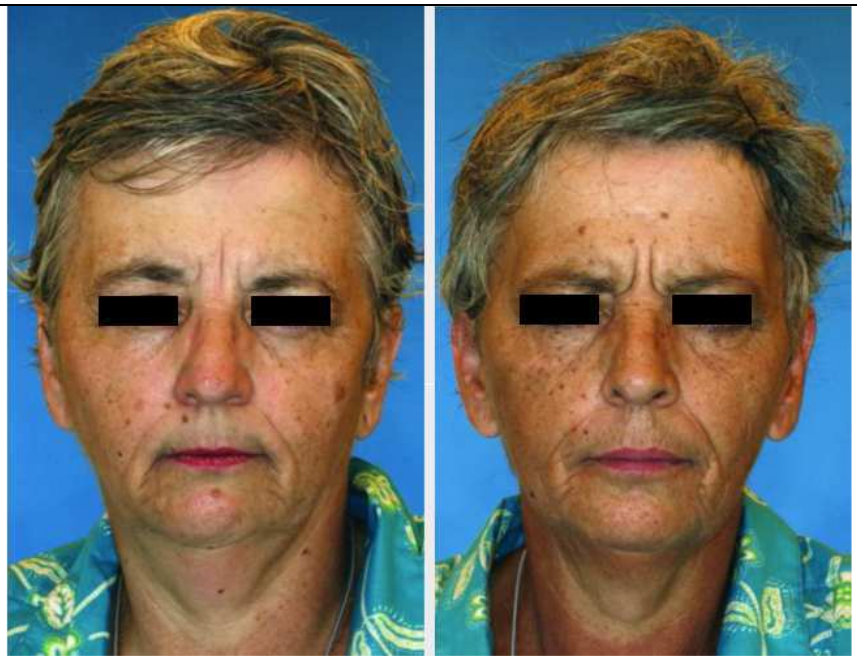
Figure 12. Illustration des effets du soleil (tirée de Guyuron et *al.*, 2009)



10 heures/semaine de plus au soleil et un IMC de 2,7 kg/m² de moins pour la jumelle de droite

L'effet du tabac sur le vieillissement cutané est également visible figure 13. Les sœurs ont 57 ans. La sœur de droite a fumé 40 ans alors que sa sœur n'a jamais fumé et a pris un traitement hormonal de substitution pendant 2 ans. Un écart d'âge perçu moyen de 8 ans a été donné par les juges.

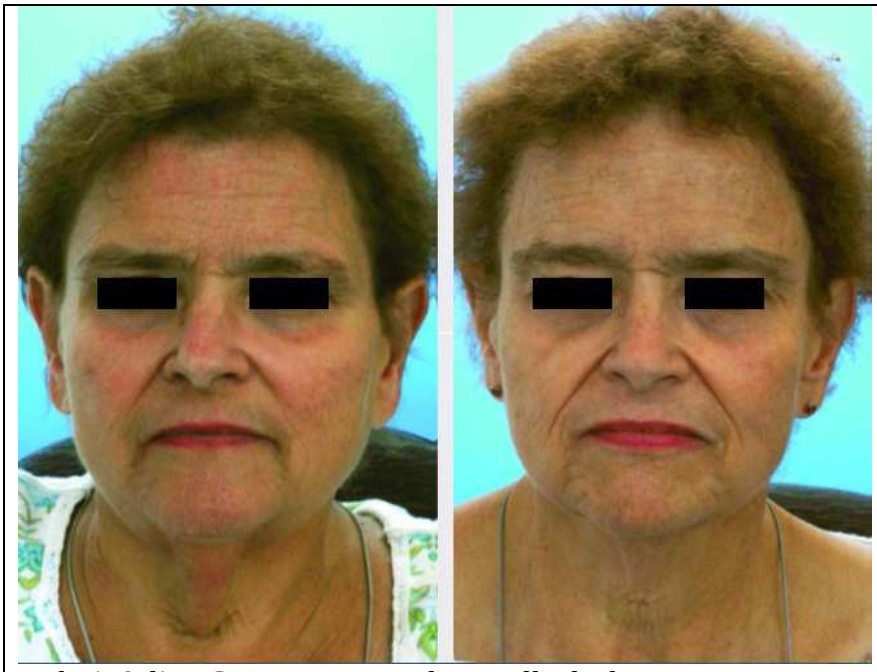
Figure 13. Illustration des effets du tabac (tirée de Guyuron et *al.*, 2009)



40 années de tabac pour la jumelle de droite
2 ans de traitement hormonal de substitution pour la sœur de gauche

L'impact de l'IMC est également illustré figure 14. Les sœurs ont 58 ans. La sœur de droite présente un IMC de près de 15 kg/m² de moins que celui de sa sœur. Un écart d'âge moyen de 5 ans a été donné par les juges.

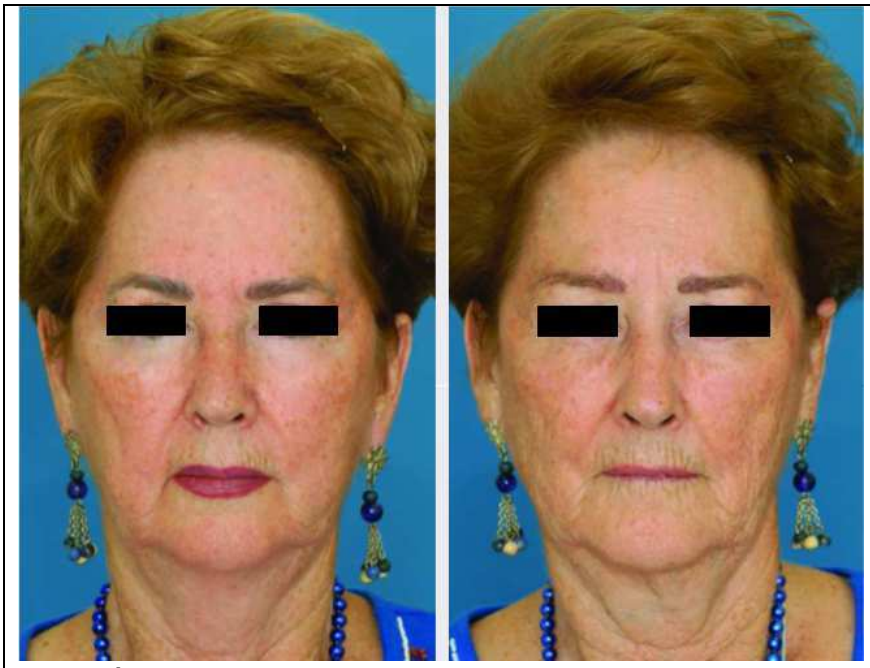
Figure 14. Illustration de l'effet de l'IMC (tirée de Guyuron et *al.*, 2009)



15 kg/m² d'IMC en moins pour la jumelle de droite

Et enfin, l'impact de la baisse des hormones sexuelles est présentée figure 15. Les sœurs ont 71 ans. La sœur de gauche a déclaré avoir pris un traitement hormonal de substitution pendant 22 ans. Un écart d'âge moyen de 7 ans a été donné entre les deux sœurs.

Figure 15. Illustration de l'effet du statut hormonal (tirée de Guyuron et *al.*, 2009)



22 années de traitement hormonal de substitution pour la jumelle de gauche

Le patrimoine génétique joue également un rôle important sur l'expression du photo-vieillissement (*Elfakir et al., 2010* ; *Ezzedine et al., 2012* ; *Le Clerc et al., 2012*). Ces données n'étant pas disponible sur l'ensemble de la cohorte, seul le phototype, reflet de la sensibilité naturelle de la peau au soleil a été pris en compte dans les analyses.

Par ailleurs, la vitamine E et la vitamine C sont des vitamines qui sont présentes dans la peau et qui peuvent jouer un rôle important dans la prévention du photo-vieillissement en stoppant la propagation de la peroxydation des acides gras (Gerrish et Gensler, 1993 ; Steenvoorden et van Henegouwen, 1997 ; Boelsma et *al.*, 2001 ; Sies et Stahl, 2004 ; Shapira, 2010). La vitamine E située dans les membranes cellulaires piège les radicaux générés et stoppe la transmission en chaîne de la réaction. La vitamine C joue un rôle essentiel dans la régénération de la vitamine E oxydée.

2.4.1 Données démographiques, socio-économiques et de style de vie

Le questionnaire rempli par le volontaire lors de leur inclusion dans l'étude (1994-1995) a permis de recueillir l'âge, la région d'habitation (code postal), le sexe, l'activité physique (irrégulière, 1 heure/jour ou > 1 heure/jour), le tabagisme (non fumeur, ex-fumeur, fumeur), le niveau d'éducation (primaire, secondaire, supérieur) et le statut hormonal (non ménopausée, ménopausée sans traitement hormonal de substitution, ménopausée avec traitement hormonal de substitution). Pour la région d'habitation, la France a été ensuite divisée arbitrairement en deux grandes parties : le Nord et le Sud, les zones Aquitaine, Limousin, Auvergne et Rhône-Alpes servant de frontières aux régions administratives pour le sud de la France. Les données anthropométriques (poids, taille, …) ont été mesurées lors du premier bilan clinique (1995-1996). Puis, l'indice de masse corporelle a été calculé en divisant le poids (kg) par la taille élevée au carré (m^2). L'IMC a ensuite été catégorisé en trois classes (<25 kg/m^2, 25-30 kg/m^2, ≥ 30 kg/m^2).

2.4.2 Phototype

La classification du phototype, reflet de la protection naturelle de la peau contre le soleil, a été mise au point progressivement et de façon empirique dans le milieu des années 70 par Fitzpatrick (Fitzpatrick, 1975) afin de fournir un outil simple, permettant d'estimer le risque individuel relatif à l'exposition solaire et d'énoncer des principes de protection adaptés (soustraction à l'exposition, usage d'écran…). La classification utilisée pour cette étude découle de celle proposée par Césarini en 1977 établie à partir d'un échantillon de Français (Césarini, 1977). Elle repose d'une part sur deux paramètres dynamiques : la fréquence de survenue de coups de soleil et l'intensité du bronzage, et d'autre part sur des données phénotypiques, reflet de la variabilité génétique des individus : la couleur de la peau en hiver, la couleur naturelle des cheveux à 20 ans et la présence de tâches de rousseur. Ces cinq paramètres fournissent une règle d'affectation utilisée pour déterminer le phototype des sujets en huit classes. Comme les niveaux I et VI étaient rares dans notre population, ils ont été regroupés avec les niveaux II and V, respectivement. Le phototype de Césarini a été déterminé en même temps que le photo-vieillissement lors du bilan clinique d'inclusion.

Tableau 2. Phototype de Césarini

Couleur des cheveux	Couleur De la peau	Taches de rousseur	Coup de soleil	Bronzage	Phototype
Blanc	Albinos	Non	Constant	Non	**0**
Roux	Laiteux	Nombreuses	Constant	Non	**I**
Blond	Clair	Nombreuses	Constant	Hâle léger	**II**
Blond	Clair	Quelques	Fréquent	Clair ou foncé	**IIIa**
Châtain	Mate	Quelques	Fréquent	Clair ou foncé	**IIIb**
Brun	Mate	Non	Rare	Foncé	**IV**
Brun	Mate	Non	Exceptionnel	Très foncé	**V**
Noir	Noir	Non	Absent	Noir	**VI**

2.4.3 Exposition au soleil

Un questionnaire sur l'exposition et la protection solaire a été développé spécifiquement pour l'étude SU.VI.MAX par un groupe de huit experts composés à la fois de dermatologues et d'épidémiologistes. Ce questionnaire portait sur l'exposition récente au soleil et au cours de la vie. Ce questionnaire a été envoyé à l'ensemble des volontaires de la cohorte en 1997 et 2001. Lors de la première enquête en 1997, 64% des questionnaires ont été renvoyés et 1332 volontaires qui n'avaient pas répondu lors de la première enquête ont répondu en 2001. Les données étaient donc disponibles pour 8084 volontaires. Ce questionnaire a été largement analysé et a donné lieu à différentes publications sur les comportements d'exposition (*Guinot et al., 2001a*, Ezzedine et al., 2007 ; Ezzedine et al., 2008 ; *Latreille et al., 2008*). En particulier, une série de scores ont été proposés (*Guinot et al., 2001a*) et une typologie de comportement solaire a été établie (*Latreille et al., 2008*). Dans nos analyses, différents scores ont été testés ainsi que la variable sur l'estimation global de l'exposition au soleil au cours de la vie posée dans le questionnaire (globalement durant votre vie, estimez-vous avoir été : pas ou peu, modérément ou beaucoup exposé(e) au soleil). Cette dernière a été conservée dans les analyses car elle fournissait des résultats similaires et est d'utilisation plus facile.

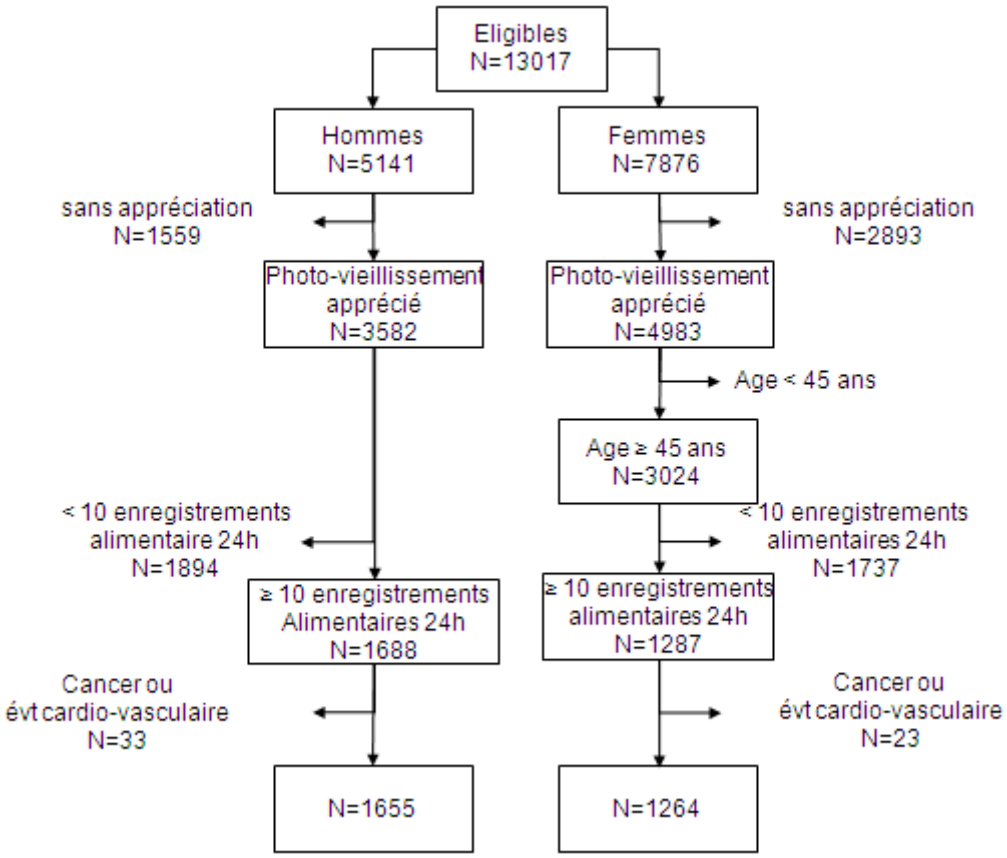
2.4.4 Apport en vitamine E et vitamine C

Les apports alimentaires renseignés dans les enregistrements 24h ont permis à l'aide de la table de composition alimentaire de SU.VI.MAX de calculer les apports alimentaires en vitamine E et C.

2.5 Population étudiée

Dans notre étude, parmi les participants dont la sévérité du photo-vieillissement avait été appréciée, nous avons sélectionné les hommes et les femmes de 45 à 60 ans qui avaient renseigné au moins 10 enregistrements 24h dans une période de 2 ans et demi après l'inclusion (Astorg et *al.*, 2004). Mennen et co-auteurs (2002) ont en effet montré que ce nombre était suffisant chez la population française pour estimer les apports habituels individuels des principales classes d'acides gras (AGS, AGMI et AGPI) avec une bonne précision. Les participants qui avaient développé un cancer ou présenté un événement cardiovasculaire pendant les deux en et demi d'étude de l'alimentation n'ont pas été inclus dans l'analyse (Figure 16).

Figure 16. Participants de la cohorte SU.VI.MAX retenus pour l'analyse



2.6 Méthodes statistiques

2.6.1 Introduction

Dans les études épidémiologiques qui examinent le lien entre les apports en nutriments et l'état de santé, la prise en compte dans l'analyse de l'apport énergétique total est importante car ce dernier peut être en lui-même un facteur de risque de l'état de santé. L'apport énergétique total est corrélé positivement avec la plupart des nutriments, soit parce que ceux-ci contribuent comme les macronutriments (lipides, glucides, protéines, éthanol) directement au calcul de l'apport énergétique, soit parce que les individus qui ont un apport énergique élevé mangent également en plus grandes quantités la plupart des aliments, et donc également plus de nutriments non énergétiques comme les vitamines, les minéraux et les fibres. Par ailleurs, l'apport énergétique total varie d'un individu à l'autre principalement en raison de trois facteurs prinpaux : l'activité physique, la corpulence, et l'efficacité métabolique (capacité de l'organisme à utiliser plus ou moins d'énergie pour absorber les nutriments et garder la température corporelle constante) (Willett, 1998). Les individus dont l'apport énergétique est supérieur ou est inférieur à la dépense énergétique présenteront une balance énergétique déséquilibrée qui entrainera une prise ou une perte de poids. Ainsi, sans ajustement sur l'apport énergétique total, un lien significatif entre les lipides et le risque de l'état de santé pourrait être le simple reflet d'une association avec l'activité physique, la corpulence ou le métabolisme. Il est donc primordial de s'assurer qu'une association observée entre les lipides et l'état de santé est indépendante de l'apport énergétique total.

Nous présenterons ci-après les principaux modèles nutritionnels qui ont été proposés ces dernières années pour essayer de séparer l'effet de l'apport énergétique total de l'effet spécifique d'un nutriment, puis nous introduirons les méthodes de régression logistique envisageable pour analyser ce type de données.

2.6.2 Modèles de nutrition

Quatre modèles nutritionnels qui permettent la prise en compte de l'apport énergétique ont été proposés dans la littérature : la méthode standard, la méthode des densités (Jovanovic et *al.*,

1994), la méthode des résidus (Willett et Stampfer, 1986) et la méthode de partition (Howe et *al.*, 1986). Parmi ces quatre modèles, les trois premiers sont des modèles de substitution isocalorique et le dernier est un modèle d'addition (ou de restriction) qui ne suppose pas que l'apport énergétique total soit fixé. Une revue de ces modèles peut être trouvée dans les articles de Willett et co-auteurs (1997) et Thiébaut et co-auteurs (2004).

2.6.2.1 Méthode standard (standard method)

Dans la méthode standard, l'apport énergétique total « NRJ_{total} » est pris en compte en l'incluant dans le modèle en plus de l'apport du nutriment « *Nutriment* » :

$$g(Y|Nutriment, NRJ_{total}) = \alpha_0 + \alpha_1 Nutriment + \alpha_2 NRJ_{total}$$

où la fonction g(.) dépend de la distribution de la variable réponse Y (normale, binomiale, multinomiale…) (McCullagh et Nelder, 1989).

Dans ce modèle, lorsque l'apport en nutriment et en énergie totale sont exprimés en kcal, α_1 correspond à l'effet d'augmenter de 1 kcal l'apport en nutriment en maintenant l'apport énergétique constant. Ce qui revient dans le cadre d'un macronutriment (par exemple, les lipides), à substituer 1 kcal d'apport aux autres macronutriments (glucides, protéines, éthanol). De même, α_2 n'est pas le risque associé à augmenter de 1 kcal l'apport énergétique total mais correspond à l'effet d'augmenter de 1 kcal l'apport des autres macronutriments quand le nutriment d'intérêt reste constant.

L'inconvénient majeur de ce modèle est d'introduire deux variables la plupart du temps très fortement corrélées, l'apport en nutriment et l'apport énergétique total, ce qui peut entrainer des difficultés d'estimation des paramètres des modèles et rendre difficile l'interprétation des résultats. Trois autres modèles ont donc été proposés pour palier ce problème de colinéarité.

2.6.2.2 Méthode des densités (nutrient density method)

La densité nutritionnelle d'un macronutriment correspond à la part d'énergie apportée par ce nutriment. Elle est calculée en divisant l'apport en nutriment par l'apport énergétique total : $\frac{Nutriment}{NRJ_{total}}$. La densité nutritionnelle peut s'exprimer soit en pourcentage d'énergie soit par exemple en apport pour 1000 kcal.

La méthode des densités peut soit inclure dans le modèle uniquement le terme de densité nutritionnelle : $g(Y|Nutriment, NRJ_{total}) = \beta_0 + \beta_1 \frac{Nutriment}{NRJ_{total}}$, soit inclure également l'apport énergétique total : $g(Y|Nutriment, NRJ_{total}) = \beta_0 + \beta_1 \frac{Nutriment}{NRJ_{total}} + \beta_2 NRJ_{total}$.

Dans le premier cas, l'interprétation du rapport $\frac{Nutriment}{NRJ_{total}}$ est difficile car une augmentation de celui-ci peut correspondre à un accroissement de l'apport en nutriment ou à une diminution de l'apport énergétique total. Dans le second cas, où l'apport énergétique total est fixé, une variation de la densité nutritionnelle $\frac{Nutriment}{NRJ_{total}}$ correspond alors à la seule variation de l'apport en nutriment.

Le coefficient β_1 de la densité nutritionnelle s'interprète, si la densité nutritionnelle est exprimée en pourcentage, comme l'effet d'augmenter l'apport en nutriment (par exemple, les lipides) de 1% de l'apport énergétique total quand l'apport énergétique total reste constant et donc à diminuer l'apport des autres macronutriments de 1%. Nous sommes ici comme dans la méthode standard dans le cadre d'un effet de substitution d'un nutriment par d'autres nutriments.

Le coefficient β_2 de l'apport énergétique total correspond, si celui-ci est exprimé en kcal, à l'effet d'augmenter de 1 kcal l'apport énergétique total lorsque la densité nutritionnelle reste constante, donc à augmenter l'apport en nutriment d'une quantité égale à $\frac{Nutriment}{NRJ_{total}}$ kcal et d'augmenter l'apport des autres macronutriments d'une quantité égale à $\frac{Autres\ Nutriments}{NRJ_{total}}$ kcal.

Dans ce modèle, la même quantité de nutriment ingéré n'a pas le même effet selon la quantité totale d'énergie ingérée, ce qui ne pose pas de problème en soi car il est attendu qu'une même quantité de nutriment consommée puisse jouer un rôle différent selon la corpulence, l'activité physique ou l'efficacité métabolique d'un individu.

Cette méthode a différents avantages. Les densités nutritionnelles sont calculées simplement sans avoir besoin de recourir à un modèle statistique comme dans la méthode des résidus que

nous verrons ci-après. Les nutritionnistes ont l'habitude de les utiliser comme mesures de la composition de l'alimentation et on les retrouve dans les recommandations nationales. Enfin, même s'il peut persister une certaine corrélation entre les deux paramètres, les problèmes de colinéarité rencontrés dans la méthode standard se retrouvent en général diminués car la corrélation est plus faible.

2.6.2.3 Méthode des résidus (residual method)

Cette méthode a été proposée par Willett et Stampfer (1986) comme une alternative à la méthode des densités pour pouvoir éliminer totalement la corrélation entre apport nutritionnel et apport énergétique total.

La première étape consiste à réaliser une régression linéaire simple de l'apport en nutriment sur l'apport énergétique total afin de calculer les apports résiduels :

$$Résidus_{nutriment} = Nutriment - (\hat{\alpha}_0 + \hat{\alpha}_1 NRJ_{total})$$

Les résidus de cette régression représentent la différence entre l'apport en nutriment d'un individu et l'apport en nutriment prédit par son apport énergétique total : $Nutriment_{individu\ i} - (\hat{\alpha}_0 + \hat{\alpha}_1 NRJ_{total,\ individu\ i})$.

L'apport résiduel est ensuite introduit dans le modèle avec l'apport énergétique total :

$$g(Y|Résidus_{Nutriment}, NRJ_{total}) = \gamma_0 + \gamma_1 Résidus_{nutriment} + \gamma_2 NRJ_{total}$$

Comme les apports résiduels sont par définition de moyenne nulle et peuvent présenter des valeurs négatives. Il peut donc être préférable d'ajouter une constante afin de se retrouver avec des apports alimentaires proches de ceux du nutriment considéré. Par exemple, la valeur prédite pour l'apport énergétique total moyen observé dans notre population = $\hat{\alpha}_0 + \hat{\alpha}_1 \times moyenne(NRJ_{total})$ peut être ajoutée, ou encore la valeur prédite pour un apport énergétique fixé à 2000 kcal = $\hat{\alpha}_0 + \hat{\alpha}_1 \times 2000$. L'ajout de cette constante ne modifie que la constante du modèle γ_0.

L'interprétation du coefficient γ_1 de l'apport résiduel est identique à l'interprétation du coefficient α_1 de l'apport en nutriment dans la méthode standard. En effet augmenter de 1 kcal l'apport résiduel en nutriment correspond à augmenter de 1 kcal la différence

$Nutriment - \hat{\alpha}_0 - \hat{\alpha}_1 NRJ_{total}$ alors que l'apport énergétique total reste constant donc d'augmenter de 1 kcal l'apport en nutriment. Nous sommes donc à nouveau dans un effet de substitution.

Le coefficient γ_2 de l'apport énergétique total mesure l'effet de l'augmentation de 1 kcal de l'apport énergétique total quand l'apport résiduel ($Nutriment - \hat{\alpha}_0 - \hat{\alpha}_1 NRJ_{total}$) reste constant, ce qui revient donc à augmenter les apports en nutriment de $\hat{\alpha}_1$, la pente de la régression de l'apport en nutriment sur l'apport énergétique total. Mais si l'apport énergétique total augmente de 1 kcal et celui du nutriment de $\hat{\alpha}_1$ kcal alors l'apport des autres macronutriments augmente également de (1 - $\hat{\alpha}_1$) kcal.

L'avantage de cette méthode réside dans l'absence de corrélation entre les apports résiduels et l'apport énergétique total qui permet de résoudre les problèmes d'estimation rencontrés dans la méthode standard. Par ailleurs, cette méthode apporte une idée de la variabilité de l'apport en nutriment indépendamment de l'apport énergétique total puisque la variance des apports résiduels correspond à la variabilité de l'apport en nutriment entre sujets ayant un même apport énergétique total.

Malgré l'indépendance entre l'apport résiduel en nutriment et l'apport énergétique total, il est préférable de conserver ce terme dans les modèles non linéaires tels que la régression logistique, en particulier lorsque l'apport énergétique total est un facteur de risque, car cela peut sinon biaiser l'estimation de l'effet du nutriment (Gail et *al.*, 1984).

2.6.2.4 Méthode de partition (energy partition method)

La dernière méthode couramment utilisée, la méthode de partition, est une alternative à la méthode standard et à la méthode des résidus. Elle a été proposée par Howe et co-auteurs (1986). L'apport énergétique du nutriment d'intérêt (par exemple les lipides) est introduit dans le modèle ainsi que l'énergie apportée par les autres nutriments :

$$g(Y|Nutriment, Autres\ nutriments) = \delta_0 + \delta_1 Nutriment + \delta_2 Autres\ nutriments$$

Dans ce modèle, le coefficient δ_1 peut être interprété, si les apports sont exprimés en kcal, comme l'effet d'augmenter de 1 kcal l'apport du nutriment quand l'énergie apportée par les

autres nutriments reste constant, donc l'effet d'additionner 1 kcal provenant du nutriment à l'apport énergétique total. De même, le coefficient δ_2 peut être interprété comme l'effet d'augmenter de 1 kcal l'apport des autres nutriments, quand l'énergie apportée par le nutriment reste constant. δ_2 mesure alors l'effet d'additionner 1 kcal provenant des autres nutriments à l'apport énergétique total.

En théorie, l'apport énergétique du nutriment n'est pas corrélé avec l'apport énergétique des autres nutriments, mais il est fréquent qu'en réalité une corrélation existe entre les deux paramètres car ces nutriments sont souvent fournis par les mêmes aliments. La corrélation est toutefois moindre qu'entre l'apport en nutriment et l'apport calorique total, mais elle peut engendrer les mêmes problèmes que ceux rencontrés avec la méthode standard.

2.6.2.5 Transformation des variables alimentaires et choix du modèle

Dans la pratique, l'apport en nutriment est souvent transformé en variable catégorielle. En effet, cette transformation permet à la fois d'exprimer des risques relatifs par rapport à différentes catégories d'apport en nutriment, de s'affranchir de l'hypothèse forte d'une relation linéaire entre l'apport en nutriment et l'état de santé, et enfin de limiter l'influence des valeurs extrêmes. Les catégories peuvent être définies soit selon des valeurs critiques connues du nutriment d'intérêt, soit selon la méthode des quantiles (par exemple, la médiane, les quartiles ou encore des quintiles). L'intérêt de la méthode des quantiles est qu'elle permet d'obtenir une répartition équilibrée des individus dans chacune des catégories et d'étudier relativement facilement des tendances. Dans notre cas et compte tenu de nos effectifs, les apports en nutriment ont été catégorisés grâce aux quartiles.

Le choix entre les différents modèles est guidé à la fois par le schéma expérimental et par les hypothèses testées. Dans le cadre de restriction calorique ou de supplémentation, le modèle de partition est particulièrement adapté car on suppose que l'apport énergétique total peut varier. Dans le cadre de prévention primaire, où l'alimentation d'un individu est supposée être modifiée pour diminuer les risques de maladie, les recommandations porteront en premier lieu sur des changements dans la composition de l'alimentation plutôt que sur le contenu total de l'alimentation car l'apport énergétique total dont un individu a besoin est relativement fixe. Les modèles de substitution isocalorique permettent de se placer dans le cadre de cette hypothèse de changement de la composition de l'alimentation. Parmi les trois modèles de substitution, le modèle standard est à éviter en cas de forte colinéarité entre le nutriment

d'intérêt et l'apport énergétique total. Entre la méthode des densités et la méthode des résidus, les résultats fournis sont en général très proches lorsque le nutriment d'intérêt est analysé de manière catégorielle (Brown et *al.*, 1994 ; Willett et *al.*, 1997 ; Hu et *al.*, 1999 ; Thiébaut et *al.*, 2004). Par allieurs, la méthode de densité est souvent préférée à la méthode des résidus car les recommandations nutritionnelles sont la plupart du temps exprimées en pourcentage d'apport énergétique. Dans notre cas, la méthode des densités a été retenue pour l'ensemble des analyses.

2.6.3 Modèles de régression logistique

La régression logistique est largement utilisée en épidémiologie car elle permet de calculer la probabilité d'un événement de santé en fonction des valeurs de différentes variables (Agresti, 1996 ; Hosmer et Lemeshow, 2000). Elle permet d'étudier la liaison entre une variable réponse (critère de jugement) qualitative Y (dichotomique, catégorielle ou ordinale) et un ensemble de p variables X_i explicatives qui peuvent être qualitatives et/ou quantitatives.

2.6.3.1 Régression logistique binaire simple

Dans le cas d'une régression logistique binaire simple où la variable réponse Y est dichotomique (par exemple, malade *vs* non malade) et une seule variable X (par exemple, exposé *vs* non exposé) est explicative, le modèle de régression logistique s'écrit :

$$\pi(x) = P(Malade = 1|x) = F(x)$$

Où, x est une valeur de X *et f* est la fonction logistique : $F(x) = \frac{e^{\beta_0+\beta_1 x}}{1+e^{\beta_0+\beta_1 x}}$

La fonction logistique est bien adaptée à la modélisation de probabilités puisqu'elle varie entre la valeur 0 et 1 selon une courbe en S (sigmoïde), et qu'elle permet de plus d'associer à chaque coefficient une statistique d'odds ratio (rapport des chances) qui est très utilisée en épidémiologie car interprétable en termes d'accroissement ou diminution du risque.

L'odds (cote) d'être malade (Y = 1) pour les personnes exposées (X=1) est égale à :

$$\frac{\pi(1)}{1-\pi(1)} = e^{\beta_0+\beta_1}$$

celle d'être malade (Y = 1) pour les personnes non exposées (X=0) est égale à : $\frac{\pi(0)}{1-\pi(0)} = e^{\beta_0}$

L'odds ratio (rapport de cotes) noté OR est alors défini par : $OR = \frac{\pi(1)/(1-\pi(1))}{\pi(0)/(1-\pi(0))} = \frac{e^{\beta_0+\beta_1}}{e^{\beta_0}} = e^{\beta_1}$

Il correspond donc au risque d'être malade chez des personnes exposées par rapport au risque d'être malade chez des personnes non exposées.

Le modèle logistique s'écrit souvent en introduisant la transformation Logit de $\pi(x)$: $g(\pi(x)) = \log\left[\frac{\pi(x)}{1-\pi(x)}\right] = \beta_0 + \beta_1 x$, le modèle logistique apparait alors comme un modèle linéaire.

L'estimation des paramètres du modèle est faite par la méthode du maximum de vraisemblance et différentes méthodes permettent de tester l'apport de la variable X au modèle : le test de Wald, la méthode du rapport de vraisemblance et le test du score.

2.6.3.2 Régression logistique binaire multiple

Le modèle de régression logistique binaire multiple généralise la régression logistique binaire simple au cas où plusieurs variables sont explicatives $X_{1,\ldots,}$ $X_{p.}$ Si l'on note $x = (x_1,\ldots, x_n)$ une valeur de $X=(X_1,\ldots, X_p)$, le modèle s'écrit alors :

$$\pi(x) = P(Y = 1 | X = x) = \frac{e^{\beta_0+\beta_1 x_1+\ldots+\beta_1 x_p}}{1+e^{\beta_0+\beta_1 x_1+\ldots+\beta_1 x_p}}$$

La régression logistique binaire multiple permet de tester les effets conjoints des différentes variables explicatives et également les éventuelles interactions entre les variables explicatives en ajoutant ces termes d'interaction dans le modèle. Une interaction entre deux variables X_1 et X_2 peut être testée en ajoutant la variable $X_{12} = X_1X_2$ dans le modèle. Cette interaction existe si la relation de l'une des variables explicatives avec la variable réponse Y dépend de la valeur de l'autre variable explicative. Une interaction entre deux variables est appelée une interaction du premier ordre. De la même façon, une interaction entre 3 variables peut être prise en compte (interaction d'ordre deux), entre 4 variables (interaction d'ordre trois), etc. Cependant l'interprétation des interactions d'ordre élevé est souvent délicate et nécessite des

échantillons de taille assez grande et bien répartis pour avoir une bonne estimation des coefficients des variables d'interaction.

2.6.3.3 Régression logistique ordinale à odds proportionnels

Le modèle de régression logistique peut-être étendu au cas où plusieurs variables sont explicatives et où la variable réponse Y à J modalités (> 2) est ordinale.

Le modèle le plus couramment utilisé est le modèle logit cumulatif à odds proportionnels (proportional odds model) McCullagh (1980).

Ce modèle relie linéairement le logit cumulé $\log\left[\frac{P(Y \leq j|x_1,...,x_p)}{P(Y> j|x_1,...,x_p)}\right]$ aux variables explicatives $X_{1,...,}$ X_p et il s'écrit : $\log\left[\frac{P(Y \leq j|x_1,...,x_p)}{P(Y> j|x_1,...,x_p)}\right] = \alpha_j + \beta_1 x_1 + ... + \beta_p x_p$

où, $P(Y \leq j|x_1,...,x_p)$ est la probabilité cumulée que la réponse Y soit inférieure ou égale à j, α_j est l'intercept associé à la modalité j qui satisfait la condition $\alpha_1 \leq \alpha_2 \leq \ldots \leq \alpha_{J-1,}$ et les β_1, β_2, …, β_p sont les paramètres associés aux différentes variables X.

Le modèle est appelé à « odds proportionnels » car $\log(OR)$, β_k, associé à la variable X_k ne dépend pas de la valeur *j* de Y et permet d'obtenir un seul OR par variable explicative. Ce modèle repose donc sur l'hypothèse d'égalité des pentes qui doit donc être testée.

2.6.3.4 Régression logistique ordinale à odds partiellement proportionnels

Lorsque cette hypothèse d'égalité des pentes est rejetée, un modèle à odds partiellement proportionnels peut être utilisé. Ce modèle autorise certaines variables qui ne respectent pas cette hypothèse à présenter des log(*OR*) différents selon la valeur *j* des modalités et de conserver l'hypothèse de proportionnalité des risques pour les autres variables (Peterson et Harrell, 1990) :

$$\log\left[\frac{P(Y \leq j|x_1,...,x_p)}{P(Y> j|x_1,...,x_p)}\right] = \alpha_j + \beta_1 x_1 + ... + \beta_k x_k + \beta_{k+1,j} x_{k+1} + ... + \beta_{p,j} x_p$$

Ici, l'hypothèse d'égalité des pentes n'est pas rejetée pour les variables X_1 à X_k et est rejetée pour les variables X_{k+1} à X_p.

L'approche GEE (generalized estimating equation: Liang et Zeger, 1986) de la procédure GENMOD du logiciel SAS ® (SAS institute INc., Cary, NC, version 9.1.3) permet d'ajuster un modèle à « chances partiellement proportionnelles » (Stokes et *al.*, 2000 ; Droesbeke et *al.*, 2005).

2.6.3.5 Choix entre un modèle de régression logistique ordinale à odds proportionnels et à odds partiellement proportionnels

Dans la pratique, dans le cadre d'une variable réponse ordinale à J modalités, un modèle de régression logistique ordinale à odds proportionnels est d'abord testé à l'aide de la statistique du Score (par exemple procédure LOGISTIC du logiciel SAS ®). Lorsque cette hypothèse est rejetée, un modèle à odds partiellement proportionnels peut alors être envisagé. Dans ce modèle, l'interaction entre chaque variable explicative et les différents types de logit est testée. Si un terme d'interaction est significatif, cela signifie que l'effet de la variable explicative n'est pas le même selon le type de logit. Dans ce cas, l'hypothèse de proportionnalité des risques est rejetée pour la variable explicative et l'on conserve l'interaction dans le modèle. Par contre si le terme d'interaction n'est pas significatif, la proportionnalité des risques n'est pas rejetée pour la variable explicative et l'interaction est alors supprimée du modèle. Dans les analyses conduites, cette procédure a été suivie.

3. Résultats

3.1 AGMI et photo-vieillissement cutané

Dietary monounsaturated fatty acids intake and risk of skin photoaging
Latreille J, Kesse-Guyot E, Malvy D, Andreeva V, Galan P, Tschachler E, Hercberg S, Guinot C, Ezzedine K
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Résumé

Le vieillissement cutané est un enjeu important en termes de santé publique puisqu'il est intimement lié à la survenue des cancers cutanés (Yaar et Gilchrest, 2007 ; Richmond-Sinclair et *al.*, 2010). Ces dernières années, de nombreuses études ont été menées pour mettre en évidence l'éventuel effet photoprotecteur sur la peau de différents micronutriments issus de l'alimentation (Sies et Stahl, 2004) mais relativement peu d'attention a été portée aux lipides. Lorsque nous nous sommes intéressés aux liens éventuels entre le photo-vieillissement et les AGMI, seulement deux études épidémiologiques (Purba et *al.*, 2001 ; Cosgrove et *al.*, 2007) avaient déjà entrepris d'étudier ce lien.

Notre étude porte sur un échantillon de 1264 femmes et 1655 hommes, âgés de 45 à 60 ans, qui avaient répondu à au moins 10 enregistrements alimentaires de 24h et pour lesquels le photo-vieillissement cutané avait été apprécié visuellement à l'inclusion. Le choix du modèle de nutrition a été guidé par l'idée qu'une modification de la composition de l'alimentation peut prévenir le vieillissement cutané. De ce fait, un modèle de substitution isocalorique a été retenu pour l'analyse des liens. Les méthodes des densités et des résidus testés tour à tour ont donné des résultats très similaires. Les apports recommandés en AGMI étant fourni en pourcentage d'apport énergétique total, le modèle de densité a été sélectionné. Des analyses séparées ont été réalisées pour les hommes et les femmes. Les apports en AGMI ont été exprimés en pourcentage d'apport énergétique total, puis catégorisés selon les quartiles. Une série de régressions logistiques ordinales à odds partiellement proportionnels a été ensuite effectuée pour étudier les liens entre ces apports et la sévérité du photo-vieillissement.

Après ajustement sur les éventuels facteurs de confusion, un lien inverse significatif a été mis en évidence entre les apports en AGMI et le photo-vieillissement chez les hommes. Plus les

apports sont élevés et plus le risque de photo-vieillissement est faible : 4 ème quartile (Q4, les plus gros consommateurs en pourcentage d'apport énergétique total) *versus* le 1er quartile (Q1) : ORA=0,76, $IC_{95\%}$ [0,57-1,00], p = 0,03. Lorsque les analyses ont été réalisées en fonction des sources d'AGMI, seul un lien inverse avec les AGMI des huiles végétales a été mis en évidence pour chacun des sexes (pour les femmes : 0,63 [0,44-0,90], p = 0,01 ; pour les hommes : 0,55 [0,40-0,76], p = 0,0004). Parmi les trois huiles les plus consommées (tournesol, olive et arachide), les apports en huile d'olive ont été les seuls à être significativement liés avec le photo-vieillissement (pour les femmes : 0,69 [0,50-0,95], p = 0,03; pour les hommes : 0,58 [0,43-0,77], p = 0,0002). Aucune association significative n'a été trouvée avec les autres sources d'AGMI (produits laitiers, viandes et charcuterie).

Les résultats de cette étude ont permis de mettre en évidence un éventuel rôle protecteur de la consommation d'huile d'olive dans la prévention du photo-vieillissement.

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Dietary Monounsaturated Fatty Acids Intake and Risk of Skin Photoaging

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Abstract

Background: Intake of monounsaturated fatty acids has been reported to reduce oxidative stress, insulin resistance and related inflammatory processes and may thus protect from skin photoaging. The objective of this study was to investigate the association between the risk of photoaging, monounsaturated fatty acids intake and the sources of monounsaturated fatty acids.

Methodology/Principal Findings: A cross sectional study was conducted within the framework of the SUVIMAX cohort. The survey included 1264 women and 1655 men aged between 45 and 60 years old. Dietary monounsaturated fatty acids intakes were estimated by dietary source through at least ten 24-h diet records completed during the first 2.5 years of the follow-up period. Severity of facial skin photoaging was graded by trained investigators at baseline during a clinical examination using a 6-grade scale illustrated by photographs. A lower risk of severe photoaging was associated with higher intakes of monounsaturated fatty acids from olive oil in both sexes. Strikingly, no association was found with intake of monounsaturated fatty acids from animal sources whether from dairy products, meat or processed meat.

Conclusion/Significance: These findings support the beneficial effect of dietary olive oil or healthy diet habits associated with olive oil consumption on the severity of facial photoaging.

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Introduction

In the past century, life-expectancy has increased in most developed countries [1]. Changes to the appearance of the skin represent a visible sign of tissue alteration that occurs with age [2]. More specifically, skin aging is an important public health issue as it may result in the development of a large range of morbidities including non melanoma skin cancers [3].

Skin aging is driven by both intrinsic and extrinsic factors. Intrinsic aging, also referred to as chronological skin aging, is an ineluctable process [4], due to genetically determined loss of cell function with age. Intrinsic skin aging is characterized by fine wrinkles, and dry, thin and pallid skin [5,6]. Extrinsic skin aging overlays intrinsic aging and is dependent on environmental and behavioral factors, in particular sun exposure. Extrinsic aging is characterized by solar elastosis, actinic keratosis, pigmentation and vascular abnormalities. The ultimate stage of this process is skin cancer, namely basal cell carcinomas and squamous cell carcinomas [7–9]. The main factor responsible for extrinsic aging is ultraviolet radiation and is thus referred to as skin photoaging. Skin damage, which may in part be reversible, is mainly driven by the production of reactive oxygen species (ROS) and related inflammation occurring in response to cumulated or intermittent intense sun exposure. Exposure to UVB damages DNA directly through generation of cyclobutane pyrimidine dimers and 6-4 photoproducts in keratinocytes and melanocytes, whereas UVA damages more indirectly through generation of ROS, leading to lipid peroxidation, activation of transcription factors (NF-kB and AP-1) and DNA strand breaks.

Numerous studies have focused on the possible role of diet in the capacity of the skin to resist damage induced by UV radiation [10]. Although the skin is a major fat storage organ in humans, data on the impact of lipid intake on skin physiology are limited. Low fat intake has been proposed to protect from photodamage [11]. In particular, monounsaturated fatty acids (MUFA) have been reported to reduce oxidative stress, insulin resistance and related inflammation [12–15].

In this context, we have performed an analysis within the framework of the SU.VI.MAX cohort designed to explore possible associations between the severity of facial skin photoaging, MUFA intake and the sources of MUFA. Photoaging was measured using a 6-grade scale specially developed and validated for assessing the

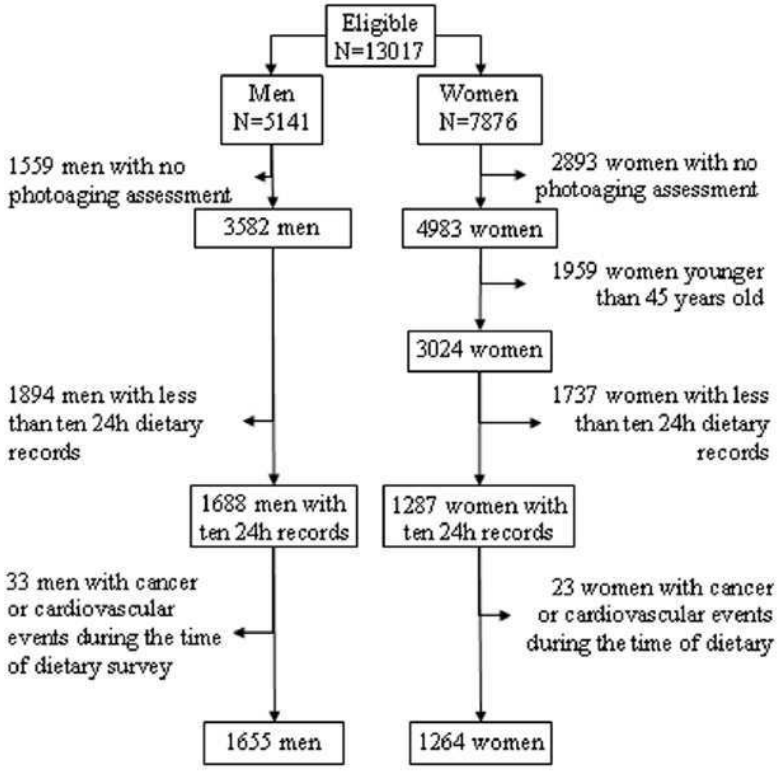

Figure 1. Flow chart of participants from the SU.VI.MAX study cohort retained in the analysis.
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overall severity of photodamage, including pigmentation abnormalities, wrinkling and tissue slackening [16].

Materials and Methods

Study Population

Subjects were participants in the SU.VI.MAX (*Supplémentation en Vitamines et Minéraux Antioxydants*) study, a double blind, placebo-controlled primary prevention trial evaluating the effect of antioxidant supplementation (a mixture of vitamin C, vitamin E, β-carotene, zinc, and selenium) on the incidence of ischemic heart diseases and of cancers in a population of adult men and women. A total of 13,017 volunteers, 7876 women and 5141 men were included in 1994–1995 with a planned follow-up of eight years. Men were aged 45–60 years and women 35–60 years at enrolment. The design, objectives and methodology of the study have been described *in extenso* elsewhere [17]. All subjects gave their informed written consent to the study. The study was approved by *ad hoc* ethical committees, the "*Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale*" (CCPPRB no. 706, Cochin Hospital, Paris, France), and the "*Commission Nationale de l'Informatique et des Libertés*" (CNIL no. 334641). The study was registered at clinicaltrials.gov as NCT00272428 [18].

Dietary Assessment

In order to take into account possible seasonal and weekly intra-individual variations in dietary intake, subjects were asked to complete a 24-h dietary record every two months from the inclusion to the end of the study for a total of six records per year (two weekend days and four weekdays per year). Dietary data were collected using the Minitel Telematic Network, a small terminal that was widely used as an adjunct to the telephone in France at the beginning of the study in the 1990s. This 24h record included about 900 items relating to food and drink for each of three meals (breakfast, lunch, and dinner) and for four other possible occasions for food intake (snacks). For each item, the subjects were requested to indicate the portion size consumed. To improve the quality of data collected, subjects received an instruction manual at the start of the study, including photographs of three portion sizes of about 250 foods and drinks. The use of the manual has been validated elsewhere [19]. The questionnaire also included questions on the type of oil or fat used for seasoning and cooking. Other details of these diet diaries have been published previously [17,20]. Subjects who completed at least ten records over a period of 2.5 years after inclusion were selected for this analysis. Ten records were considered to be sufficient to estimate the individual intake of monounsaturated fatty acids (FA) with acceptable accuracy [21]. Finally, all subjects who developed a cancer or a cardiovascular event during the course of the dietary survey (2.5 years) were not included in the analysis (Fig. 1).

Food Composition Table

Food composition was determined using the SU.VI.MAX food composition table [22] with respect to energy and MUFA. This table was compiled from existing tables, notably the French food composition table [23] and recent updates [24], the USDA National Nutrient Database [25] and the British McCance & Widdowson's food composition table [26], as well as from original

Table 1. Demographic, medical and behavioral characteristics according to photoaging severity.

		Women (n = 1264)					Men (n = 1655)				
		Photoaging grades					Photoaging grades				
		1–2	3	4	5–6		1–2	3	4	5–6	
Characteristics		**(n = 193)**	**(n = 555)**	**(n = 402)**	**(n = 114)**	**P-value**	**(n = 212)**	**(n = 744)**	**(n = 574)**	**(n = 125)**	**P-value**
Age (years)		48.8 (3.5)	50.6 (4.1)	53.2 (4.0)	56.5 (3.7)	<0.0001	48.3 (4.2)	51.2 (4.4)	53.9 (3.9)	57.9 (2.6)	<0.0001
BMI (kg/m^2) (%)	<25	76	79	72	72	0.13	54	50	50	53	0.60
	[25–30[	16	15	22	20		40	44	44	38	
	>=30	8	5	6	8		6	6	6	8	
Phototype* (%)	I-II	3	3	6	5	0.18	2	2	1	5	0.0001
	IIIa	14	12	10	9		12	7	8	6	
	IIIb	52	53	49	52		37	45	40	35	
	IV	29	25	29	27		45	41	42	42	
	V–VI	1	5	5	6		4	4	9	13	
Lifetime sun	None – Low	8	9	10	11	0.88	13	10	13	8	0.26
exposure* (%)	Moderate	58	58	59	55		59	60	61	58	
	High	33	28	29	30		28	29	26	34	
Overall physical	Irregular	29	24	25	16	0.10	22	24	21	14	0.21
activity* (%)	<1 h/day	34	34	36	31		27	24	23	19	
	≥1 h/day	37	42	40	54		52	53	56	66	
Smoking habits* (%)	Never smokers	62	66	63	75	0.24	34	38	35	35	0.67
	Former smokers	26	25	27	17		55	52	55	50	
	Smokers	11	8	9	8		11	11	10	15	
Hormonal status and	Non-menop	74	59	37	13	<0.0001					
MHT intake*,‡ (%)	Menop with MHT	20	30	46	56						
	Menop without MHT	6	12	16	31						
Educational	Elementary school	22	23	23	25	0.38	27	25	25	31	0.0181
level* (%)	Secondary school	45	41	48	47		36	33	36	47	
	University or equivalent	33	35	29	27		37	41	39	22	
Geographic	North of France	67	66	72	75	0.12	71	67	65	77	0.0267
location† (%)	South of France	33	34	28	25		29	33	35	23	

Values are expressed as means (SD) or percentages. Differences in demographic, medical and behavioral characteristics between photoaging grades were examined using analysis of variance for continuous variables (age) and the chi^2 test for categorical variables.
*Due to possible missing values the sum of the cell frequencies can be smaller than the total indicated in the top of the columns.
†France has been arbitrarily divided in north and south using the northern frontier of Aquitaine, Limousin, Auvergne, and Rhône-Alpes regions.
‡MHT: menopausal hormone therapy.
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publications. In addition, vitamin E and vitamin C intake was estimated using the SU.VI.MAX food composition table. The MUFA intake from each food category for each individual was then estimated as follows. Each complex dish was first broken down into each of its constitutive simple food items (for example, pies into butter, milk…) using a recipe table validated by dietitians. Then, simple food items were grouped into food group, such as vegetable oils, dairy products, meats and processed meats.

Outcome Variable

The severity of facial skin photoaging was assessed at baseline by trained investigators using a six-grade ordinal scale, each grade being depicted by three photographs to illustrate the diversity and the range of manifestations within each grade [4,16]. Due to the restricted age range of our population (middle-aged individuals) grades 1 and 6 were rarely present, thus these extreme grades were grouped with grades 2 and 5, respectively. The outcome variable was thus expressed in four grades of severity (grades 1–2, 3, 4 and 5–6).

Covariates

Data on age, geographical location (postcode), smoking habits (never, former, current), physical activity (irregular, less than 1h of walking per day, more than one hour of walking per day), educational level (primary school, secondary school or higher education), and hormonal status (non-menopausal, menopausal with use of menopausal hormone therapy, menopausal without use of menopausal hormone therapy) were collected through a self-administrated questionnaire at inclusion. Height and weight were measured using standardised procedures in subjects wearing undergarments. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters, squared), and categorized into three groups: <25 kg/m^2, 25–30 kg/m^2, ≥30 kg/m^2. For geographical location, France was arbitrarily

Table 2. Dietary factors according to photoaging severity.

	Women (n = 1264)					Men (n = 1655)				
	Photoaging grades					Photoaging grades				
	1–2	3	4	5–6		1–2	3	4	5–6	
Dietary factors	**(n = 193)**	**(n = 555)**	**(n = 402)**	**(n = 114)**	**P-value**	**(n = 212)**	**(n = 744)**	**(n = 574)**	**(n = 125)**	**P-value**
Monounsaturated fat (% TEI)	15.5 (2.4)	15.5 (2.8)	14.8 (3)	15.1 (3.4)	0.0106	14.8 (2.9)	14.7 (2.9)	14.5 (2.8)	13.9 (2.8)	0.0005
Monounsaturated fat from										
Dairy (%TEI)	4.2 (1.7)	4.2 (1.7)	4.3 (1.7)	4.4 (1.5)	0.73	4.1 (1.5)	4.0 (1.6)	4.0 (1.8)	4.0 (1.5)	0.61
Meat (%TEI)	1.1 (0.8)	1.0 (0.9)	1.0 (0.8)	1.0 (0.9)	0.12	1.3 (1.1)	1.2 (0.9)	1.1 (0.9)	1.1 (0.8)	0.28
Processed meats (%TEI)	1.1 (1.1)	1.1 (1.3)	1.0 (1.3)	1.1 (1.4)	0.35	1.6 (1.5)	1.4 (1.5)	1.4 (1.4)	1.4 (1.6)	0.31
Vegetable oils (%TEI)	3.9 (2.5)	3.7 (2.3)	3.6 (2.0)	3.7 (2.0)	0.14	3.4 (1.9)	3.4 (2.1)	3.3 (2.1)	2.8 (2.0)	0.01
Olive oil (g/day)	6.4 (4.4)	5.8 (4.8)	5.5 (4.4)	5.8 (4.6)	0.04	7.4 (5.8)	7.2 (5.2)	7.0 (5.4)	5.6 (4.1)	0.003
Peanut oil (g/day)	1.8 (1.6)	1.8 (1.9)	1.6 (1.6)	1.7 (1.4)	0.45	2.4 (1.8)	2.2 (2.1)	2.1 (2.0)	2.1 (1.6)	0.29
Sunflower oil (g/day)	4.9 (3.6)	5 (4.3)	4.9 (3.9)	4.9 (3.5)	0.93	6.1 (4.3)	6.1 (4.6)	6.1 (5.5)	6.1 (4.4)	0.90
Energy intake (MJ/day)	7.7 (2.2)	7.7 (2.6)	7.6 (2.4)	7.2 (2.5)	0.23	10.5 (3.1)	10.4 (3.2)	10.4 (3.2)	10.8 (2.4)	0.42
Vitamin E (mg/day)	11.7 (5.1)	11.6 (5.6)	11.4 (5.8)	11.4 (4.4)	0.73	13.5 (5.0)	13.7 (5.9)	13.7 (6.8)	14.1 (5.4)	0.49
Vitamin C (mg/day)	83.0 (47.5)	89.1 (54.2)	88.8 (55.5)	96.4 (51.5)	0.17	95.5 (56.2)	91.2 (57.1)	92.3 (62.2)	88.5 (56.3)	0.50

Values are medians (IQR). Differences in dietary factors between photoaging grades were assessed using the Kruskal-Wallis test. TEI, Total energy intake.
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divided into North and South areas using the northern frontier of Aquitaine, Limousin, Auvergne, and Rhône-Alpes regions. In addition, skin phototype was determined at baseline according to the classification proposed by Césarini: I, II, IIIa, IIIb, IV, V, VI [27]. Levels I and VI, which were rare in our population, have been grouped with levels II and V respectively. In addition, lifetime sun exposure was collected using the following question: "How would you describe the intensity of your skin's exposure to the sun during your lifetime?" none/mild, moderate, or severe [28,29].

For the present analysis, we included 1264 women and 1655 men aged between 45 and 60 years old, from both placebo and intervention groups, with data for dietary intake and skin photodamage (Fig. 1).

Statistical Analyses

Separate analyses were conducted for each sex. First, nutrient density was calculated by expressing MUFA as a percentage of total energy intake (TEI) and this nutrient density was then categorized into quartiles. Individual MUFA densities from the main food sources and the intake of the three most frequently consumed oils containing large amounts of MUFA were similarly categorized into quartiles.

Due to the ordinal nature of the outcome variable [30], a partial proportional odds model (PPOM) was used to study the relationship between photoaging and MUFA density independent of total energy intake [31]. The model was adjusted for total energy intake, vitamin E and vitamin C intake, age, and other possible confounders (covariates). Results are expressed as estimated odds ratios (ORs) with their 95% confidence intervals (95% CI) for each quartile with respect to the first quartile as the reference. In addition, a trend for linearity was tested by assigning each subject the median value of their quartile, this value being modeled as a continuous variable. In addition, to study the contributions of specific sources of MUFA independent of total MUFA intake, similar models were performed with respect to the dietary origin of MUFA adjusted on the same covariates, as well as for total MUFA density. Finally, associations between the severity of photoaging and the three most consumed oils (olive oil, sunflower oil and peanut oil) were investigated using the same methods.

All tests were two-sided and type I error was set at $P<0.05$. Statistical analyses were carried out using SAS® software release 9.1.3 (SAS Institute, Cary, NC, USA).

Results

The distribution of the women and men enrolled according to photoaging severity and by demographic, medical and behavioral variables is presented in Table 1. As expected, the severity of skin photoaging was strongly linked to age in both sexes, with non-menopausal women presented less severe photoaging. In men, a higher severity of skin photoaging was associated with a lower education level, a higher phototype and a higher latitude (North of France). In both sexes, daily intakes of MUFA were lower among the most severe grades of photoaging than among the lowest grades of photoaging (Table 2). Similar associations were found for intake of MUFA from vegetable oils and olive oil.

After adjustment for possible cofounders, a significant association was found in men between severity of photoaging and dietary intake of MUFA (Table 3). Higher intake of MUFA was associated with a lower risk of severe photoaging (highest *vs* lowest quartile of MUFA: AOR = 0.76, 95%CI (0.57–1.00), p = 0.03). For both sexes, a higher consumption of MUFA provided by vegetable oils was found to be associated with a lower risk of severe photoaging (for women: 0.63 (0.44–0.90), p = 0.01; for men: 0.55 (0.40–0.76), p = 0.0004). No association was found with MUFA intake from dairy products, meats and processed meats. Finally, of the three most frequently consumed oils (sunflower, olive and peanut oil), a significant association was found for olive oil. A higher intake of olive oil was significantly associated with a lower risk of severe photoaging (for women: 0.69 (0.50–0.95), p = 0.03; for men: 0.58 (0.43–0.77), p = 0.0002). In our population, olive oil was the main source of vegetable oil MUFA (59% and 51%, respectively), whereas sunflower and peanut oils provided only 15% and 13% of vegetable oil MUFA.

Table 3. Risk of photoaging according to lipid intakes.

Fat intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-value*
Women (n = 1204)					
Monounsaturated fat (% TEI)	<13.9	[13.9–15.3[	[15.3–16.7[	≥16.7	
AOR [95% CI]†	1.00 (ref)	0.93 (0.70–1.23)	0.86 (0.65–1.15)	0.88 (0.65–1.19)	0.37
Monounsaturated fat from					
Dairy (%TEI)	<3.5	[3.5–4.3[	[4.3–5.2[	≥5.2	0.12
AOR [95% CI]‡	1.00 (ref)	1.10 (0.84–1.44)	1.13 (0.84–1.51)	1.27 (0.95–1.70)	
Meat (%TEI)	<0.7	[0.7–1.0[	[1.0–1.5[	≥1.5	0.17
AOR [95% CI]‡	1.00 (ref)	1.00 (0.77–1.31)	0.88 (0.67–1.16)	0.84 (0.63–1.12)	
Processed meats (%TEI)	<0.6	[0.6–1.1[	[1.1–1.8[	≥1.8	0.62
AOR [95% CI]‡	1.00 (ref)	0.98 (0.74–1.29)	0.85 (0.65–1.13)	0.95 (0.71–1.26)	
Vegetable oils (% TEI)	<2.7	[2.7–3.7[	[3.7–4.9[	≥4.9	0.009
AOR [95% CI]‡	1.00 (ref)	0.99 (0.75–1.32)	0.92 (0.67–1.25)	0.63 (0.44–0.90)	
Olive oil (g/day)	<3.8	[3.8–5.8[	[5.8–8.4[	≥8.4	
AOR [95% CI]†	1.00 (ref)	0.87 (0.66–1.15)	0.89 (0.65–1.20)	0.69 (0.50–0.95)	0.03
Peanut oil (g/day)	<1.0	[1.0–1.7[	[1.7–2.7[	≥2.7	
AOR [95% CI]†	1.00 (ref)	1.02 (0.77–1.34)	0.91 (0.67–1.24)	0.94 (0.69–1.27)	0.59
Sunflower oil (g/day)	<3.2	[3.2–4.9[	[4.9–7.3[	≥7.3	
AOR [95% CI]†	1.00 (ref)	1.19 (0.90–1.57)	1.12 (0.82–1.52)	1.13 (0.79–1.61)	0.67
Men (n = 1566)					
Monounsaturated fat (% TEI)	<13.2	[13.2–14.6[	[14.6–16.0[	≥16.0	
AOR [95% CI]†	1.00 (ref)	0.89 (0.69–1.16)	0.76 (0.58–0.99)	0.76 (0.57–1.00)	0.03
Monounsaturated fat from					
Dairy (%TEI)	<3.2	[3.2–4.0[	[4.0–4.8[	≥4.8	
AOR [95% CI]‡	1.00 (ref)	0.87 (0.67–1.13)	1.10 (0.85–1.42)	1.09 (0.84–1.42)	0.28
Meat (%TEI)	<0.7	[0.7–1.1[	[1.1–1.6[	≥1.6	
AOR [95% CI]‡	1.00 (ref)	1.16 (0.90–1.48)	0.99 (0.77–1.28)	1.00 (0.77–1.30)	0.76
Processed meats (%TEI)	<0.8	[0.8–1.4[	[1.4–2.3[	≥2.3	
AOR [95% CI]‡	1.00 (ref)	1.04 (0.81–1.33)	0.98 (0.75–1.27)	1.09 (0.83–1.44)	0.62
Vegetable oils (% TEI)	<2.4	[2.4–3.3[	[3.3–4.4[	≥4.4	
AOR [95% CI]‡	1.00 (ref)	0.71 (0.54–0.92)	0.61 (0.46–0.81)	0.55 (0.40–0.76)	0.0004
Olive oil (g/day)	<4.7	[4.7–7.1[	[7.1–10.0[	≥10.0	
AOR [95% CI]†	1.00 (ref)	0.84 (0.64–1.09)	0.81 (0.62–1.06)	0.58 (0.43–0.77)	0.0002
Peanut oil (g/day)	<1.4	[1.4–2.2[	[2.2–3.3[	≥3.3	
AOR [95% CI]†	1.00 (ref)	1.10 (0.85–1.42)	0.85 (0.66–1.11)	0.80 (0.61–1.06)	0.09
Sunflower oil (g/day)	<4.0	[4.0–6.1[	[6.1–8.7[	≥8.7	
AOR [95% CI]†	1.00 (ref)	0.84 (0.65–1.09)	0.92 (0.70–1.21)	0.95 (0.68–1.33)	0.99

TEI, Total energy intake,
*Probability of Wald test for linear trend.
†AOR [95% CI]: Adjusted odds ratio and 95% confidence interval adjusted for age, educational level, smoking status, overall physical activity, body mass index, hormonal status, lifetime sun exposure, phototype, geographic location, vitamin E and C intakes and energy.
‡Adjusted for the same covariates plus total monounsaturated fat intake (%TEI).
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Discussion

In this study we report a significant association between total intake of MUFA and skin photoaging in men but not in women. When the individual contribution of each source of MUFA was considered, higher intakes of MUFA from vegetable oil were however found to be negatively associated with severe skin photoaging independently of environmental factors known to cause premature and accelerated skin aging in both sexes, whereas intake of MUFA from animal products (dairy products, meat and processed meats) was not significantly associated with skin photoaging. In particular, a higher consumption of olive oil was inversely correlated with the severity of skin photoaging. Olive oil was the only one of the three vegetable oil sources of MUFA usually consumed in our study population (olive oil, sunflower oil and peanut oil) to present such a protective effect. These findings are consistent with previous studies which have addressed individual aspects of this relationship [32–34]. Hence, Purba et al. [33] reported a negative association between total MUFA intake, olive oil intake and skin aging, whereas Nagata et al. [34]

found a positive association between MUFA intake and skin elasticity. In contrast, another study did not find any association between oleic acid consumption and wrinkled appearance and even reported a higher risk of senile dryness in higher consumers [32]. However, in these two last studies, fatty acids were considered as a whole, without taking into account their specific origin.

The observed negative association between olive oil intake and severe photoaging may be due to its specific fatty acid profile with a high amount of MUFA and a low ratio of n-6 PUFA/n-3 PUFA [35,36]. Indeed, MUFA is far less susceptible to peroxidation than PUFA. In contrast to olive oil, we did not find dairy products to be negatively associated with skin photodamage although they provide comparably high amounts of MUFA to olive oil. However, dairy products are also a rich source of saturated fatty acids, which are known to be associated with insulin resistance and an increase of inflammatory processes [37]. Another hypothesis would be that squalene and polyphenols contained in olive oils may play a role in preventing photodamage [35,36]. Squalene is to a large extent sequestered in the skin (sebum is reported to contain 12%), where it is believed to exert a major protective effect against free radical damage and skin dryness [36]. Polyphenols are also known to be powerful radical scavengers. Both squalene and polyphenols have been assumed to be primarily responsible for the beneficial effects of the Mediterranean diet. Finally, as expected, the consumption of olive oil in our population was also positively associated with high consumption of fruits, vegetables, fish and tea, and negatively associated with sweet products, butter and milk. In that sense, the consumption of olive oil could also be considered as a marker of a healthy diet [38].

Our study has both strengths and limitations. The strengths encompass the assessment of dietary intake based on a mean of ten computerized 24-hour diet records in order to take into account weekly and seasonal intra-individual variability in the intake of the monounsaturated fatty acids, which may be considerable [21]. Furthermore, a validated manual containing a photographic guide was provided to each subject in order to facilitate estimation of portion size among seven proposed portion sizes [19]. Our study was also a well-characterized sample of middle-aged women and men living in the community evaluated. Finally, due to the number of statistical tests performed, we cannot exclude that some significant associations found may be due to chance, in spite of the overall coherence between findings in men and in women. Moreover, the cross-sectional design of our study does not allow us to address the causality of the associations observed.

In conclusion, our findings provide support for a beneficial role of olive oil in preventing severe facial photoaging. This result should be supported by further mechanistic studies taking into account the relationship between a diet rich in MUFA, subcutaneous fat and the overall aging process, of which skin photodamage may be a readily observable surrogate marker. Finally, our findings provide a useful insight into the beneficial effect of olive oil, as the main source of dietary fat, as promoted in a diversified diet, although the only way to demonstrate that olive oil can prevent photodamage is to perform an interventional study.

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Author Contributions

Conceived and designed the experiments: JL KE SH CG. Performed the experiments: PG EKG. Analyzed the data: JL. Wrote the paper: JL CG KE EKG DM. Provided advice and critically reviewed the manuscript: PG VA ET SH.

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3.2 AGPI n-3 et photo-vieillissement cutané

Association of n-3 polyunsaturated fatty acids dietary intake with severity of skin photoaging in middle-aged Caucasian population

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Résumé

Les AGPI n-3 à longues chaînes (AGPI-LC n-3), en particulier l'acide eicosapentaénoïque (EPA, C20:5 n-3) et l'acide docosahexaénoïque (DHA, C22:6 n-3) sont connus pour moduler l'inflammation (Calder, 2009 ; Galland, 2010). Chez l'homme, une supplémentation à dose relativement élevée de ces acides gras (entre 4 et 10 grammes) a permis de mettre en évidence un effet protecteur à la fois contre les dommages induits par les UVB et dans la modulation de l'expression de marqueurs génotoxiques précoces des cancers cutanés (Orengo et *al.*, 1992 ; Rhodes et *al.*, 1994 ; Rhodes et *al.*, 2003). Le rôle des AGPI n-3 sur l'expression du vieillissement cutané n'a été étudié que dans une seule étude épidémiologique publiée récemment (Nagata et *al.*, 2010).

Notre étude porte sur les liens entre AGPI n-3 et photo-vieillissement cutané étudiés comme pour les AGMI à l'aide d'une série de régressions logistiques ordinales à odds partiellement proportionnels chez les 1264 femmes et 1655 hommes, âgés de 45 à 60 ans, qui avaient répondu à au moins 10 enregistrements alimentaires de 24h et pour lesquels le photo-vieillissement cutané avait été apprécié à l'inclusion.

Après ajustement sur les éventuels facteurs de confusion, un lien inverse significatif a été mis en évidence entre les apports en ALA et le photo-vieillissement chez les hommes : 4 ème quartile (Q4, les plus gros consommateurs en pourcentage d'apport énergétique total) *versus* le 1er quartile de ALA : AOR=0,65, $IC_{95\%}$ [0,49-0,87], p = 0,004). Un lien significatif a été également mis en évidence entre les apports en EPA et le photo-vieillissement chez les femmes (0,69 [0,52-0,91], p = 0,04). Les ALA et les DPA provenant de différentes sources,

les analyses ont été réalisées en tenant compte de l'origine de ces acides gras, contrairement aux EPA et DHA fournis essentiellement par les poissons et les crustacés.

Pour les hommes, une consommation plus élevée en ALA fournis par les huiles végétales et les fruits et légumes était associée avec un risque moindre de présenter un photo-vieillissement élevé (huiles végétales : 0,72 [0,53-0,99], p = 0,04 et fruits et légumes : 0,73 [0,53-0,98], p = 0,04, respectivement). Chez les femmes, les apports en ALA des huiles végétales tendent également à être inversement liés au photo-vieillissement (0,77 [0,56-1,07], p = 0,06). Aucun lien n'a été trouvé avec les ALA des produits laitiers pour chacun des sexes. Aucun lien n'a été également mis en évidence avec les DPA des différentes sources.

Cette étude a permis de mettre en évidence un lien entre la consommation de ALA et l'expression du photo-vieillissement cutané qui n'avait pas été étudié précédemment dans les études sur le vieillissement cutané (Purba et *al.*, 2001 ; Cosgrove et *al.*, 2007 ; Nagata et *al.*, 2010). Ces résultats suggèrent un éventuel rôle bénéfique de la consommation quotidienne d'AGPI n-3 sur l'expression du photo-vieillissement.

*Cover Letter

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Paris, January 25th 2013,

Dear Pr Akimichi Morita,

We would like to submit the enclosed manuscript *"Association of n-3 polyunsaturated fatty acids intake with severity of skin photoaging in middle-aged Caucasian population"* for publication in the *Journal of the Dermatological Science.* We believe this paper will be of interest to your readers since it is the first study, to te best of our knowledge, that has reported a link between dietary intake of n-3 polyunsaturated fatty acids (n-3 PUFAs) and skin aging.

We examined the association between dietary n-3 PUFA intake (overall and according to food sources) and facial skin photoaging after adjustment for potential confunders. We found that severe photoaging was inversely associated with higher intake of α-linolenic acid (ALA) in men and with higher intake of eicosapentaenoic acid (EPA) in women. These findings suggest a possible beneficial effect of n-3 PUFA on skin aging.

The manuscript has been reviewed by a native English speaker prior to submission. The data in the manuscript are original and the manuscript is not under consideration for publication elsewhere. None of the contents have been published previously except in abstract form. All authors have read and approved all versions of the manuscript, its content, and its submission to the *Journal of the* Dermatological Science. We propose Myrto Trakatelli and Djavad Mossalayi as recommended reviewers.

We look forward to hearing from you and thank you for your consideration.

Yours faithfully,

Julie Latreille

*Manuscript
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Association between dietary intake of n-3 polyunsaturated fatty acids and severity of skin photoaging in a middle-aged Caucasian population

Running head: n-3 PUFA intake and skin photoaging

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Conflict of interest:

The authors declare no conflict of interest. No funding sources

SUMMARY

Background: Intake of long-chain n-3 polyunsaturated fatty acid (PUFAs) supplementation has been reported to be associated with reduced UVB-erythemal sensitivity, but their relationship to photoaging has not been studied to date.

Objective: To investigate associations between daily n-3 PUFA intake and the severity of skin photoaging.

Methods: A cross-sectional study was conducted on 2919 subjects aged 45-60 years from the SU.VI.MAX cohort. At baseline, trained investigators graded the severity of facial skin photoaging using a validated 6-grade scale during a clinical examination. Intake of α-linolenic (ALA), eicosapentaenoic (EPA), docosapentaenoic (DPA), and docosahexaenoic acids (DHA) were evaluated by dietary source using ten 24-h dietary record questionnaires during the first 2.5 years of the follow-up period.

Results: After adjustment for possible confounders, severe photoaging was found to be inversely associated with higher intake of ALA in men and with higher intake of EPA in women. When considering the different food sources of ALA for men, an inverse association appeared between severe photoaging and ALA from vegetable oils, as well as with ALA from fruit and vegetables, whereas no association was observed for ALA from dairy products. In women, ALA from vegetable oils also tended to be inversely linked to photoaging.

Conclusions: These findings suggest a possible benefit effect of n-3 PUFAs on skin aging. Nonetheless, further epidemiological studies are necessary to confirm our results and to gain additional insights into underlying mechanisms.

Key words: dietary intake; α-linolenic acid; eicosapentaenoic acid; docosapentaenoic acid; docosahexaenoic acid; skin photoaging

1. Introduction

Skin photoaging results from a combination of intrinsic and extrinsic aging factors [1]. Intrinsic aging is an inevitable process due to the natural degeneration of cell functioning that occurs with age and is genetically determined. Clinically, intrinsic aging is characterised by fine wrinkles and by dry, thin and pallid skin. On the contrary, extrinsic aging is dependent on environmental and behavioural factors and may be considered reversible [2]. It is characterized by solar elastosis, actinic keratosis, pigmentation and vascular abnormalities [1, 3]. Ultraviolet radiation, the main factor responsible for extrinsic aging, leads to production of reactive oxygen species (ROS) in the skin and associated inflammation, which alter the functioning of the skin [4]. As a consequence, photoprotection by micronutrients from the diet, such as carotenoids, ascorbate and tocopherol or polyphenolic compounds has received much attention over the last years [5]. In contrast, the possible involvement of lipid intake in photoaging has been poorly investigated, although long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs), in particular eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) have been suggested to modulate skin inflammation[6]. Consistently, supplementation with high doses of the n-3 PUFAs, EPA and DHA acids has been found to protect from UVB-induced damage and to modulate the expression of early genotoxic markers of human skin cancer [7-9].

α-Linolenic acid (ALA) is the precursor of the n-3 PUFA family and is considered as an essential fatty acid because it cannot be synthetized by mammals. LC n-3 PUFAs (eicosapentaenoic (EPA), docosapentaenoic (DPA), and docosahexaenoic acids (DHA) are derived from ALA, but the extent of conversion in humans is low [10]. Therefore in humans, the diet constitutes the main source of n-3 PUFAs. ALA is provided by

different food sources (vegetable oils, green leafy vegetables, nuts and animal fats) whereas LC n-3 PUFAs are mainly derived from foods from marine source [11].

In this context, we have examined, within the framework of the French SU.VI.MAX cohort, possible associations between the severity of skin photoaging and the n-3 PUFAs dietary intakes, as well as the dietary sources of n-3 PUFAs.

2. Materials and methods

2.1. Study population

Subjects were participants in the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) study, a randomized, double-blind, placebo-controlled, primary prevention trial evaluating the effect of daily antioxidant vitamins (C, E and β-carotene) and minerals (selenium and zinc) at nutritional doses on the incidence of cancer and ischemic heart disease. A total of 13,017 volunteers, 7876 women aged 35-60 years, and 5141 men aged 45-60 years old, were included in 1994–1995 with a planned follow-up of eight years. Details on study design, recruitment, and baseline characteristics of the subjects have been reported previously [12]. The study was approved by *ad hoc* ethical committees, the *"Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale"* (CCPPRB no. 706, Cochin Hospital, Paris, France), and the *"Commission Nationale de l'Informatique et des Libertés"* (CNIL no. 334641).

2.2. Outcome variable

Each participant was examined at baseline by trained investigators to rate the severity of facial skin photoaging using a validated 6-grade ordinal scale of photodamage [13]. Each grade is depicted by three reference photographs to illustrate the diversity and the range of pigmentation anomalies, wrinkling and slackening. As grades 1 and 6 were rarely present in our middle-aged population, they were grouped with grades 2 and 5, respectively. The outcome variable was thus one of four severity grades (1-2: mild or mild/moderate, 3: moderate, 4: moderate/severe and 5-6: severe or very severe).

2.3. Dietary assessment

To assess usual dietary intake taking into account for potential seasonal and weekly variations, participants were asked to fill in a 24-h dietary record every 2 months, from the inclusion to the end of the study, i.e. a total of six records per year (2 weekend days and 4 weekdays per year). The dietary data were collected using the Minitel Telematic Network (the ancestor of internet), a small computerized terminal widely used in France at the beginning of the study, in the 90's. An instruction manual of photographs containing a guide for food classification was provided to facilitate estimation of portion size (seven proposed portion sizes). Photos of portion sizes were previously validated using 780 subjects in a pilot study [14]. Each 24-h dietary record included about 900 items relative to food and drinks for each of three meals (breakfast, lunch, and dinner) and of four other food intake occurrences. It also included questions on the type of oil or fat used for seasoning and cooking. Additional details on the 24-h dietary record have been published previously [11, 12]. All the

subjects who completed at least 10 records over a period of 2.5 years after inclusion were retained for this analysis [11]. Ten records have been considered to be sufficient to estimate the individual intake of PUFAs with adequate accuracy [15].

2.4. Food composition table

The energy and nutrient content of declared food intake was determined from the SU.VI.MAX food composition table. This table was developed from the French food composition table; the US Department of Agriculture National Nutrient Database; and the British McCance & Widdowson's food composition table [16]. For each participant, the n-3 PUFAs intake from each food category (meats, processed meats, poultry, eggs, dairy products, fish and seafood, vegetable oil, fruit and vegetables, sweet products, nuts, cereal products) was estimated by a two-step process. Firstly, each complex dish was broken down into each constituent simple food item using a recipe table validated by dietitians (for example, chocolate cake was broken down as follows: egg, sugar, butter, black chocolate, flour, and salt). Secondly, simple food items were grouped into food categories.

2.5. Covariates

Demographic, medical and behavioural characteristics were collected through a self-administrated questionnaire at enrolment: age, global physical activity (none, less than 1h of walking per day, more than one hour of walking per day), geographical location (North and South areas using the northern frontier of Aquitaine, Limousin, Auvergne, and Rhône-Alpes regions), smoking habits (never, former, current), educational level (primary school, secondary school or higher education) and hormonal

status (non-menopausal, menopausal with or without menopausal hormone therapy). Height and weight were measured using standardized procedures on subjects wearing their undergarments. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters, squared), and has been categorized in three classes: <25 kg/m², [25-30[kg/m², ≥ 30 kg/m². Skin sun sensitivity was evaluated using the classification proposed by Césarini (phototypes: I, II, IIIa, IIIb, IV, V, VI) [17]. As levels I and VI were rare in our population, they have been grouped with levels II and V, respectively. Lifetime sun exposure was assessed from a 3-grade question included in a self-reported questionnaire: ''How would you describe the intensity of your skin exposure to the sun during your lifetime? none/mild, moderate, or severe" [18, 19].

2.6. Statistical analyses

Fatty acid intake was expressed as percentage of total energy intake (TEI), and these nutrient densities were categorized afterwards into quartiles according to their distributions. The associations between nutrient densities and each covariate were tested with chi² tests for categorical covariates and analyses of variance for continuous covariates. Next, the relationships between nutrient densities and the outcome of interest (grading of skin photoaging) were studied using a series of partial proportional odds models [20]. Each model was adjusted for total energy intake [21], vitamin E and vitamin C intake, age, and other possible confounders (covariates). The results are expressed as estimated odds ratios (ORs) with their 95% confidence intervals (95% CI) for each quartile, the first quartile being used as the reference. Moreover, linear trends were tested by assigning each participant the median value for their quartile, this value being modelled afterwards as a continuous variable. In order to evaluate the

contribution of each specific source of n-3 PUFAs, similar models were tested according to the dietary origin of n-3 PUFAs adjusted for the same set of covariates, plus the total n-3 PUFAs density.

Regarding the study sample, all subjects who developed a cancer or a cardiovascular event during the time of dietary survey (2.5 years) were not included in the analysis. Moreover, women younger than 45 years old were excluded from the analysis in order to keep comparable classes of age for men and women. In conclusion, the present analysis included all participants aged between 45 and 60 years old at inclusion in the cohort and for whom data for dietary intakes and skin photodamage severity grading were completed (1264 women and 1655 men) (Fig. 1).

Separate analyses were conducted for men and women, all tests were two-sided and type I error was set at $P < 0.05$. Statistical analyses were carried out using SAS® software release 9.1.3 (SAS Institute, Cary, NC, USA).

3. Results

Statistical indicators of dietary n-3 PUFA intake distribution are presented in Table 1. ALA was the main source of n-3 PUFAs, followed by DHA, EPA and DPA. The mean intake of ALA (0.39% of TEI/day for women and 0.36% for men) was lower than the current French recommendation (1% of TEI). The mean intake of EPA (118 mg/day for women and 144 for men) was also lower than the French recommendation (250 mg/day), whereas the mean intake of DHA (210 mg/day for women and 248 mg for men) was close to the recommended intake (250 mg/day) [22].

The association between n-3 PUFA intake as a percentage of total energy intake and the different covariates evaluated are shown in Table 2. A significant association was found between total energy intake and intake of LC n-3 PUFAs in both men and women, with the lowest total energy intake having the highest intake of LC n-3 PUFAs. Geographical location and smoking habits were significantly associated with ALA intake. In both sexes, high consumers of ALA were more likely to live in the South of France and to be non smokers.

After adjustment for possible confounders, a significant association was found between ALA intake and the severity of photoaging in men, whereas no significant association was found for long-chain n-3 PUFAs (Table 3). A higher intake of ALA was associated with a lower risk of severe photoaging (highest vs lowest quartile of ALA: AOR=0.65, 95%IC [0.49-0.87], p = 0.004). In women, no significant association was found with intake of ALA, but women with the highest intakes of long-chain n-3 PUFAs tended to present a lower severity of photoaging (0.75 (0.57-0.99), p = 0.08). In particular, a significant decrease in photoaging risk was found in women with higher

intakes of EPA (0.69 (0.52-0.91), p = 0.04) and a similar tendency with DPA (0.77 (0.58-1.02), p = 0.10) (Table 4).

The analyses were then reiterated according to the source of PUFAs. Given that ALA is provided by different food sources, we decided to study all sources giving more than 10% of total ALA intake: dairy products (29.7% for women and 29.4% for men, respectively), fruit and vegetables (14.9% and 12.6%, respectively) and vegetable oils (14.8% and 14.3%, respectively). For men, the highest consumption levels of ALA, provided by vegetable oils and by fruit and vegetables were found to be associated with a lower risk of severe photoaging (0.72 (0.53-0.99), p = 0.04 and 0.73 (0.53-0.98), p = 0.04, respectively) (Table 3). In women, ALA from vegetable oils also tended to be inversely linked to photoaging (0.77 (0.56-1.07), p = 0.06) (Table 4). No association with photoaging was found for ALA intake from dairy products in either men or women.

In contrast to ALA, EPA and DHA were mostly derived from fish and shellfish. DPA was also derived from marine sources (39.4% for women and 35.8% for men), but also from meat (24.3% and 25.8%, respectively) and processed meat (17.5% and 20.3%, respectively). No association was found between photoaging and the different food sources of DPA in either men or women.

4. Discussion

In this large study, we found inverse associations between severity of photoaging and ALA intake in men, and between severity of photoaging and EPA intake in women independently of environmental factors known to play a role in skin aging such as smoking habits and sun exposure. In contrast, no significant association was found between photodamage and DPA or DHA. To the best of our knowledge, none of the studies that have investigated the relationship between dietary factors and skin aging have specifically addressed potential associations between photoaging and ALA intake [23-25].

When considering the different food sources of ALA, an inverse association was found between severe photoaging and ALA from vegetable food sources (vegetable oil, fruits and vegetables). This finding is consistent with those of Purba *et al.* [24] who studied the association between specific dietary consumption categories and actinic skin damage. They reported an inverse association between actinic skin damage and dietary intake of vegetables and olive oil. In contrast to ALA consumption from overall vegetable food sources, we did not find ALA intake from dairy products to be associated with skin photodamage. It should be emphasized that dairy products are not only a major source of ALA but also a significant provider of saturated fatty acids, which are recognized to play a major role in inflammatory processes [6].

Contrary to our findings, Nagata et co-workers did not report a significant association between long-chain n-3 PUFA (EPA+DHA) intake and skin aging in a population of 716 Japanese women, aged 20–74 years [25]. Strikingly, in their sample, the lowest intake of long-chain n-3 PUFAs were higher than those found in the highest quartile of our Western population. Thus, in our French population, subjects in the

lowest quartile of EPA and DHA consumption had a median intake below 100 mg, whereas in the Japanese population the median intake of EPA and DHA in the lowest quintile was 366 mg/day. In consequence, the high levels of long-chain n-3 PUFAs intake in the Japanese sample may have a protective effect in all subjects.

Our study has both strengths and limitations. The strengths of our study include the large sample size of this community-dwelling middle-aged subjects as well as the methods used to evaluate photoaging and to assess dietary intakes. Indeed, Skin was evaluated using the Larnier skin photoaging photographic scale. This method is a reproducible, quantitative method of measuring overall photoaging. The assessment of dietary intakes was also based on ten computerized 24-hour records. This specific tool allowed us to take into account weekly and seasonal intra-individual variability. The use of a validated photographic repertory for estimating portion sizes also increased the precision of the dietary data [14]. Our study also has some limitations, notably that the cross-sectional and observational design of our study does not allow us to address the causality of the associations observed. Moreover, the participants implied by an interventional trial are likely to be more health-conscious and nutritionally aware than the average population.

In summary, our findings suggest a beneficial role of n-3 PUFA intake in preventing photoaging. These findings, which differ between men and women, could be related to gender-specific skin physiology as well as gender-specific sun exposure and protection habits [18, 26]. The inverse association between n-3 PUFAs and severe skin aging may be due to the protective effect of these nutrients on inflammation previously reported in experimental and clinical studies [7-9, 27, 28]. These protective effects should be put in a wider perspective as these nutrients may also be considered

as surrogate markers of a healthy and diversified diet including large intakes of vegetable and marine food products. Nevertheless, further epidemiological studies are necessary to confirm our results and to gain further insights into underlying mechanisms of photoaging and social and behavioural patterns.

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Abbreviations used

ALA	α-linolenic acid
AOR	adjusted odds ratio
CI	confidence interval
EPA	Eicosapentaenoic acid
DPA	docosapentaenoic acid
DHA	docosahexaenoic acid
PUFA	polyunsaturated fatty acid
OR	odds ratio
ROS	reactive oxygen species
TEI	total energy intake
UVB	ultraviolet B

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Fig. 1. Flow chart of participants of the SU.VI.MAX study retained in the analysis.

Cancer/CV: subjects diagnosed with cancer or cardiovascular disease during the dietary evaluation period.

Figure(s)
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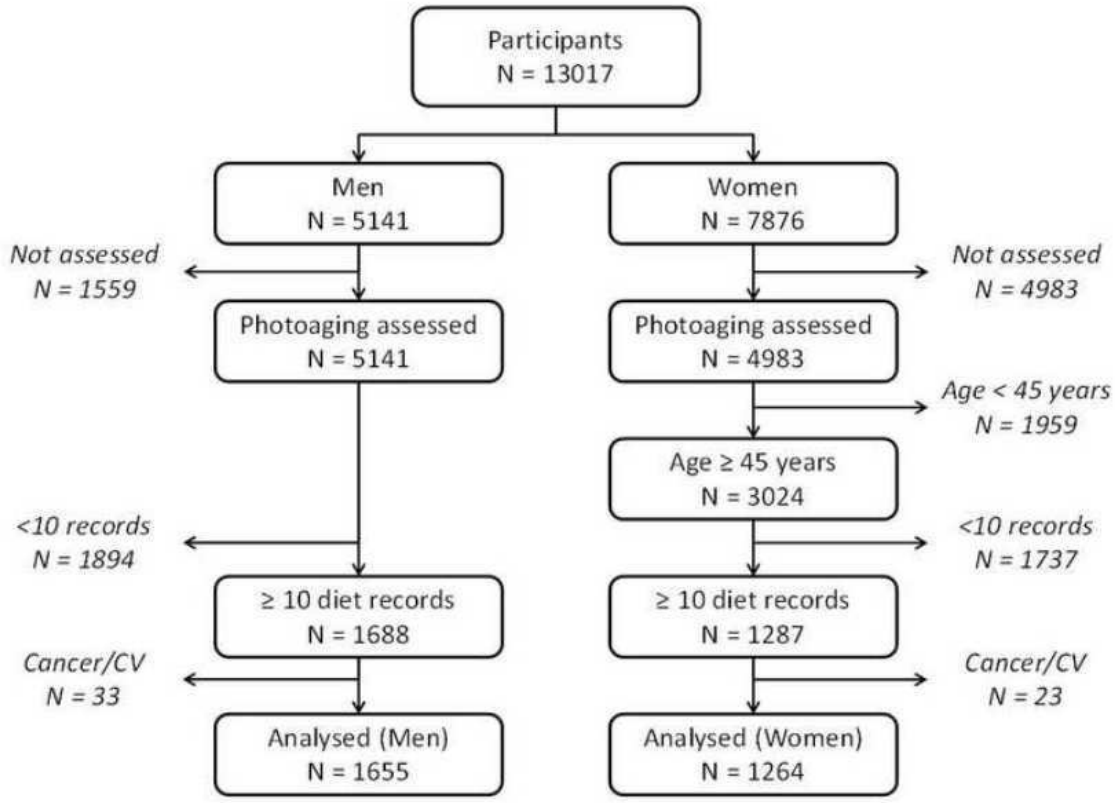

Table(s)

Table 1 Statistical indicators of the distribution of n-3 PUFA intake according to gender

n-3 PUFA intake	Min	P5	P25	Median	P75	P95	Max	Mean	STD
Women (n=1264)									
18:3 n-3 ALA (mg/d)	203	426	600	731	890	1260	2491	772	271
LC n-3 PUFA (mg/d)	5	86	168	306	515	972	1842	386	292
20:5 n-3 EPA (mg/d)	1	19	47	92	164	295	641	118	95
22:5 n-3 DPA (mg/d)	1	20	33	48	73	128	244	58	34
22:6 n-3 DHA (mg/d)	3	35	84	161	284	556	1077	210	171
18:3 n-3 ALA (%TEI)	0.16	0.29	0.34	0.37	0.42	0.57	1.13	0.39	0.09
LC n-3 PUFA (%TEI)	0.00	0.04	0.09	0.16	0.27	0.49	1.15	0.20	0.15
20:5 n-3 EPA (%TEI)	0.00	0.01	0.02	0.05	0.08	0.16	0.33	0.06	0.05
22:5 n-3 DPA (%TEI)	0.00	0.01	0.02	0.03	0.04	0.07	0.15	0.03	0.02
22:6 n-3 DHA (%TEI)	0.00	0.02	0.04	0.08	0.15	0.28	0.68	0.11	0.09
Men (n=1655)									
18:3 n-3 ALA (mg/d)	320	550	760	910	1110	1500	3954	959	307
LC n-3 PUFA (mg/d)	26	109	228	377	619	1101	2479	467	323
20:5 n-3 EPA (mg/d)	1	25	64	117	195	352	953	144	111
22:5 n-3 DPA (mg/d)	5	29	46	66	91	148	282	74	37
22:6 n-3 DHA (mg/d)	15	46	109	195	332	617	1363	248	188
18:3 n-3 ALA (%TEI)	0.17	0.27	0.31	0.34	0.38	0.51	1.64	0.36	0.09
LC n-3 PUFA (%TEI)	0.01	0.04	0.09	0.14	0.24	0.43	1.31	0.18	0.13
20:5 n-3 EPA (%TEI)	0.00	0.01	0.02	0.04	0.07	0.14	0.53	0.06	0.04
22:5 n-3 DPA (%TEI)	0.00	0.01	0.02	0.02	0.03	0.06	0.13	0.03	0.01
22:6 n-3 DHA (%TEI)	0.01	0.02	0.04	0.07	0.13	0.24	0.66	0.09	0.07

Min: minimum value; P5: 5th percentile (5% of the population has lower intakes than the P5 value); P25: 25th percentile (25% of the population has lower intakes than the P25 value); Median (50% of the population has lower intakes than the P25 value); P75: 75th percentile (75% of the population has lower intakes than the P75 value); P95: 95th percentile (95% of the population has lower intakes than the P95 value); Max: maximum value; STD: standard deviation; ALA: α-linolenic acids; LC n-3 PUFA: long chain n-3 polyunsaturated fatty acids; EPA: eicosapentaenoic acids; DPA: docosapentaenoic acids; DHA: docosahexaenoic (DHA) acids; TEI: Total energy intake.

Table(s)

Table 2 Relationship between n-3 PUFA intake as a percentage of total caloric intake and selected covariates

		Women (n=1264)				Men (n=1655)			
		ALA density		LC n-3 PUFA density		ALA density		LC n-3 PUFA density	
Characteristics		Q1	Q4 [a]	Q1	Q4 [a]	Q1	Q4 [a]	Q1	Q4 [a]
Age in years, mean (std)		51.8 (4.6)	51.7 (4.6)	51.4 (4.5)	51.6 (4.6)	52.7 (4.7)	52.3 (4.7)	51.9 (4.7)	52.7 (4.8)
Energy in Kcal, mean (std)		1853 (466)	1861 (455)	1912 (442)	1785 (452) **	2554 (585)	2500 (542)	2606 (552)	2406 (536)***
Photoaging grades [b], %	1-2 (Mild and mild/moderate)	14	17	14	16	11	14	14	12
	3 (Moderate)	42	46	43	45	42	46	47	42
	4 (Moderate/severe)	35	30	34	29	37	34	29	40
	5-6 (Severe/very severe)	9	7	8	10	11	6	9	7
Educational level [b],%	Elementary school	26	20	25	18 *t*	28	23	28	20 *t*
	Secondary school	43	48	45	48	36	37	36	34
	University or equivalent	31	31	30	34	36	40	36	45
Smoking habits [b], %	Never smokers	62	66*	62	63	28	39***	34	37
	Former smokers	24	27	26	28	55	53	53	54
	Smokers	14	7	11	9	16	7	12	9
Geographic location [c], %	North of France	76	65**	66	74	77	60**	63	69
	South of France	24	35	34	26	23	40	37	31
BMI (kg/m²), %	< 25	78	77	80	72	50	53	53	48
	[25 – 30[	15	19	15	22	45	40	40	45
	≥30	7	5	5	6	5	8	7	7
Phototype [b], %	I-II	3	5	5	3	1	2	1	3
	IIIa	11	9	14	8	5	10	9	6
	IIIb	52	52	46	56	42	44	42	43
	IV	25	28	29	25	43	36	39	40
	V-VI	6	4	6	5	6	7	6	6
Lifetime sun exposure question [b], %	None – Low	10	10	10	10	9	12	11	13
	Moderate	58	59	60	53	57	59	58	54
	High	28	28	29	32	27	24	27	29
Physical activity [b], %	None	26	26	26	26	22	21	20	21
	less than 1h of walking/day	34	37	35	34	26	22	22	23
	more than 1h of walking/day	40	37	39	40	53	57	58	55
Hormonal status [b], %	Not menopausal	50	50	55	51	-	-	-	-
	Menopausal with MHT	37	35	32	37	-	-	-	-
	Menopausal without MHT	13	14	14	13	-	-	-	-

[a] We only present the values for the extreme quartiles (Q1 and (Q4) but statistical testing was performed taking into account all four categories of nutrient densities; [b] Percentages do not add up to 100% because of missing values; [c] France has been arbitrarily divided into North and South using the northern frontier of Aquitaine, Limousin, Auvergne, and Rhône-Alpes regions; ALA: α-linolenic acid; LC n-3 PUFA: Long-chain n-3 polyunsaturated fatty acids (sum of eicosapentaenoic (20:5n-3), docosapentaenoic (22:5n-3) and docosahexaenoic acids (22:6n-3)); std: standard deviation; MHT: menopausal hormone therapy; Probability of chi² tests or ANiOVA: ***t*** $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, other comparisons are non significant.

Table(s)

Table 3 Risk of photoaging according to levels of n-3 PUFA, total and by source, intake in men (n=1655)

Fat intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-value[a]
18:3 n-3 ALA, % TEI [b]	<0.31	[0.31 – 0.34[	[0.34 – 0.38[	≥0.38	0.004
AOR (95% CI) [c]	1	0.82 (0.63-1.06)	0.74 (0.56-0.96)	0.65 (0.49-0.87)	
18:3 n-3 ALA from					
Dairy products, %TEI	<0.080	[0.080 – 0.100[	[0.100 – 0.122[	≥0.122	
AOR (95% CI) [d]	1	0.84 (0.65-1.09)	1.16 (0.90-1.50)	1.06 (0.81-1.39)	0.42
Fruit and vegetables, % TEI	<0.030	[0.030 – 0.041[	[0.041 -0.055[	≥0.055	
AOR (95% CI) [d]	1	0.85 (0.65-1.10)	0.78 (0.59-1.03)	0.73 (0.53-0.98)	0.04
Vegetable oils, % TEI	<0.031	[0.031 – 0.046[	[0.046 -0.064[	≥0.064	
AOR (95% CI) [d]	1	0.78 (0.60-1.01)	0.62 (0.47-0.81)	0.72 (0.53-0.99)	0.04
LC n-3 PUFA, % TEI	<0.088	[0.088 – 0.145[	[0.145 – 0.237[	≥0.237	
AOR (95% CI) [c]	1	0.89 (0.69-1.14)	0.88 (0.69-1.14)	0.90 (0.70-1.16)	0.54
20:5 n-3 EPA, % TEI	<0.024	[0.024 – 0.045[	[0.045 – 0.074[	≥0.074	
AOR (95% CI) [c]	1	1.01 (0.79-1.3)	0.91 (0.71-1.17)	1.05 (0.82-1.36)	0.77
22:5 n-3 DPA, %TEI	<0.018	[0.018 – 0.025[	[0.025 – 0.035[	≥0.035	
AOR (95% CI) [c]	1	0.92 (0.71-1.18)	0.84 (0.65-1.08)	0.93 (0.72-1.20)	0.65
22:5 n-3 DPA from					
Fish and seafood, %TEI	<0.003	[0.003 – 0.007 [	[0.007 – 0.017[	≥0.017	
AOR [95% CI] [e]	1	1.04 (0.80-1.35)	1.06 (0.79-1.43)	1.32 (0.90-1.92)	0.16
Meat, %TEI	<0.003	[0.003 – 0.006 [	[0.006 – 0.008[	≥0.008	
AOR [95% CI] [e]	1	1.01 (0.79-1.29)	1.02 (0.79-1.31)	1.02 (0.79-1.32)	0.98
Processed Meat, %TEI	<0.002	[0.002 – 0.004 [	[0.004 – 0.007[	≥0.007	
AOR [95% CI] [e]	1	1.07 (0.84-1.37)	0.94 (0.73-1.22)	1.06 (0.81-1.38)	0.97
22:6 n-3 DHA, %TEI	<0.043	[0.043 – 0.075[	[0.075 – 0.127[	≥0.127	
AOR (95% CI) [c]	1	0.92 (0.72-1.19)	0.84 (0.65-1.08)	0.9 (0.70-1.17)	0.46

[a] Probability of Wald test for linear trend. [b] For each fatty acid, the first line describes the cut-off values of each quartile. [c] AOR [95% CI]: Adjusted odds ratio and 95% confidence interval adjusted for age, educational level, smoking status, physical activity, body mass index, hormonal status, lifetime sun exposure, phototype, geographic location, vitamin E and C intake and energy. [d] Adjusted for the same covariates plus total ALA intake (%TEI).

[e] Adjusted for the same covariates plus total DPA intake (%TEI). ALA: α-linolenic acid; TEI:

Total energy intake; EPA: eicosapentaenoic acid (20:5n-3); DPA: docosapentaenoic acid (22:5n-3); DHA: docosahexaenoic acid (22:6n-3); LC n-3 PUFA: Long-chain n-3 polyunsaturated fatty acids (sum of EPA, DPA and DHA).

Table(s)

Table 4 Risk of photoaging according to levels of n-3 PUFA, total and by source, intake in women (n=1264)

Fat intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-value [a]
18:3 n-3 ALA, % TEI [b]	<0.34	[0.34 – 0.37[	[0.37 – 0.42[	≥0.42	
AOR (95% CI) [c]	1	0.96 (0.72-1.28)	1.07 (0.80-1.43)	0.82 (0.61-1.10)	0.17
18:3 n-3 ALA from					
Dairy products, %TEI	<0.090	[0.090 – 0.110[	[0.110 – 0.131[	≥0.131	
AOR (95% CI) [d]	1	0.93 (0.71-1.22)	1.04 (0.78-1.39)	1.18 (0.87-1.58)	0.17
Fruits and vegetables, % TEI	<0.038	[0.038 – 0.053[	[0.053 -0.072[	≥0.072	
AOR (95% CI) [d]	1	1.24 (0.92-1.68)	1.11 (0.81-1.52)	1.14 (0.80-1.62)	0.62
Vegetable oils, % TEI	<0.036	[0.036 – 0.052[	[0.052 –0.072[	≥0.072	
AOR (95% CI) [d]	1	1.08 (0.82-1.44)	0.78 (0.58-1.05)	0.77 (0.56-1.07)	0.06
LC n-3 PUFA, % TEI	<0.091	[0.091 – 0.160[	[0.160 – 0.268[	≥0.268	
AOR (95% CI) [c]	1	0.81 (0.61-1.07)	0.73 (0.55-0.96)	0.75 (0.57-0.99)	0.08
20:5 n-3 EPA, % TEI	<0.026	[0.026 – 0.049[	[0.049 – 0.085[	≥0.085	
AOR (95% CI) [c]	1	0.71 (0.53-0.94)	0.73 (0.56-0.97)	0.69 (0.52-0.91)	0.04
22:5 n-3 DPA, %TEI	<0.018	[0.018 – 0.025[	[0.025 – 0.038[	≥0.038	
AOR (95% CI) [c]	1	0.82 (0.62-1.09)	0.66 (0.5-0.87)	0.77 (0.58-1.02)	0.10
22:5 n-3 DPA from					
Fish and seafood, %TEI	<0.003	[0.003 – 0.008[	[0.008 – 0.021[	≥0.021	
AOR [95% CI] [e]	1	0.99 (0.74-1.32)	0.99 (0.71-1.39)	0.97 (0.61-1.53)	0.93
Meat, %TEI	<0.003	[0.003 – 0.005[	[0.005 – 0.008[	≥0.008	
AOR [95% CI] [e]	1	1.20 (0.92-1.58)	1.1 (0.83-1.45)	1.09 (0.82-1.45)	0.79
Processed Meat, %TEI	<0.002	[0.002 – 0.004[	[0.004 – 0.006[	≥0.006	
AOR [95% CI] [e]	1	0.90 (0.68-1.19)	0.92 (0.7-1.21)	0.96 (0.73-1.26)	0.62
22:6 n-3 DHA, %TEI	<0.045	[0.045 – 0.084[	[0.084 – 0.151[	≥0.151	
AOR (95% CI) [c]	1	0.80 (0.60-1.05)	0.79 (0.60-1.04)	0.80 (0.60-1.05)	0.24

[a] Probability of Wald test for linear trend. [b] For each fatty acid, the first line describes the cut-off values of each quartile. [c] AOR [95% CI]: Adjusted odds ratio and 95% confidence interval adjusted for age, educational level, smoking status, physical activity, body mass index, hormonal status, lifetime sun exposure, phototype, geographic location, vitamin E and C intake and energy. [d] Adjusted for the same covariates plus total ALA intake (%TEI).

[e] Adjusted for the same covariates plus total DPA intake (%TEI). ALA: α-linolenic acid; TEI:

Total energy intake; EPA: eicosapentaenoic acid (20:5n-3); DPA: docosapentaenoic acid (22:5n-3); DHA: docosahexaenoic acid (22:6n-3); LC n-3 PUFA: Long-chain n-3 polyunsaturated fatty acids (sum of EPA, DPA and DHA).

3.3 Autres travaux réalisés sur la cohorte SU.VI.MAX

En marge de ce travail de thèse, j'ai participé sur la période de 1998 à 2012 à une série de travaux réalisée sur la cohorte SU.VI.MAX et que je présenterai succinctement dans les pages qui suivent : recherche d'une typologie du comportement alimentaire qui a fait l'objet de mon mémoire de master 2 (*Guinot et al., 2001b*), étude du comportement d'exposition au soleil (*Guinot et al., 2001a*), caractérisation de la sensibilité naturelle de la peau au soleil (*Guinot et al., 2005a*), sensibilité de la peau auto-déclarée (*Guinot et al., 2006a*), lien entre phototype, statut en vitamine D et densité minérale osseuse (*Guinot et al., 2006b*), impact d'une supplémentation en vitamines et minéraux antioxydants sur le risque de cancer cutané (*Hercberg et al., 2007*), lien entre les variants du gène du récepteur de la mélanocortine de type 1 (MC1R) et la couleur de la peau (*Latreille et al., 2009*), impact des variants du gène MC1R sur la sévérité du photo-vieillissement cutané du visage (*Elfakir et al., 2010*), éventuel effet résiduel ou retardé de la supplémentation sur la survenue de cancer cutané 5 ans après l'arrêt de la supplémentation (*Ezzedine et al., 2010*), recherche des facteurs de risques des lentigines et des tâches de rousseur chez des femmes caucasiennes (*Ezzedine et al., 2012*), étude d'association génomique (GWAS) sur le photo-vieillissement (*Le Clerc et al., 2012*) et lien entre les variants du gène MC1R et les rides du sommeil (*Jdid et al., 2013*).

3.3.1 Recherche d'une typologie du comportement alimentaire

Use of multiple correspondence analysis and cluster analysis to study dietary behaviour: Food consumption questionnaire in the SU.VI.MAX cohort
Guinot C, Latreille J, Malvy D, Preziosi P, Galan P, Hercberg S, Tenenhaus M.
Eur J Epidemiol, 2001, 17, 505-16
Article présenté en annexe 2

Résumé

Les effets de différents aliments et nutriments sur le développement de maladies ont été souvent étudiés mais peu d'attention a été accordée à l'effet des habitudes alimentaires. L'objectif principal de cette recherche était d'établir une typologie de comportement alimentaire d'un large échantillon d'hommes et de femmes à l'échelon national.

Un questionnaire de fréquence alimentaire ayant été rempli par 2923 femmes et 2180 hommes de la cohorte SU.VI.MAX a été utilisé pour mener à bien cette recherche. Les personnes devaient estimer en moyenne le nombre de fois où ils avaient consommé un aliment sur la période des six ou douze derniers mois. La typologie recherchée a été réalisée à partir des fréquences mensuelles des apports alimentaires dichotomisées par rapport à la médiane. Les analyses ont été réalisées par sexe. Dans un premier temps, une analyse des correspondances multiples a été effectuée afin d'obtenir une vue d'ensemble des données en représentant graphiquement les variables et les individus. Les proximités entre les individus et les modalités des variables ont pu être observées sur les différents plans principaux. Les facteurs principaux interprétables ont été retenus, ce qui a permis pour la suite de travailler sur des coordonnées factorielles moins nombreuses que les variables de départ. Une "dissection" du nuage de points des individus selon l'orientation les trois premiers facteurs a ensuite permis d'aboutir à une typologie à huit classes. La même démarche a été réalisée sur la consommation de produits allégés.

Cette recherche a permis la définition pour chaque sexe de huit types de comportement alimentaire et quatre types de comportement de consommation de produits allégés.

Le but de ce travail était d'une part de proposer une méthodologie originale pour définir une typologie (*Guinot et al., 2012*), et d'autre part de fournir une typologie de comportement alimentaire permettant l'étude des liens avec les facteurs de santé et de détecter d'éventuels comportements alimentaires à risque afin de formuler des recommandations de santé publique.

3.3.2 Etude du comportement d'exposition au soleil

Sun exposure behaviour of a general adult population in France
Guinot C, Malvy D, Latreille J, Préziosi P, Galan P, Vaillant L, Tenenhaus M, Hercberg S, Tschachler E
Skin and Environment – Perception and Protection (J. Ring, S. Weidinger, U. Darsow, éditeurs), 10th EADV Congress, Munich, 2001. ISBN 88-323-1411-8, Monduzzi editore S.p.A., Bologne, 2001, p. 1099-106
Article présenté en annexe 3

Résumé

Les rayons ultraviolets sont connus pour jouer un rôle prépondérant dans l'accélération du vieillissement cutané et le développement des tumeurs cutanées. Néanmoins, l'augmentation de la durée des vacances, la facilité des voyages et la mode du bronzage ont entraîné ces cinquante dernières années une plus grande exposition au soleil. Dans le but de pouvoir décrire l'exposition et la protection solaire des volontaires de la cohorte SU.VI.MAX, des scores ont été construits à partir des données d'un questionnaire auto-administré développé spécifiquement pour l'étude SU.VI.MAX.

Huit dermatologues et épidémiologistes ont contribué à l'élaboration du questionnaire. Celui-ci comporte deux parties, la première partie sur les habitudes d'exposition et de protection solaire dans l'année qui vient de s'écouler, et la deuxième partie sur les habitudes d'exposition appréciées globalement au cours de la vie. Ce questionnaire a été envoyé aux 12741 volontaires de la cohorte par courrier en février 1997. Soixante dix pour cent des questionnaires ont été renseignés et récupérés, parmi lesquels 91% étaient exploitables. Au final, les données de 4825 femmes et 3259 hommes ont été analysées. Des scores quantifiant l'exposition et la protection au soleil ont ensuite été construits. Une méthode de recherche de typologie de variables a d'abord été utilisée pour sélectionner des groupes de variables homogènes. Les groupes ont été construits de manière à maximiser l'inertie expliquée par la première composante principale de chaque groupe. Autrement dit, les groupes de variables ont été construits de telle façon que les variables appartenant au même groupe soient aussi corrélées que possible entre elles. Ensuite, une analyse en composantes principales (ACP) a été réalisée sur chacun des groupes afin d'obtenir un score.

Trois scores ont été construits à partir des informations sur l'année précédente : « Intensité de l'exposition au soleil », « Utilisation de produits de protection » et « Intensité des coups de soleil ». De même, neuf scores caractérisant le comportement d'exposition face au soleil au cours de la vie ont été construits : « Intensité d'exposition au soleil au cours de la vie », « Intensité des coups de soleil dans l'enfance », « Intensité des coups de soleil à l'âge adulte », « Nudisme », « Exposition aux UV artificiels », « Pratique d'un sport de montagne exposant particulièrement la peau au soleil », « Pratique d'un sport nautique exposant particulièrement la peau au soleil », « Pratique d'un hobby exposant particulièrement la peau au soleil » et « Exercice d'une profession exposant particulièrement la peau au soleil ».

Cette étude a permis de décrire le comportement d'exposition et de protection face au soleil d'un large échantillon d'hommes et de femmes d'âge moyen. Des différences de comportement ont pu être mises en évidence entre les sexes, entre les phototypes et entre les classes d'âge. Les scores construits pourront être utilisés par la suite comme facteurs d'ajustement dans des travaux portant sur la cohorte ainsi que cela a été fait pour l'étude des facteurs de risques des lentigines et des éphélides (*Ezzedine et al., 2012*).

3.3.3 Caractérisation de la sensibilité de la peau au soleil

Sun-reactive skin Type in 4912 French Adults participating in the SU.VI.MAX Study
Guinot C, Malvy D, Latreille J, Ezzedine K, Galan P, Tenenhaus M, Ambroisine L, Hercberg S, Tschachler E
Photochem Photobiol, 2005, 81, 934-40
Article présenté en annexe 4

Résumé

Le phototype, reflet de la protection naturelle de la peau contre le soleil, a été mis au point de façon empirique afin de fournir un outil permettant d'estimer le risque individuel relatif à l'exposition solaire et d'énoncer des principes de protection adaptés. Le phototype proposé par Césarini est basé sur deux caractéristiques dynamiques : « la réaction de la peau après exposition au soleil » et « la fréquence des coups de soleil » ainsi que sur trois caractéristiques phénotypiques : « la couleur de la peau en hiver », « la couleur naturelle des cheveux à 20 ans » et « la fréquence des éphélides ». L'objectif de ce travail était de décrire les fréquences

de ces caractéristiques chez les volontaires de la cohorte et de mieux comprendre les associations entre ces caractéristiques.

Les données ont été recueillies lors de l'examen clinique réalisé sur la cohorte au cours de l'année 1998. Les caractéristiques de 4912 volontaires, 2868 femmes (âgées de 35 à 60 ans) et 2044 hommes (45 à 60 ans), ont été évaluées par des médecins entraînés au préalable par un dermatologue expérimenté. Le phototype avait été déterminé au préalable lors du bilan médical réalisé en 1995 sur 4201 de ces volontaires. Afin de visualiser les liens entre les différentes caractéristiques du phototype, une analyse des correspondances multiples (ACM) a été réalisée. Cette méthode a permis en outre d'étudier les liens avec le sexe et de construire un score quantifiant la sensibilité de la peau au soleil.

Un « effet Guttman » a été mis en évidence sur le premier plan factoriel selon une forme en « U » caractéristique d'un phénomène unidimensionnel. La première composante a donc été retenue pour construire un score qui résume la sensibilité face au soleil. Cette étude a également permis de mettre en évidence une sensibilité cutanée de la peau face au soleil moins importante chez les hommes que chez les femmes.

3.3.4 Sensibilité de la peau auto-déclarée

Self-reported skin sensitivity in a general adult population in France: data of the SU.VI.MAX cohort
Guinot C, Malvy D, Mauger E, Ezzedine K, Latreille J, Ambroisine L, Tenenhaus M, Préziosi P, Morizot F, Galan P, Hercberg S, Tschachler E
J Eur Acad Dermatol, 2006, 20, 380-90
Article présenté en annexe 5

Résumé

Cette étude a été réalisée afin d'examiner la fréquence de la sensibilité cutanée auto-déclarée chez un large échantillon de la population française générale et d'étudier les associations entre les différentes manifestations rapportées. Un questionnaire sur la sensibilité cutanée a été développé à partir des résultats de différentes études menées sur la région d'Ile-de-France. Ce questionnaire explore différents types de sensibilité : type I (rougeurs liées à la réactivité vasculaire de la peau), type II (manifestations cutanées liées à des conditions environnementales particulières), type III (manifestations cutanées liées à l'application de

certaines substances), type IV uniquement pour les femmes (survenue de boutons liés au cycle menstruel). Par ailleurs, les manifestations liées à la non-application des produits cosmétiques habituels pour les femmes et celles liées aux rasages pour les hommes ont aussi été étudiées.

La sensibilité de la peau du visage a été rapportée par 61% des femmes (n=5074) et 32% des hommes (n=3448), la fréquence décroissant avec l'âge. A classe d'âge comparable, la fréquence des types I, II et III étaint plus élevée chez les femmes (78, 72 et 58% respectivement) que chez les hommes (56, 48 et 28%). La fréquence du type IV était rapportée par 49% des femmes et les réactions cutanées après rasage par 41% des hommes. Une analyse factorielle des correspondances multiples a permis de metttre en évidence des liens entre le phototype et les manifestations de sensibilité cutanée. Les phototypes clairs ont déclaré plus fréquemment des réactions associées au type I et des rougeurs et des sensations de chaleurs liées aux conditions environnementales (type II).

La sensibilité de la peau est un phénomène qui concerne un grand nombre de personnes, les hommes comme les femmes, et qui décline avec l'âge. Ce questionnaire, publié en anglais, qui permet d'identifier des sujets qui présentent différents types de sensibilité cutanée pourrait être utilisé dans des études spécifiques, sur des populations plus homogènes afin de pouvoir en étudier les mécanismes biologiques sous-jacents.

3.3.5 Lien entre phototype, statut en vitamine D et densité minérale osseuse

Phototype, statut en vitamine D et densité minérale osseuse chez des femmes à risque d'ostéoporose [Phototype, vitamin D status and bone mineral density among women at risk of osteoporosis]
Guinot C, Ezzedine K, Mauger E, Ambroisine L, Latreille J, Bertrais S, Preziosi P, Galan P, Chapuy MC, Arnaud S, Meunier PJ, Tschachler E, Hercberg S, Malvy S
Rev Med Interne, 2006, 27, 369-74
Article présenté en annexe 6

Résumé

Le but de cette étude était de déterminer les facteurs associés à de la densité minérale osseuse (DMO) mesurée au niveau du col du fémur chez un groupe de femmes d'âge moyen considérées à risque d'ostéoporose, c'est-à-dire défini par un statut précaire en

vitamine D [25(OH)D3 < 78 nmol/L] et hyperparathyroïdisme [parathormone circulante > 36 pg/mL]).

Cette étude a été conduite en deux temps chez 122 femmes de la cohorte. L'impact sur la DMO de différentes variables, dont l'âge, l'indice de masse corporelle (IMC), le statut en vitamine D, la consommation d'alcool, l'intensité d'exposition au soleil et le phototype a été testé à l'aide de modèles de régression linéaire.

Aucun lien significatif n'a été retrouvé entre la DMO et les variables documentant le statut en vitamine D, le taux de parathormone et le phototype. Cependant, les phototypes les plus clairs étaient plus souvent associés avec les valeurs les plus basses de DMO. Par contre, la valeur moyenne de la DMO diminuait significativement avec l'âge et augmentait avec l'indice de masse corporelle et le niveau d'activité physique.

Les conclusions de cette étude étaient les suivantes. Quel que soit leur phototype, les femmes à statut précaire en vitamine D devraient adopter une supplémentation en vitamine D associée à un apport adapté en calcium tout au long de l'année, sans négliger l'adoption de mesures de protection solaire et le maintien d'un niveau satisfaisant d'activité physique, pour améliorer leur densité osseuse et prévenir le risque fracturaire.

3.3.6 Impact d'une supplémentation en antioxydants sur le risque de cancer cutané

Antioxidant supplementation increases the risk of skin cancer in women but not in men
Hercberg S, Ezzedine K, Guinot C, Preziosi P, Galan P, Bertrais S, Estaquio C, Briançon S, Favier A, Latreille J, Malvy D
J Nutr, 2007, 137, 2098-105
Article présenté en annexe 7

Résumé

La prévalence des cancers cutanés, mélanomes et non mélanomes, n'a cessé d'augmenter ces dernières années. L'exposition aux rayonnements ultraviolets, en favorisant la production de radicaux libres, est la cause principale de survenue de ces cancers. La peau possède pourtant un important système de défense antioxydant qui peut se retrouver dépassé lorsque

l’exposition au soleil est excessive. L’impact d’une supplémentation en vitamines et en minéraux antioxydants à dose nutritionnelle sur la diminution du risque de cancer cutané a été étudié chez l’ensemble des 13017 participants de l’étude SU.VI.MAX.

Le traitement a été alloué de façon aléatoire : groupe placebo (n=6481) et groupe d’intervention (n = 6536). La supplémentation consistait en une prise quotidienne d’une capsule d’antioxydants (120 mg vitamine C, 30 mg vitamine E, 6 mg bêta-carotène, 100 µg sélénium et 20 mg zinc). Les participants ont été suivis pendant 8 ans. Le critère de jugement était la survenue d’un premier évènement de tous types de cancer cutané : mélanome, non mélanomes (carcinome cutané spino-cellulaire et baso-cellulaire), et autres types de cancers cutanés. Lorsqu'un cancer cutané était suspecté, le diagnostic et le rapport histo-pathologique étaient récupérés soit auprès du corps médical soit directement auprès des participants. Tous les événements ont été validés à l’aveugle par un comité d'experts.

L’analyse a été réalisée en « intention de traiter ». Les taux cumulés des cancers cutanés ont été tout d’abord représentés pour chacun des sexes et des groupes à l’aide de la méthode de Kaplan-Meier, puis comparés grâce au test du Logrank. L’effet de la supplémentation sur la survenue de cancers cutanés a ensuite été testé en ajustant sur différents facteurs de confusion grâce au modèle de Cox. Les résultats ont été exprimés sous la forme de rapports des risques instantanés (Hazard Ratio : HR) avec leurs intervalles de confiance à 95% (IC95%).

Un total de 157 cas de cancer cutané (81 chez les femmes et 76 chez les hommes) a été diagnostiqué : 25 mélanomes (16 chez les femmes et 9 chez les hommes), 115 carcinomes baso-cellulaires (57 chez les femmes et 58 chez les hommes), 13 carcinomes spino-cellulaires (4 chez les femmes et 9 chez les hommes) et 4 cas de Maladie de Bowen chez les femmes. Chez les femmes, le nombre total de cancers cutanés était significativement plus élevé à la date de fin de l’étude dans le groupe d’intervention que dans le groupe placebo (51 *versus* 30 ; p=0,02). Cette différence était significative pour les mélanomes (13 *versus* 3 ; p=0,01) et non significative pour les non mélanomes (38 *versus* 27 ; p=0,15). Aucune différence n’a été mise en évidence chez les hommes. Le modèle de Cox a permis de mettre en évidence après ajustement un risque plus élevé de survenue de tous types de cancer cutané dans le groupe d’intervention chez les femmes. Cet effet a été ensuite retrouvé pour la survenue de mélanomes mais pas pour les non mélanomes.

Malgré l'effectif modéré des évènements survenus dans notre étude, nos résultats suggèrent que la supplémention en antioxydants aurait un effet différent sur la survenue des cancers cutanés chez les femmes et les hommes, et pourrait être délétère pour les femmes.

3.3.7 Effet de la supplémentation sur la survenue de cancer cutané 5 ans après l'arrêt de la supplémentation

Incidence of skin cancers during 5-year follow-up after stopping antioxidant vitamins and mineral supplementation
Ezzedine K, Latreille J, Kesse-Guyot E, Galan P, Hercberg S, Guinot C, Malvy D
Eur J Cancer, 2010, 46, 3316-22
Article présenté en annexe 8

Résumé

Ce travail fait suite à l'étude précédente (*Hercberg et al., 2007*). L'objectif était ici d'étudier un éventuel effet résiduel ou retardé de la supplémentation sur la survenue de cancers cutanés 5 ans après l'arrêt de la supplémentation.

A la fin de l'étude d'intervention (01/09/2012), 11054 sujets ont été suivis en post-supplémentation pendant une durée de 5 ans. Tous les problèmes majeurs de santé étaient notifiés tous les 6 mois grâce à l'envoi de questionnaires aux participants, et par toutes les informations supplémentaires envoyées spontanément par les volontaires. Le critère de jugement était comme lors de l'étude précédente, la survenue d'un premier évènement de tous types de cancer cutané. Lors de la déclaration d'un cancer, le type et le stade du cancer était certifié par un rapport anatomopathologique.

Les taux cumulés des cancers cutanés ont été tout d'abord représentés pour chacun des groupes à l'aide de la méthode de Kaplan-Meier. Puis, un éventuel effet résiduel ou retardé de la supplémentation en antioxydants pendant la période de post-intervention a été testé à l'aide d'une série de modèles de Cox où le traitement a été défini comme une variable dépendante du temps et en ajustant sur les covariables. Aucun effet résiduel ou retardé de la supplémentation n'a été mis en évidence pour les deux sexes. Cinq ans après l'arrêt de la supplémentation, les femmes ne présentent plus de risque significativement plus élevé de développer un cancer cutané. Des résultats similaires avaient été observés dans des études d'intervention sur le

cancer du poumon, l'incidence des tumeurs augmentant rapidement dès le début de la période d'intervention. L'hypothèse avancée était que la supplémentation en antioxydants avait eu un effet sur la croissance de tumeurs préexistantes plutôt que sur l'apparition de nouvelles tumeurs. Dans ces différentes études, le risque de cancer diminuait après l'arrêt de la supplémentation. Ces résultats suggèrent un lien causal entre la prise d'antioxydants et la révélation de tumeurs cutanées. Tant que ce lien n'a pas été invalidé, la prise systématique d'une supplémentation en antioxydants semble donc être déconseillée chez les individus à risque, tels que ceux qui se sont exposés au soleil de façon excessive au cours de la vie.

3.3.8 Lien entre variants du gène MC1R et couleur de la peau

MC1R gene polymorphism affects skin color measured by reflectance in a population of French adult women
Latreille J, Ezzedine K, Elfakir A, Ambroisine L, Gardinier S, Galan P, Hercberg S, Gruber F, Rees J, Tschachler E, Guinot C
Photochem Photobiol, 2009, 85, 1451-58
Article présenté en annexe 9

Résumé

Le gène du récepteur de la mélanocortine de type 1 (MC1R) est le premier gène qui a été identifié comme jouant un rôle important dans les variations normales de la pigmentation de la peau et des cheveux chez les humains. Ce gène présente de nombreux variants chez les caucasiens et différents variants fonctionnels de ce gène ont été rapportés liés à une couleur de peau claire appréciée la plupart du temps à l'aide d'échelles cliniques. Le but de ce travail était d'étudier le lien entre ces variants observés chez un échantillon de femmes adultes caucasiennes et la couleur de la peau mesurée objectivement par spectrophotométrie.

Une étude génétique ancillaire transversale a été conduite dans le cadre de l'étude SU.VI.MAX sur un échantillon de 570 femmes volontaires (âge à l'inclusion : 44-70 ans) ayant respecté des consignes cosmétiques. La couleur de la peau a été mesurée sur la face interne de l'avant-bras à l'aide d'un spectrophotomètre CM2600d de Minolta (Osaka, Japon) dans des conditions environnementales contrôlées (température du laboratoire : 21 ± 3° ; humidité relative du laboratoire : 37 ± 5 %). Cet appareil permet de mesurer les pourcentages de réflectance de la lumière sur la peau entre les longueurs d'onde 400 et 700 nm, avec un pas de 10 nm. Le lien entre les 9 variants du MC1R les plus fréquents dans l'échantillon et la

couleur de la peau a été d'abord étudié en regroupant les variants selon la diminution de fonction du récepteur : diminution « majeure » *versus* « mineure ». Puis, l'effet individuel de chaque variant a été testé. Les liens entre la couleur de la peau et les variants ont ensuite été examinés à l'aide d'une analyse en composantes principales (ACP) et les zones de confiance ont été obtenues à l'aide d'une méthode de rééchantillonnage (Bootstrap partiel).

Les femmes porteuses des variants R151C, D294H, D84E et R160W présentaient des pourcentages de réflectance significativement plus élevés à la fin du spectre visible (région rouge du spectre), indiquant un niveau plus faible de mélanine fonctionnelle. En revanche, aucun effet n'a été mis en évidence pour les autres variants comparés au type sauvage. Nos résultats soutiennent donc l'hypothèse selon laquelle les variants du MC1R n'altèreraient pas nécessairement la couleur de la peau, mais pourraient aussi augmenter sa sensibilité aux radiations UV.

3.3.9 Lien entre variants du gène MC1R et sévérité du photo-vieillissement cutané

Functional MC1R gene variants are associated with an increased risk for severe photoaging of facial skin
Elfakir A, Ezzedine K, Latreille J, Ambroisine L, Jdid R, Galan P, Hercberg S, Gruber F, Malvy D, Tschachler E, Guinot C
J Invest Dermatol, 2010, 130, 1107-15
Article présenté en annexe 10

Résumé

Faisant suite du travail précédent *(Latreille et al., 2009*), l'effet des variants du MC1R sur la sévérité du photo-vieillissement cutané du visage a été étudié.

Plusieurs images digitales de haute qualité du visage de chaque volontaire avaient été prises de façon standardisée. Ces photographies ont ensuite été examinées par un dermatologue afin de noter la gravité du photo-vieillissement global du visage selon une échelle photographique en 6 grades validée (Larnier et *al.,* 1994).

Le lien entre les variants du MC1R et l'expression du photo-vieillissement cutané a été d'abord étudié en regroupant les neuf variants les plus fréquents selon les catégories :

homozygotes de type sauvage, un seul variant ou deux variants. Puis, les variants ont été regroupés selon la diminution de fonction du récepteur : diminution « majeure » ou *versus* « mineure » (homozygote de type sauvage, seulement un ou deux variants mineurs, un seul variant majeur, deux variants majeurs). Pour finir, l'effet individuel de chaque variant a été testé. Les effets du polymorphisme du MC1R sur la gravité du photo-vieillissement (« léger » grades 1-3 vs « sévère » grades 4-6) ont été testés à l'aide de régressions logistiques en ajustant sur des facteurs de confusion.

Après ajustement sur l'âge, la présence de variants du MC1R a été trouvée associée à un risque significativement accru de photo-vieillissement sévère. Les femmes porteuses de deux variants du MC1R ont près de 2,33 fois plus de risque de présenter un photo-vieillissement sévère ($IC_{95\%}$ [1,17 – 4,63]) par rapport à des femmes homozygotes de type sauvage, ce risque atteignant 5,61 [1,43-21,96] lors de la présence simultanée de deux variants majeurs.

Les liens trouvés entre certains variants du MC1R et la sévérité du photo-vieillissement suggèrent que le polymorphisme du gène MC1R joue un rôle prépondérant dans l'expression du photo-vieillissement cutané. Cependant, des recherches des populations différentes et des échantillons de taille plus importante sont encore nécessaires pour clarifier la contribution exacte de la perte de fonction du récepteur des différents variants du MC1R dans l'expression du photo-vieillissement. Ce travail a fait l'objet d'une publication allégée en français (*Latreille et al., 2011*).

3.3.10 Facteurs de risques des lentigines et des éphélides

Freckles and solar lentigines have different risk factors in Caucasian women
Ezzedine K, Mauger E, Latreille J, Jdid R, Malvy D, Gruber F, Galan P, Hercberg S, Tschachler E, Guinot C
J Eur Acad Dermatol Venereol, 2012, doi: 10.1111/j. 1468-3083.2012.04685.x
Article présenté en annexe 11

Résumé

Toujours dans la suite des études menées précédemment (*Latreille et al., 2009* ; *Elfakir et al., 2010*) une nouvelle analyse a été conduite afin d'étudier l'impact de variables phénotypiques,

comportementales et génétiques (gène du MC1R) sur les taches de rousseur et la sévérité des lentigines solaires chez des femmes caucasiennes.

La sévérité des lentigines solaires a été évaluée à partir des photographies du visage par un dermatologue, puis un score de sévérité des lentigines solaires a été construit à l'aide d'une analyse en composantes principales. Les taches de rousseur ayant tendance à disparaitre avec l'âge, les antécédents auto-déclarés de taches de rousseur ont été utilisés comme variable d'intérêt. L'exposition au soleil a été caractérisée quant à elle à l'aide d'une typologie en 6 classes définie au préalable. Les facteurs de risque ont été étudiés à l'aide d'une série de régressions logistiques. La fréquence des coups de soleil et la présence de variants fonctionnels du gène MC1R ont été trouvées associées de façon indépendante avec les antécédents des taches de rousseur. Concernant les lentigines, en plus de l'âge cinq facteurs ont été trouvés liés de façon indépendante à la sévérité des lentigines solaires : la couleur de la peau, la capacité à bronzer, les antécédents des taches de rousseur, l'exposition au soleil et la prise de contraceptifs oraux ou de traitements progestatifs.

Ces résultats vont dans le sens des travaux publiés ultérieurement en apportant un nouvel éclairage sur les différences entre les lentigines solaires qui sont des marqueurs du photo-vieillissement cutané et les taches de rousseur qui sont principalement déterminées par des facteurs génétiques.

3.3.11 Etude GWAS sur l'expression du vieillissement cutané

A genome-wide association study in Caucasian women points out for a role of the STXBP5L gene in facial photoageing
Le Clerc S, Taing L, Ezzedine K, Latreille J, Labib T, Coulonges C, Bernard A, Melak S, Carpentier W, Malvy D, Jdid R, Galan P, Hercberg S, Morizot F, Guinot C, Tschachler E, Zagury JF
J Invest Dermatol, 2012, doi:10.1038/jid.2012.458
Article présenté en annexe 12

Résumé

Une étude d'association génomique (GWAS) a été réalisée dans la continuité de l'étude gène centré précédente afin d'explorer plus largement l'impact des facteurs génétiques sur

l’expression du vieillissement cutané (*Latreille et al., 2009* ; *Elfakir et al., 2010* ; *Ezzedine et al., 2012*).

La sévérité du photovieillissement global (Larnier et *al.,* 1994) et la sévérité d'une série de signes de vieillissement ont été évaluées par un dermatologue à partir de trois photographies numériques du visage de chaque volontaire à l'aide d'échelles ordinales spécifiques avec illustrations photographiques. Puis, trois scores ont été calculés à l’aide d’une analyse en composantes principales : lentigines, rides et relâchement. Ces indicateurs ont ensuite été utilisés comme critères de jugement dans les analyses. Par ailleurs, l'ADN extrait de l'échantillon de sang prélevé pour chaque femme a été analysé grâce à une puce à haut-débit Illumina Human Omni1-Quad contenant 1140000 marqueurs génétiques.

Parmi les marqueurs, 91000 étaient des variants du nombre de copies et seront analysés par la suite, 118000 n'ont montré aucune variation, 55000 présentaient des erreurs de génotypage et 2000 étaient situés sur le chromosome Y. Après les contrôles de qualité, il restait 874000 SNPs. Les stratifications ont été examinées en utilisant la méthode Eigenstrat : 18 sujets ont été exclus de la suite de l'analyse. Les relations entre les génotypes et les indicateurs de vieillissement ont été étudiés séparément par des régressions linéaires ajustées sur l'âge, le tabagisme, l'exposition solaire au cours de la vie, le statut hormonal, et les deux principaux vecteurs propres.

Un signal a dépassé le seuil de Bonferroni ($p = 6\ 10^{-9}$) et a été associé avec le grade de photovieillissement. Il a été également trouvé corrélé avec les scores de rides et de relâchement. Ce SNP intronique est situé dans un gène exprimé dans la peau. Ce gène, qui n'a pas été décrit auparavant dans le contexte du vieillissement, peut constituer un bon gène candidat pour l'étude des mécanismes moléculaires du vieillissement cutané.

3.3.12 Variants du gène MC1R et rides du sommeil

MC1R major variants are a risk factor of sleep lines in Caucasian women.
Jdid R, Ezzedine K, Latreille J, Galan P, Hercberg S, Malvy D, Tschachler E, Guinot C
J Eur Acad Dermatol Venereol, 2013, doi: 10.1111/jdv.12119
Article présenté en annexe 13

Résumé

Dans le prolongement de ces précédents travaux (*Latreille et al., 2009* ; *Elfakir et al., 2010, Ezzedine et al., 2012, Le Clerc et al., 2012*) une nouvelle analyse a été conduite afin d'étudier les facteurs de risques phénotypiques, comportementaux et génétiques (gène MC1R) des rides du sommeil chez des femmes caucasiennes.

De façon indépendante, deux dermatologues ont examiné trois photographies numériques du visage de 542 volontaires (face et profils), âgées de 44 à 70 ans, afin d'identifier les rides du sommeil et d'évaluer la sévérité de différents signes de vieillissement cutané. Les facteurs de risques ont été étudiés à l'aide d'une série de régressions logistiques.

Soixante femmes (11%) présentaient des rides du sommeil. Elles présentaient en général plusieurs rides du sommeil. Ces rides étaient localisées sur le front, le long du nez, sur les joues et sous les yeux, et plus rarement sur le menton. Comme attendu, les rides du sommeil étaient associées à l'âge, et les femmes avec des rides du sommeil présentaient également un vieillissement cutané plus important. Après ajustement sur les facteurs de confusions éventuels, la présence de deux variants majeurs du gène MC1R a été trouvée associée avec une augmentation du risque de présence des rides du sommeil (ORa [IC95] : 8.25 [2.62-25.97]).

Les données sur les rides du sommeil sont peu nombreuses dans la littérature et cette étude est la première à être réalisée sur un échantillon relativement large de femmes. Nos résutlats suggèrent que le polymorphisme du gène MC1R pourrait jouer un rôle important dans l'apparition des rides du sommeil.

4. Discussion, perspectives et conclusion

4.1 Discussion

La discussion portera principalement sur les résultats issus de l'étude des liens entre les acides gras et le photo-vieillissement cutané. Nous présenterons ensuite les différentes perspectives de ces travaux avant de conclure.

Dans la première étude, nous avons mis en évidence, chez une large population d'hommes et de femmes vivant en France, un lien inverse entre la sévérité du photo-vieillissement et les apports en AGMI provenant des huiles végétales, et plus spécifiquement ceux fournis par l'huile d'olive. Par contre, aucun lien statistiquement significatif n'a été trouvé avec les apports en AGMI des produits animaux (produits laitiers, viandes, et charcuterie) *(Latreille et al., 2012).* Nos résultats sont en accord avec les résultats de l'étude menée par Purba et co-auteurs (2001) qui rapportent également une association inverse entre le vieillissement actinique de la peau du dos de la main et les apports en AGMI, et en huile d'olive (Tableau 3). Nagata et co-auteurs (2010) ont également mis en évidence un lien significatif entre les apports en AGMI et l'élasticité de la peau mais n'ont pas trouvé de lien avec la sévérité des rides de la patte d'oie. Cependant, ils n'ont pas tenu compte dans leur analyse des différentes sources d'AGMI. Contrairement à nos résultats, Cosgrove et co-auteurs (2007) n'ont pas mis en évidence de lien entre apports en acide oléique et les rides et rapportent un risque plus élevé de sècheresse cutanée sénile quand la consommation en acide oléique augmente. Cependant, comme Nagata et co-auteurs (2001), ces auteurs n'ont pas étudié les liens avec les différentes sources alimentaires des AGMI. Par ailleurs, la diversité de consommation des AGMI n'était pas comparable entre les différentes populations étudiées pour des raisons probables d'habitudes alimentaires culturelles. Ainsi, dans l'étude de Nagata et co-auteurs menée au Japon (2010), la médiane du quintile inférieur des apports en AGMI était de 16,3 g/jour et celle du quintile supérieur égale à 26,1 g/jour tandis que dans notre étude, la médiane du quintile inférieur était égale à 19,6 g/jour et à 41,6 g/j pour le quintile supérieur. De plus, dans notre étude et celle de Purba et *al.*, une partie de la population vivait dans le bassin méditerranéen qui est connu pour être une région à forte consommation d'huile d'olive. Dans la littérature, seules ces trois études épidémiologiques ont examiné les liens entre AGMI et vieillissement cutané.

Le lien inverse mis en évidence dans notre étude entre l'huile d'olive et la sévérité du photo-vieillissement cutané peut s'expliquer par le profil lipidique particulier de l'huile d'olive, avec un contenu élevé en AGMI et également un faible ratio en AGPI n-6/AGPI n-3 (Owen et *al.*, 2000 ; Viola et Viola, 2009). Les AGMI sont en effet moins sensibles à la peroxydation lipidique que les AGPI. L'absence de lien entre le photo-vieillissement cutané et les produits laitiers, qui sont pourtant des sources importantes d'AGMI, peut être due aux quantités d'AGS également fournies par ces aliments. En effet, les AGS sont rapportés pour être associés à la résistance à l'insuline et au processus inflammatoire, ce qui pourrait de ce fait annuler les effets bénéfiques des AGMI (Riccardi et *al.*, 2004 ; Galland, 2010). L'effet bénéfique de l'huile d'olive peut également être lié à des composants mineurs de cette huile, tels que le squalène et les polyphénols, qui pourraient également jouer un rôle important sur la prévention du photo-vieillissement (Owen et *al.*, 2000 ; Viola et Viola, 2009). Le squalène, un hydrocarbure, est un des principaux composés des lipides de surface de la peau (le sébum en contient environ 12%). Il serait un filtre naturel de l'espèce majeure de ERO qu'est l'oxygène singulet, serait peu sensible à la peroxydation lipidique, et protégerait ainsi la peau contre les rayonnements UV (Kelly, 1999 ; Huang et *al.*, 2009 ; Viola et Viola, 2009). De même, des propriétés anti-oxydantes et anti-inflammatoires ont été attribuées aux polyphénols, ce qui leur permettraient également de défendre la peau du stress oxydant (Owen et *al.*, 2000 ; Cicerale et *al.*, 2010). Enfin, l'association trouvée avec l'huile d'olive peut être également le reflet d'une alimentation globalement plus saine, car comme attendu, les apports en huile d'olive étaient corrélés positivement avec les apports en poisson, en fruits et légumes, et en thé, et corrélés négativement avec les apports en aliments sucrés, en beurre, en lait, ou encore en alcool fort (Willett et *al.*, 1995).

Tableau 3. Etudes épidémiologiques portant sur le lien entre lipides et vieillissement cutané

Type d'étude Participants ***Référence***	**Sexe, effectif, âge (années) et pays**	**Variables d'intérêt**	**Recueil des données alimentaires Lipides et/ou aliments sources étudiés**	**Résultats significatifs pour les lipides ou les aliments sources**
Etude transversale Participants de l'étude IUNS[1] « Food Habits in Later Life » *Purba et al., 2001*	H[2] et F[3], n = 453, >=70, Australie et Europe (Grèce et Suède)	• Vieillissement actinique de la peau du dos de la main (échelle photographique à 6 niveaux, Beagley-Gibson, 1980)	QFA[4] semi-quantitatif • Lipides totaux, • AGS[5], • AGMI[6], • AGPI[7], • Aliments sources	• Apports en lipides totaux et en AGMI corrélés négativement avec le vieillissement de la peau • Apports en AGS corrélés positivement avec le vieillissement de la peau • Apports en légumes, légumes secs, poissons, huile d'olive et céréales corrélés négativement avec le vieillissement cutané • Apports en viandes, produits laitiers et produits sucrés corrélés positivement avec le vieillissement cutané
Etude transversale Participants de l'étude NHANES I[8] *Cosgrove et al., 2007*	F, n=4025, 40 à 74, Etats-Unis	• Peau ridée (oui/non) • Sécheresse sénile (oui/non) • Atrophie de la peau (oui/non) (visage et corps)	Rappel alimentaire de 24 heures réalisé par un spécialiste de la nutrition • Lipides totaux, • AGS, • AO[9], • LA[10]	• Apports en lipides totaux associés positivement avec le risque d'atrophie et l'aspect ridé de la peau • Apports en AO associés positivement avec la sécheresse sénile • Apports en LA associés négativement avec le risque de sécheresse sénile et atrophie de la peau
Etude transversale Participants d'un check up de santé (hôpital de Gifu au Japon) *Nagata et al., 2010*	F, n=716, 20 à 74, Japon	• Capacitance de la peau de l'avant-bras, u.a[11], cornéomètre CM825, • Taux de sébum du front, µg/cm², sébumètre SM810, • Elasticité de la peau du bras, coefficient U_r/U_f [12], cutomètre SEM575 • Rides de la patte d'oie (échelle à 6 niveaux de Daniell, 1971)	QFA semi-quantitatif • Lipides totaux, • AGS, • AGMI, • AGPI, • AGPI-LC[13] n-3 (EPA[14]+DHA[15]), • Aliments sources	• Apports en lipides totaux, AGS et AGMI corrélés positivement avec l'élasticité de la peau • Apports en AGS corrélés négativement avec la sévérité des rides de la patte d'oie

Tableau 3. Suite

Type d'étude Participants ***Référence***	**Sexe, effectif, âge (années) et pays**	**Variables d'intérêt**	**Recueil des données alimentaires Lipides et/ou aliments sources étudiés**	**Résultats significatifs pour les lipides ou les aliments sources**
Etude transversale Participants de l'étude SUVIMAX[16] *Latreille et al., 2012*	H et F, n = 2919, 45 à 60, France	• Photo-vieillissement cutané au niveau du visage (échelle photographique à 6 niveaux, Larnier et *al.*, 1994)	Dix enregistrements alimentaires de 24 heures • AGMI, • Sources d'AGMI : - Huiles végétales, - Produits laitiers, - Viande, - Charcuterie	• Apports en AGMI associés négativement avec le risque de photo-vieillissement du visage chez les hommes • Apports en AGMI provenant des huiles végétales (huile d'olive) associés négativement avec le risque de photo-vieillissement chez les hommes et les femmes
Etude transversale Participants de l'étude SUVIMAX *Soumis pour publication en 2013 dans la revue Journal of Dermatological Science*	H et F, n = 2919, 45 à 60, France	• Photo-vieillissement cutané au niveau du visage (échelle photographique à 6 niveaux, Larnier et *al.*, 1994)	Dix enregistrements alimentaires de 24 heures • ALA[17], • Sources de ALA : - Produits laitiers - Fruits et légumes - Huiles végétales • AGPI-LC n-3 (EPA+DHA+DPA[18]) • EPA, • DPA, • Sources d'AGMI : - Poissons et fruits de mer, - Viande, - Charcuterie, • DHA	• Apports en EPA associés négativement avec le risque de photo-vieillissement chez les femmes • Apports en ALA associés négativement avec le risque de photo-vieillissement chez les hommes • Apports en ALA provenant des huiles végétales et des fruits et légumes associés négativement avec le risque de photo-vieillissement chez les hommes • Apports en ALA provenant des huiles végétales tendent à être associés négativement avec le photo-vieillissement chez les femmes

[1]IUNS International Union of Nutritional Sciences, [2]H : homme, [3]F : femme, [4]QFA : Questionnaire de fréquence alimentaire, [5]AGS : acide gras saturés, [6]AGMI : acides gras monoinsaturés, [7]AGPI : acides gras polyinsaturés, [8]NHANES I : First National Health and Nutrition Examination Survey, [9] AO : acide oléique, [10] LA : acide linoléique (C18:2 n-6), [11]u.a. : unité arbitraire, [12]U_r/U_f : capacité de la peau à revenir à sa position initiale après déformation, [13] AGPI-LC : acides gras polyinsaturés à longues chaînes, [14] EPA : acide eicosapentaénoïque (C20:5 n-3), [15] DHA : acide docosahexaénoïque (C22:6 n-3), [16] SU.VI.MAX SUpplémentation en Vitamines et Minéraux AntioXydants, [17] ALA : acide α-linolénique (C18:3 n-3) , [18] DPA : acide docosapentaénoïque (C22:5 n-3)

Nous avons également mis en évidence un lien entre sévérité du photo-vieillissement cutané et apports en EPA chez les femmes (soumis dans le Journal of Dermatological Science en 2013). Le lien entre les AGPI-LC n-3 et le vieillissement cutané a été étudié par Nagata et co-auteurs (2010). Contrairement à notre étude, Nagata et son équipe n'ont pas mis en évidence de lien entre les apports en AGPI-LC n-3 (EPA + DHA) et le vieillissement cutané chez leur population de 716 femmes japonaises, âgées de 20 à 74 ans. Cependant dans leur population, les apports les plus faibles en AGPI-LC n-3 (quintile inférieur) étaient supérieurs aux apports les plus élevés (quintile supérieur) observés dans notre population française, et de ce fait peut-être déjà suffisants pour observer un effet protecteur. Par ailleurs, la taille relativement faible de leur échantillon et la présence d'individus jeunes (20-74 ans, moyenne ± écart-type : 43.3 ± 8.2) peuvent expliquer l'absence de résultat significatif. Purba et co-auteurs (2001) n'ont pas étudié directement le lien avec les apports en AGPI-LC n-3, mais ils rapportent un lien inverse entre la consommation de poisson, principale source des AGPI–LC n-3, et le vieillissement actinique du dos de la main. Dans de notre étude, nous avons également mis en évidence un lien inverse entre sévérité du photo-vieillissement cutané et apports en ALA chez les hommes ce qui n'avait jamais été étudié auparavent. Un lien inverse a été mis en évidence avec les ALA fournis par les produits végétaux : huiles végétales et fruits et légumes chez les hommes et une tendance avec les huiles végétales chez les femmes. Parmi les différentes huiles végétales, l'huile d'olive était, comme pour les AGMI, celle qui contribuait le plus aux apports en ALA (51%), les huiles comme l'huile de noix et de colza, très riches en ALA, étant peu consommées.

Le lien inverse entre apports en AGPI n-3 et la sévérité du vieillissement cutané peut être dû à l'effet de ces acides gras sur l'inflammation déjà rapporté dans des études expérimentales sur les animaux et dans quelques études cliniques (Takemura et al., 2002 ; Black et Rhodes, 2006 ; Galland, 2010). Il peut également être le reflet d'une alimentation saine liée à une consommation élevée de fruits et légumes, huile d'olive et poisson.

Des résultats différents ont été observés entre les hommes et les femmes. Ces écarts pourraient être liés à des différences structurelles, physiologiques et fonctionnelles de la peau entre les sexes dues principalement à l'influence hormonale. En effet, l'épaisseur de la peau est globalement plus importante chez l'homme (16% de plus) que chez la femme, elle contient plus de capillaires que la peau des femmes, sa production de sébum est également plus importante et elle serait également plus hydratée. Enfin, les hormones stéroïdes sexuelles

influent également le métabolisme, l'accumulation et la distribution du tissu adipeux (Tchernof et *al.*, 2000 ; Cohen-Letessier et Bombal, 2002 ; Mayes et Watson, 2004 ; De Maddalena et *al.*, 2012). Ces écarts pourraient également être dus à des différences de comportement et de protection face au soleil. En effet, nous avons montré lors de notre étude sur le comportement et l'exposition au soleil que les femmes déclaraient plus fréquemment utiliser des produits de protections solaires et plus régulièrement tout au long de l'exposition que les hommes (*Guinot et al., 2001a*).

Parmi les points forts de ces études, on peut mettre en avant tout d'abord la taille importante de l'échantillon, issu de la population générale, avec à la fois des hommes et des femmes, et enfin la tranche d'âge étudiée (45 à 60 ans). En effet, cette tranche d'âge permet d'observer les différentes manifestations cliniques du vieillissement cutané car c'est généralement à partir de la quarantaine que certains signes tels que le relâchement et les troubles pigmentaires commencent à apparaitre (*Guinot et al., 2001a*). L'utilisation pour évaluer la sévérité du photovieillissement d'une échelle ordinale photographique développée, testée, et validée par Larnier et co-auteurs (1994) est également un élément important de cette étude. En effet, cette échelle combine à la fois les troubles pigmentaires, les rides et le relâchement ce qui permet ainsi de considérer le vieillissement cutané dans son ensemble. Par contre, on peut regretter de ne pas avoir pu prendre des photographies du visage des individus dans des conditions standardisées ce qui dans ce contexte épidémiologique à l'époque était impossible et reste difficilement réalisable sur de tels échantillons. L'estimation des apports alimentaires a été réalisée à partir de 10 enregistrements 24h remplis à différents jours et à différents moments de l'année ce qui a permis d'obtenir des données fiables et représentatives de la consommation habituelle des AGMI et des AGPI n-3 (Le Moullec et *al.*, 1996 ; Mennen et *al.*, 2002). Enfin, des facteurs de confusion connus comme la sensibilité naturelle de la peau au soleil (le phototype) et l'intensité d'exposition au soleil (auto-appréciée) ont pu être pris en compte dans les analyses.

Différents modèles de nutrition étaient envisageables afin d'étudier l'effet de l'apport en acide gras sur l'expression du photo-vieillissement cutané : des modèles de substitution (méthode standard, méthode des résidus ou méthode de densité) ou d'addition (méthode de partition). Nous sommes ici dans le cadre d'une prévention primaire où la composition de l'alimentation doit être modifiée pour diminuer le risque de photo-vieillissement, un modèle de substitution a donc été sélectionné. Ainsi, comme nous l'avons expliqué dans le chapitre 2.6 sur les méthodes statistiques, la méthode standard a été immédiatement écartée de part la forte

colinéarité existant entre les acides gras et l'apport énergétique total. Parmi les deux modèles de substitution restants, la méthode des densités a été retenue afin d'exprimer les résultats en termes de pourcentage d'apport énergétique. En effet, ceci présente l'avantage de fournir des résultats exprimés comme les nutritionnistes pour les mesures de la composition de l'alimentation que l'on retrouve également dans les recommandations nationales. La méthode des résidus a été néanmoins testée et a fourni des résultats très similaires à ceux obtenus avec la méthode des densités. Par ailleurs, les méthodes de densités et des résidus permettent de mesurer l'effet de la substitution des acides gras par les autres macronutriments à apport énergétique total constant, mais l'effet direct de l'apport en acide gras ne peut être étudié. Enfin, afin de compléter les résultats issus des modèles de substitution et apporter un éclairage supplémentaire sur l'effet du nutriment, quelques auteurs recommandent de tester la méthode de partition qui est le seul modèle qui permette une estimation directe de l'effet d'addition (Willett et *al.*, 1997 ; Thiébaut et *al.*, 2004).

D'autre part, certains auteurs préconisent d'ajuster les modèles de nutrition sur l'apport énergétique lié à l'alcool lorsque celui-ci est connu pour influencer le critère d'intérêt. Bien que l'effet de l'alcool n'est pas été mis en évidence dans les études sur le vieillissement cutané (Purba et *al.*, 2001 ; Nagata et *al.*, 2010), nous avons néanmoins décidé de tester nos modèles en ajustant sur l'apport énergétique lié à l'alcool. Comme attendu, dans notre population les hommes et les femmes présentaient des écarts importants de consommation d'alcool : l'écart inter-quartile Q1-Q3 = 3,4-11,6 %NRJ chez les hommes et 0,8-5,8 %NRJ chez les femmes. Après ajustement sur l'apport énergétique lié à l'alcool, les effets principaux trouvés pour les acides gras n'ont pas été modifiés, seuls les résultats pour les apports en huile d'arachide ont été légèrement changés chez les hommes, la tendance que nous avions trouvée (P=0,09) étant devenue significative (P=0,04). De plus, un lien entre la consommation d'alcool et le risque de photo-vieillissement a été mis en évidence chez les hommes, le risque de sévérité du photo-vieillissement augmentant avec la consommation d'alcool. En revanche chez les femmes, cet effet ne s'est pas révélé significatif. Il serait donc intéressant de pouvoir étudier l'effet de l'alcool sur le photo-vieillissement chez les hommes, de tester l'interaction avec le reste de l'alimentation, notamment l'apport en antioxydant, ainsi que de tester l'interaction avec d'autres comportements à risque comme le tabagisme l'exposition aux UV et l'utilisation de moyens de protection face au soleil.

Les principaux facteurs de confusion susceptibles d'interagir avec l'effet de l'alimentation sur le photo-vieillissement cutané ont été pris en compte dans ces travaux : le tabagisme, l'exposition solaire, l'utilisation de moyens de protection face au soleil et le phototype, appréciant la sensibilité naturelle de la peau au soleil. Nous avons retrouvé pour ces facteurs des résultats similaires à ceux obtenus lors de l'étude réalisée par Malvy et co-auteurs (2000) sur les facteurs liés à l'expression du photo-vieillissement.

Les femmes présentant un phototype IIIa, IIIb et IV ont montré un risque de photo-vieillissement avéré significativement plus faible que celles présentant les phototypes les plus clairs (I&II). Par contre, aucune différence n'a été mise en évidence entre les phototypes I&II et les phototypes les plus foncés (V), lequel présente pourtant une meilleure protection naturelle face au soleil. Ceci nous a conduit pour chacun des sexes à examiner de façon détaillée le comportement d'exposition et de protection au soleil pour chacun des phototypes et nous a permis de mettre en évidence des profiles d'exposition et de protection différentes selon les phototypes qui pourraient expliquer les liens trouvés avec l'expression du photo-vieillissement. En effet, les femmes présentant un phototype foncé (V), par rapport à celles ayant un phototype clair (I-II), ont déclaré plus fréquemment avoir été beaucoup exposées au soleil (28% *vs* 7%), avoir été exposées aux UV artificiels (11% *vs* 4%) et avoir pratiqué plus fréquemment une profession exposant particulièrement au soleil (16% *vs* 5%). De plus, elles ont déclaré utiliser moins régulièrement des moyens de protection solaire (42% *vs* 67%). Les femmes des autres phototypes présentaient, quand à elles, des profils intermédiaires en termes d'intensité d'exposition et de protection solaire. Les risques moindres de photo-vieillissement observés chez les femmes présentant les phototypes intermédiaires (IIIa à IV) par rapport à celles présentant un phototype clair (I-II) pourraient donc s'expliquer par une meilleure protection naturelle vis à vis des effets des radiations UV associée à une exposition modérée et à une bonne attitude de protection solaire. Par contre, les femmes présentant un phototype V, malgré une protection naturelle importante face au soleil, pourraient en raison de leur comportement d'exposition élevée au soleil et d'une utilisation modérée de moyens de protection présenter un photo-vieillissement proche de celui des sujets présentant des phototypes clairs.

Chez les hommes, aucune différence significative n'a été mise en évidence entre la sévérité du photovieillissement des sujets présentant un phototype clair (I-II) et ceux présentant les phototypes IIIa, IIIb ou IV. Par contre, les hommes de phototype V présentaient un risque plus élevé de photo-vieillissement que les autres phototypes. Comme chez les femmes,

l’examen du comportement d’exposition et d'utilisation de moyens de protection face au soleil a permis de constater que plus les hommes présentent une protection naturelle importante face au soleil (phototypes élevés), plus ils déclarent fréquemment s’exposer de façon intensive. En revanche chez les hommes, l’utilisation régulière de moyens de protection solaire est moins fréquente que chez les femmes, excepté chez les hommes présentant un phototype clair (I-II). Le bénéfice d'une protection naturelle importante face au soleil semble donc être annulé par un comportement d'exposition importante associée à une absence d'utilisation de moyens de protection.

Un lien entre la sévérité du photo-vieillissement cutané et l'intensité d'exposition au soleil au cours de la vie était attendu, cependant celui-ci s’est révélé non significatif. L’intrication complexe entre le phototype et le comportement d’exposition et d'utilisation de moyens de protection solaire peuvent en partie expliquer ce résultat. Les sujets présentant la meilleure protection naturelle au soleil (phototypes élevés) sont aussi ceux qui s’exposent de façon plus importante et qui se protègent le moins. De même, nous avons retrouvé le lien entre la localisation géographique et la sévérité du photo-vieillissement que Malvy et co-auteurs (2000) ont déjà rapporté : les sujets du sud de la France présentant un photo-vieillissement moins important par rapport a ceux du nord alors que cette région est réputée être soumise à un haut niveau de rayons ultraviolets ambiants. Les sujets vivant dans les régions françaises les plus ensoleillées ont probablement adopté un comportement de protection face au soleil et présentent aussi probablement plus souvent que ceux du nord un bronzage résiduel protecteur d'une année sur l'autre. Par ailleurs, cela pourrait indiquer qu’une exposition progressive, probable dans le sud de la France, pourrait également avoir un effet protecteur sur le photo-vieillissement par la production d’eumélanine.

L’effet du tabac sur le photo-vieillissement, rapporté par différents auteurs (Kadune et *al.*, 1991 ; Ernster et *al.*, 1995) était également attendu, mais aucun lien significatif n’a été mis en évidence dans notre étude. Ceci est sans doute dû à la variable disponible. En effet, nous ne disposions que d’une classification globale en trois niveaux (non fumeur, fumeur, ex-fumeur), alors que l'effet du tabac est généralement étudié grâce au nombre d'années de tabagisme ou par le nombre de paquets années (moyenne du nombre de paquets par jour multiplié par le nombre d’année pendant lequel le sujet a fumé) en tenant compte du nombre d'années d'arrêt pour les ex-fumeurs.

Le photo-vieillissement apparaît donc influencé à la fois par des facteurs génétiques (ainsi que le montre l'effet du phototype) en interaction avec des facteurs comportementaux, comme l'exposition et l'utilisation de moyens de protection face au soleil. Le rôle du polymorphisme génétique a été étudié par ailleurs dans une étude transversale réalisée sur un sous échantillon de 570 femmes issues de cette population : un risque plus élevé de photo-vieillissement sévère a été mis en évidence chez des porteurs de certains variants du gène MC1R prédisposant à une plus grande production de phaeomélanine, et du gène STXBP5 (Le Clerc et al., 2012 ; Elfakir et al., 2010). En effet il existe deux types de mélanine, l'eumélanine anti-oxydante et la phaeomélanine pro-oxydante. Le rapport entre ces deux types de mélanine, dont le gène MC1R est le principal responsable au niveau génétique, détermine en partie le phototype.

L'alimentation, en particulier la consommation d'acides gras, semble également pouvoir agir dans ce processus complexe qu'est le photo-vieillissement qui correspond au vieillissement cutané que l'on observe sur une zone non protégée (Purba et *al*., 2001 ; Nagata et *al.*, 2010 ; Cosgrove et *al.*, 2007 ; *Latreille et al., 2012*). Cependant, la liste des facteurs pouvant agir sur le vieillissement cutané ne s'arrête bien évidemment pas à ceux que nous avons pu étudier dans le cadre de cette étude. Une grande variété de facteurs physiques et chimiques peuvent agresser la peau et accroitre le vieillissement cutané. Ainsi, parmi les agents utilisés lors d'études spécifiques avec une mise en place d'un stress oxydant au niveau cutané, on compte des polluants aériens issus des dérivés de la combustion des carburants qu'ils soient d'origine automobile ou industrielle, des composants de la fumée de cigarette, des contaminants d'origine alimentaire, des substances médicamenteuses et des produits d'entretien (Athar, 2002). Par ailleurs, des rayonnements autres que les UV (infra-rouges, rayons-X, rayons gamma, mais aussi probablement la lumière visible) et la pesanteur, interviendraient également dans le processus du vieillissement de la peau (Pierard, 1996).

4.2 Perspectives

Dans la continuité de ces travaux, nous avons l'intention d'étudier les liens entre sévérité du photo-vieillissement et apports en AGPI n-6. En effet, contrairement aux AGPI n-3, les AGPI n-6 sont rapportés quand à eux pour avoir une action pro-inflammatoire (Calder, 2009). Ils produisent des dérivés qui sont la plupart du temps des inducteurs plus puissants de l'inflammation que ceux issus des AGPI n-3. Nous avons prévu dans le prolongement de l'étude des AGPI n-3 d'étudier sur notre population les liens entre la sévérité du photo-vieillissement et les apports nutritionnels en AGPI n-6 (LA et AA). L'effet des ratios des précurseurs des familles (LA/ALA) et des dérivés (AA/EPA) pourraient être également étudiés car les deux familles, AGPI n-3 et AGPI n-6, partagent les mêmes enzymes pour produire leurs métabolites et sont donc en concurrence pour la synthèse de leurs médiateurs. Ce travail pourrait être réalisé sur la population qui a été étudiée pour les AGMI et les AGPI n-3.

Un autre volet de notre recherche pourra s'attacher a étudié les liens entre la sévérité du photo-vieillissement et les apports en polyphénols. En effet, les polyphénols ont été décrits comme ayant des propriétés anti-oxydantes, anti-inflammatoires et immunomodulatrices qui pourraient protéger du photo-vieillissement. Les polyphénols représentent une grande famille de composés chimiques trouvés avant tout dans les fruits, les légumes, les noix, les graines et également dans les produits transformés comme le thé, le vin ou le chocolat. Ces quinze dernières années, un intérêt grandissant a été porté à ces composés dans la prévention des dommages photo-induits. Différentes études expérimentales menées in vitro et in vivo sur l'animal ont montré les propriétés anti-oxydantes, anti-inflammatoires et immunomodulatrices des polyphénols (Nichols et Katiyar, 2010 ; Asensi et al., 2011 ; González et al., 2011). Nous pourrons donc étudier sur le même échantillon d'individus que pour les acides gras les liens entre la sévérité du photo-vieillissement cutané et les apports alimentaires des principales classes de polyphénols. La base de données Phénol Explorer et une table de composition validée (Neveu et *al.*, 2010) sont actuellement disponibles et permettent de calculer les apports en les principales classes de polyphénols des participants de l'étude SU.VI.MAX.

Pour ces deux projets la méthodologie utilisée pour les deux recherches pourra être utilisée pour cette nouvelle problématique.

4.3 Conclusions

Ces travaux ont permis de mettre en évidence un lien entre les apports en huile d'olive, en AGPI n-3 et le photo-vieillissement chez une large population française d'hommes et de femmes d'âge moyen. Ces résultats confortent l'hypothèse selon laquelle les AGMI et les AGPI n-3 pourraient avoir un effet photoprotecteur. Ils contribuent à soutenir les recommandations en faveur d'un régime riche en huile d'olive et en AGPI n-3, comme le régime méditerranéen, dans la prévention du photo-vieillissement et du vieillissement en général. Dans la suite de ces travaux il pourrait être intéressant d'étudier le rôle éventuel des composants mineurs de l'huile d'olive (squalène et polyphénols) sur le photo-vieillissement cutané.

Liste des publications

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Annexes

Annexe 1. Regroupement des aliments simples (*en Italique*) en catégories de sources alimentaires (**en gras**)

Huile végétale
Huile d'olive, huile de tournesol, huile d'arachide, huile de maïs, huile de colza, huile de noix, huile de pépin de raisin, huile de soja, huile mélangée type Isio 4

Viandes
Bœuf : bifsteack, entrecôte, flanchet, faux filet, rôti braisé, pot au feu, steack hâché (5%, 10%, 15%, 20% matière grasse (MG) ou % MG inconnue), tournedos, côte *Veau : côte, escalope, filet, poitrine, rôti, épaule, jarret* *Mouton, agneau : côte, gigot, côtelette, épaule* *Porc : côte, filet, travers, poitrine* *Cheval : bifsteack, rôti*

Volailles et gibiers
Poulet, poule, coquelet, chapon, pintade, canard, magret, oie, caneton, dinde, caille, pigeon, faisan, lapin, lièvre, chevreuil, sanglier, biche, jambon de volaille, saucisse de volaille

Poissons et fruits de mer
Anchois, anguille, bar / loup, baudroie, brochet, cabillaud, carpe, carrelet, colin, eglefin, flétan, haddock, hareng, lieu noir, limande, lotte, maquereau, merlan, morue, mulet, perche, raie, rascasse, roussette, sardine, saumon, sole, thon rouge, truite, turbot, surimi, hoki, saumon, huître, moule, bigorneau, bulot, coquille saint-jacques, coque, praire, palourde, plateau de fruits de mer, crevette grise, crevette rose, gambas, langoustine, langouste, homard, crabe, calmar / poulpe / seiche, moule, crabe, tourteau

Charcuteries
Jambon de paris, cuit, jambon d'york, cuit, jambon fumé, jambon sec type bayonne, parme, bacon fumé, cuit, lard maigre (lardon), andouillette, crue, boudin blanc, cuit, boudin noir, cru, chair à saucisse, crue, merguez, crue, chipolata, crue, saucisse de morteaux, saucisse de toulouse, saucisse de cervelas, saucisse de francfort, saucisse sèche, saucisson de lyon, saucisson sec, saucisson à l'ail, salami, mortadelle, andouille, crue, foie gras, fromage de tête, galantine, pâté de campagne, pâté de foie de porc, rillettes, pâté / terrine autre, jambonneau, cuit, viande des grisons, chorizo sec

Produits laitiers
Fromages : beaufort, bleu, bonbel - babybel, mini-babybel, boursin, brie, camembert : 40%, 45%, 50% ou 60% matière grasse/matière sèche (MG/MS), mini-camembert 45% MG/MS, camembert allégé 25% MG/MS, cantal, carré de l'est, chabichou, chaource, cheddar, chèvre mi-sec, chèvre mou, comté, coulommiers, crottin de chèvre, edam, emmental, fromage fondu 45%, 65% ou 70% MG/MS, fromage à pâte ferme 25% MG/MS, fromage à pâte molle 25% MG/MS, gouda, maroilles, morbier, munster, neufchâtel, parmesan, picodon, pont-l'evêque, pouligny saint-pierre, pyrénées au lait de vache, pyrenées au lait de brebis, raclette, reblochon, roquefort, rouy, saint-marcellin, saint-paulin, saint-nectaire, sainte-maure, selles-sur-cher, tomme, vacherin, féta de brebis, mozzarella, mascarpone, mimolette, gruyère râpé, vache qui rit, vache qui rit allégée, saint-môret, saint-môret allégé, mini-caprice des dieux, mini-boursin, mini-roquefort, mini-emmental, mini-bresse bleu, kiri, samos 99, p'tit louis, mini-sylphide, mini-rambol noix, chavroux, mini-meule d'or, saint-gervais carré, tranches fines, mini-tartare, tranches fines allégées *Laits : lait cru entier, lait frais entier, lait frais demi-écrémé pasteurisé, lait frais écrémé, lait entier Ultra Haute Température (UHT), lait demi-écrémé UHT, lait écrémé UHT, lait concentré non sucré, reconstitué, lait concentré sucré, reconstitué, lait concentré sucré, non reconstitué, lait écrémé en poudre, lait de brebis, lait de chèvre, milk-shake, lait aromatisé, lait demi-écrémé en poudre, lait demi-écrémé à teneur garantie en vitamines, lait supplémenté en vitamines ou minéraux, lait de croissance, lait fermenté, lait autre* *Matière grasse : beurre, beurre allégé, beurre demi-sel, beurre allégé demi-sel, crème fraîche, crème fraîche allégée 15% MG* *Yaourt : yaourt nature, yaourt 0% MG nature, yaourt au bifidus nature, yaourt aromatisé, yaourt aromatisé allégé, yaourt à boire aromatisé, yaourt au lait entier nature, yaourt au lait entier aromatisé, fromage blanc 40% MG/MS nature, fromage blanc 20% MG/MS nature, fromage blanc 10% MG/MS nature, fromage blanc 0% MG/MS nature, fromage frais demi-sel nature, fromage frais 0% MG/MS nature, fromage frais 40% MG/MS aux fruits, fromage frais 0% MG/MS aromatisé, petit-suisse 40% MG/MS nature, petit-suisse 30% MG/MS nature, petit-suisse 20% MG/MS sucré, petit-suisse nature, sucré, yaourt au bifidus et acidophilus, nature, yaourt au soja, yaourt au lait de brebis, autres laits fermentés, petit-suisse aromatisé, yaourt au bifidus aromatisé, fromage frais 40% MG/MS nature*

Fruits et légumes
Abricot, banane, brugnon - nectarine, cerise, citron, clémentine, figue, fraise, framboise, fruit de la passion, grenade, kaki, kiwi, mandarine, mangue, melon, mirabelle, myrtille, nectarine, nèfle, orange, pamplemousse jaune, pamplemousse rose, papaye, pêche, poire, pomme, prune, raisin blanc, raisin noir, olive noire, olive verte, avocat, chicorée (salade), chou blanc, chou rouge, concombre, cresson, endive, frisée, laitue, mâche, pissenlit, poireau, salade verte, artichaut, asperge, aubergine, bette, brocoli, carotte, céleri-branche, céleri-rave, champignon, chou blanc, chou rouge, chou vert, choucroute, chou-fleur, choux de bruxelles, courgette, endive, epinard, fenouil, haricot beurre, haricot vert, maïs doux, marron / châtaigne, oignon, petit pois, poireau, poivron rouge, poivron vert, potiron, radis noir, radis rose, salsifis, tomate cerise, tomate (crue ou cuite, sauce), ail, cerfeuil, ciboulette / fines herbes, curry, echalotte, gingembre, persil / basilic, poivre

Céréales
Pain (complet, de mie, de seigle, biscotte, grillé, viennois, azyme, suédois, aux céréales, sans sel), croûton de pain, chapelure / panure, pâtes (ordinaires, aux oeufs, complètes), pâtes fraîches, blé. cuit, semoule, semoule de maïs (polenta), quinoa, riz (blanc, complet, sauvage), vermicelle, tapioca, flocon d'avoine, céréale pour petit déjeuner type all bran, enrichie, céréale sucrée pour petit déjeuner, enrichie, muesli aux fruits pour petit déjeuner, enrichi, pétale de maïs pour petit déjeuner non sucrée, enrichie, riz soufflé pour petit déjeuner, enrichi, blé soufflé pour petit déjeuner, enrichi, maïs soufflé pour petit déjeuner, enrichi, céréale chocolatée pour petit déjeuner, enrichie, céréale allégée pour petit déjeuner type spécial k. enrichie

Fruits à coque
Amande, noisette, noix, noix du brésil, noix de coco, cacahuète, pistache, noix de cajou

Sucrerie
Entremets, crème anglaise, crème dessert au chocolat, chantilly allégée 15% MG, meringue, croissant ordinaire, croissant au beurre, pain au lait, pain au chocolat, biscuit type petit beurre, petite galette ronde, biscuit chocolaté, barre chocolatée, chocolat au lait, chocolat au lait, avec fruits secs, chocolat noir, chocolat allégé, pâte chocolatée, chocolat blanc, cookie, beignet, barre de céréales, biscuit pour petit déjeuner, chausson aux pommes, chouquette, pâte brisée, pâte feuilletée.

Annexe 2. Article sur la typologie du comportement alimentaire

Use of multiple correspondence analysis and cluster analysis to study dietary behaviour: Food consumption questionnaire in the SU.VI.MAX cohort

Guinot C, Latreille J, Malvy D, Preziosi P, Galan P, Hercberg S, Tenenhaus M.

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Use of multiple correspondence analysis and cluster analysis to study dietary behaviour: Food consumption questionnaire in the SU.VI.MAX. cohort

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Abstract. Although the effects of individual foods or nutrients on the development of diseases and their risk factors have been investigated in many studies, little attention has been given to the effect of overall dietary patterns. The main objectives of this study were to identify dietary patterns and groups of subjects with similar food consumption habits, i.e. 'dietary profiles', using multiple correspondence analysis and cluster analysis. A food frequency questionnaire was sent to a large population-based sample (2923 women and 2180 men), recruited among the 'SUpplémentation en VItamines et Minéraux AntioXydants' (SU.VI.MAX.) cohort participants in France. The food items were dichotomised in order to focus the study on the highest levels of consumption. Multiple correspondence analysis allows the construction of principal components, which optimally summarise the data, and enables the construction of graphical displays. An interesting property of these graphical displays is that associations between food items can be observed on various projection planes, each category of each food item being located at the centre of gravity of the subjects corresponding to this category. An ascending hierarchical classification was unsuccessfully tried in order to determine clusters from these principal components. Therefore, a 'dissection' of the cloud of points was performed according to the orientation of the axes, providing a readily interpretable eight-dietary profiles typology for each sex. This statistical approach allows identification of particular dietary patterns and dietary profiles, which might be more appropriate in studies of diet-disease associations than the single food or nutrient approach that has dominated past epidemiological research.

Key words: Cluster analysis, Dietary patterns, Food consumption habits, Food frequency questionnaire, Multiple correspondence analysis, Nutritional epidemiology

Introduction

The hypothesis, that diseases not caused by severe deficiency malnutrition could nevertheless be linked to nutritional factors emerged in the 1960s. These diseases, which today comprise major public health problems in industrialised countries (notably cancers, cardiovascular diseases, obesity, osteoporosis, etc.), are clearly of multifactorial origin. Among the potentially determinant factors, dietary behaviour seems to play a important role, even more so because it is possible to act on it and thus there is the hope of reducing the risk of disease [1, 2]. Since the features of dietary behaviour are of a multidimensional nature, its description and an understanding of its impact on the possible development of chronic diseases comprise a major factor in terms of scientific knowledge and public health.

In the majority of works performed to date, the analyses of links between diet and health have taken only one 'isolated' food or nutrient into consideration. The dietary data are most often collected by means of a questionnaire on the frequency of consumption on a qualitative or 'semi-quantitative' basis and relating to a few dozen groups of foods [3]. In addition, the information about physical activity that is indispensable for understanding the nutritional balance and the relationship between diet and health is generally missing of very brief [3]. In epidemiological observation studies (ecological studies, case–control studies or prospective studies), the relationships between the consumption of foods [4] (e.g. meats, fish, cereals, fruit, sugar, fats, wine, etc.) or nutrients [5] (fats, carbohydrates, proteins, trace elements) on the one hand and either the value of nutritional status markers [6, 7] (blood levels of cholesterol, triglycerides or glucose, body fat index, markers of vitamin and mineral status) or health indicators [8, 9] (cancer, cardiovascular disease, diabetes, obesity, osteoporosis) on the other hand are analysed.

Indeed, although some adjustments are made in the best cases [9], these analyses do not include the fact that apart from its possible direct effect on nutritional status (or state of health), the consumption of a given food or nutrient may intervene indirectly by the fact that it is associated with the consumption of other foods or that it modulates the intake of other nutrients [10]. In addition, the consequences of the consumption of a given amount of a given food (or nutrient) on nutritional status or health may be totally different if the consumption of other foods (or nutrients) differs [11, 12].

Multiple correspondence analysis [13–15] is a factor analysis method which summarises a set of categorical variables into a small number of orthogonal variables called principal components. When all the categorical variables are binary, a multiple correspondence analysis is equivalent to a principal component analysis of the dummy variables describing the binary categorical variables. Graphical displays are used to summarise the proximities between the subjects and to show the associations between the categorical variables. The subjects are represented in two-dimensional graphical displays constructed using principal components as axes system. Categories of variables are located at the centre of gravity of the subjects corresponding to these categories. This last representation is a specificity of multiple correspondence analysis.

The purpose of this study was to identify dietary patterns and to identify groups of subjects with similar food consumption habits, i.e. 'dietary profiles'. This approach could possibly enable the investigation of diet-disease relationship more efficiently than the study of individual foods or nutrients as the overall pattern of a diet may have a greater effect on health than any single component. Multiple correspondence analysis and cluster analysis have been applied to food consumption frequency data collected within the context of the monitoring of subjects enrolled in the 'SUpplémentation en VItamines et Minéraux AntioXydants' (SU.VI.MAX.) cohort, comprising a large sample of adult subjects living in France and recruited from the general population.

Material and methods

Study context and background

The SU.VI.MAX. study is a randomised double-blind, placebo-controlled, primary-prevention trial designed to test the efficacy of a daily supplementation with antioxidant vitamins and minerals, at nutritional doses, in reducing the major health problems in industrialised countries, and especially the main causes of premature death [16, 17] conducted in a 'free-living' adult population sample. In this aim, 12,735 eligible volunteers (women aged 35–60 years; and men aged 45–60 years at inclusion) were recruited according to sex, age-group, smoking habits and geographic location, randomly included in 1994 and are being followed up for 8 years. The selection criteria of this study excluded subjects presenting ambiguous motivations or obsessional behaviours concerning diet and health [17]. The methods, design, and population characteristics of the study have been described elsewhere [16, 18].

Briefly, demographic and medical history, anthropometric measurements and clinical examinations were performed at onset for all participants. The variables assessment available and used in the current analysis included demographic, anthropometric, lifestyle, cigarette smoking, oral contraceptive use and menopausal status information. Smoking history was classified as current, former or never. Height and weight were obtained with subjects in standardised clothing. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in m^2). Dietary intakes were measured every 2 months by means of a specific program adapted for Minitel (French local precursor of the web), together with an iconographic support package. This information is based on consumption during the previous 24 hours, and is requested on different days of the week.

The SU.VI.MAX. study has been approved by the ethical committee for studies with human subjects (CCPPRB no. 706) of Paris-Cochin, and the 'Comité National Informatique et Liberté' (CNIL no. 334641) which advocates that all medical information be confidential and anonymous.

As a satellite study of the longitudinal survey, an optional questionnaire on food consumption frequency was sent by post to the cohort individuals in 1997 independently of the usual nutritional monitoring.

Food-frequency questionnaire

The food-frequency questionnaire concerned the frequency of consumption of various foods at various times of day, and the subjects had to estimate the average number of times they consumed the food in question during the previous 6 or 12 months. For each food, the frequency of consumption was noted at different times of day corresponding to the main meals and between meals. To remedy this dispersion of the data, the cumulative value for the day was calculated for each specified food, leading 44 consumption frequency variables for the day.

Sample description

Among the 5723 questionnaires sent back by the cohort subjects, 5003 could be analysed and related to 2923 women and 2180 men. General characteristics of the studied sub-sample are shown in Table 1.

Table 1. Socio-demographic characteristics of the studied population sample

	Women	Men
Number of subjects	2923	2180
Age class (years)[a]		
35–39	504	–
40–44	669	–
45–49	798	915
50–54	503	635
55–60	449	630
Socio-professional categories (%)		
Farmers, self employed	4.0	8.1
Managerial staff, intermediate profession	55.3	62.8
Employed, workers	19.8	12.2
Non-active subjects	22.9	16.8
Level of education (%)		
Elementary school	17.1	22.6
Secondary school	40.5	36.9
University or equivalent	42.4	40.5
Family situation (%)		
Living alone	18.7	9.2
Co-habitating	81.3	90.8
Tobacco habits (%)		
Non-smoker	56.8	33.6
Former smoker	29.5	47.5
Current smoker	13.7	18.9
Contraceptive habits (%)		
No contraception	65.1	–
Oral contraception	12.7	–
Intra-uterine device	22.2	–
Hormonal status (%)		
Menopausal	34.5	–
BMI in kg/m^2 (%)		
<18.5	3.9	0.5
18.5–25	73.3	48.2
25–30	16.8	43.4
$\geqslant 30$	6	7.8
Geographical location[b]		
Paris and Ile de France	19.6	18.1
Centre-East	8.4	8.2
North-West	9.2	9.4
North-East	7.1	7.5
West	24.6	24.4
South-West	8.1	9.2
Rhône-Alpes-Auvergne	13.4	13.5
Mediterranean location	9.5	9.8

[a] The data are presented as percentages, except for the number of subjects in the age classes.
[b] The French departments were grouped into eight geographic regions.

Data management

In the series of boxes available for a food in the questionnaire, it sometimes happened that one of the boxes was not completed. Since this event was not frequent (28% of missing values), we decided to replace the missing value by the mean observed from the available data of the food in question in each sex. This approach motivated by the rarity of the event in our data set had no significant impact on the distribution of the food in question. Among the various imputation methods, a more cautious but much more onerous imputation method would have been used. In the 'Hot Deck' method, for each non-respondent on one or more items a matching respondent is found on the basis of predetermined essential characteristics for imputation, then the missing items of the non-respondent are replaced by the respondent's values [19].

As classically reported in nutrition studies [20], most distributions of our food items were highly positively skewed. The preliminary linear correlation study revealed that the relationships between the food items were weak and the different transformations attempted for normalisation of distribution failed. Therefore, the food items were transformed by quantiles into categorical variables, in order to increase the strength of the links between the food items [21]. Since many subjects did not consume one or other of the food, the distributions were considered into two parts: consumers and non-consumers. Then, the distributions of consumers were divided using quartiles, which led to five categories for each food item. The underlying hypothesis was that links may exist only between the 'over-consumption' of some food items. From different groupings of these categories tested, it appeared that the median of the consumers was an interesting cut-off which can be used as a threshold. The distribution of each food item was eventually dichotomised: category 0 'no consumption and consumption less than the median of the consumers', and category 1 'consumption over or equal to the median of the consumers', in order to focus on the study of the highest levels of consumption [10].

Statistical methods

Separate analyses were performed for men and women in order to take into account any possible difference in dietary behaviour between the two sexes. A multiple correspondence analysis was first performed to construct principal components optimally summarising the data using the dichotomised variables [13–15]. This method allows to derive a set of coordinate values summarising the subjects and the food categories, and thus allows the associations between the subjects and the associations between the food categories to be displayed graphically (CORRESP procedure). The percentage of the variance of the principal components constructed using this method are known to underevaluate the information

summarised. Therefore, the percentage of explained variance of each component was estimated using the Benzecri formula (Benzecri option) [22]. The interpretable principal components were kept. Each principal component has been interpreted by examining the contribution of each food category to the variance of the category coordinates. The contribution of a category was considered significant when it was above the average contribution $1/p$ (where p is the total number of categories of the variables). Finally, graphical displays of the categories of food items were constructed, using the principal components, in a series of two-dimensional graphs, plotting one component against another within a set of axes systems (PLOT procedure).

An ascending hierarchical classification was tried unsuccessfully to determine the most plausible number of clusters (CLUSTER procedure, Ward's minimum variance method) from these principal components [23]. Unfortunately, the cubic clustering criterion [24] was always very negative, which means that the subjects were distributed according to a continuum in the space constituted by the components used as axes systems. Moreover, the projection of the individual data on the graphical displays obtained using different pairs of components did not show a visible cluster structure but a homogeneous data set. Consequently, it was decided to proceed to a 'dissection', according to the nomenclature proposed by Everitt [23], in a multidimensional space defined by the principal components in accordance to the orientation of these axes. This dissection led to groups of approximately equal size.

The statistical analysis was performed with SAS®, version 8.1 [24].

Results

Two types of missing values were encountered (1% of data): the first type (72%) correspond to an implied negative response, and the second (28%) to an omission that was replace by the mean observed for each sex for the food in question.

For women, the first three principal factors of the multiple correspondence analysis were kept. The graphical displays of the associations of food items for women are shown in Figures 1 and 2. The percentage of the explained variance have been calculated using Benzecri's formula: the first component amounts to 47%, with 21% for the second component and 11% for the third (total for the three components: 79%). Each component was labelled according the most contributive food items (highlighted with a dash line on the figures).

The interpretation of the axes was as follows: the first component illustrated a rich diet of the 'luxurious' type in the positive values (in beige on the figure), the second component a 'dietetic' behaviour in the negative values (in yellow), and the third component opposed a 'bread and butter' type of diet in the negative values (in red) to a over-consumption of 'alcoholic drinks' in the positive values (in brown).

For men, the first three principal factors of the multiple correspondence analysis were kept. The graphical displays obtained for men are presented in Figures 3 and 4. The percentage of the variance explained by the first component amounts to 44%, with 22% for the second component and 12% for the third (total for the three components: 78%). The interpretation of the axes was as follows: the first axis was again determined by a 'luxurious' type of behaviour in the positive values (in beige on the figure), the second axis had a 'dietetic' cannotation in the positive values (in yellow), and the third axis indicated a 'bread and butter' type of diet in the negative values (in red), but, unlike the situation found in women, the consumption of alcoholic drinks by men (in brown) was positively associated with 'luxurious' type (positive on the first axis) and negatively associated with a 'dietetic' type of behaviour (negative on the second axis).

The descriptive analysis of the typologies showed groups of foods which correspond to known behaviours. These groups of foods were used as dietary patterns to describe the dietary profile of each group of subjects: the consumption of fizzy drinks, sandwiches, cereals and mineral water was associated with behaviour considered to be 'modern', the consumption of biscuits, cakes, sweets, pastries and honey or jam was associated with behaviour described as 'greedy', the consumption of sweeteners, tea, infusions, fruit, cooked vegetables, green salad and fish was associated with behaviour described as 'healthy conscious', the consumption of chicory, tap water and soup was associated with behaviour described as 'traditional', and the consumption of coffee, meats, cooked pork meats, pulses and alcoholic drinks was associated with behaviour described as 'hedonistic'. These dietary patterns were then used for the schematic characterisation of the various groups of individuals who determine different dietary profiles.

Figure 5, which illustrates the grouping of individuals among the women, shows no visibly detectable structure to the data. Because of this, this mass of points was partitioned to produce eight groups of individuals more homogeneous according to the positive or negative value of the three axes. (Figure 6). This partition of the cloud of points led to a readily interpretable typology of dietary profiles for men and women.

A description of the socio-demographic characteristics of the eight dietary profiles of women is presented in Table 2. The eight women's dietary profiles have been described schematically, using the dietary patterns, in the following manner:

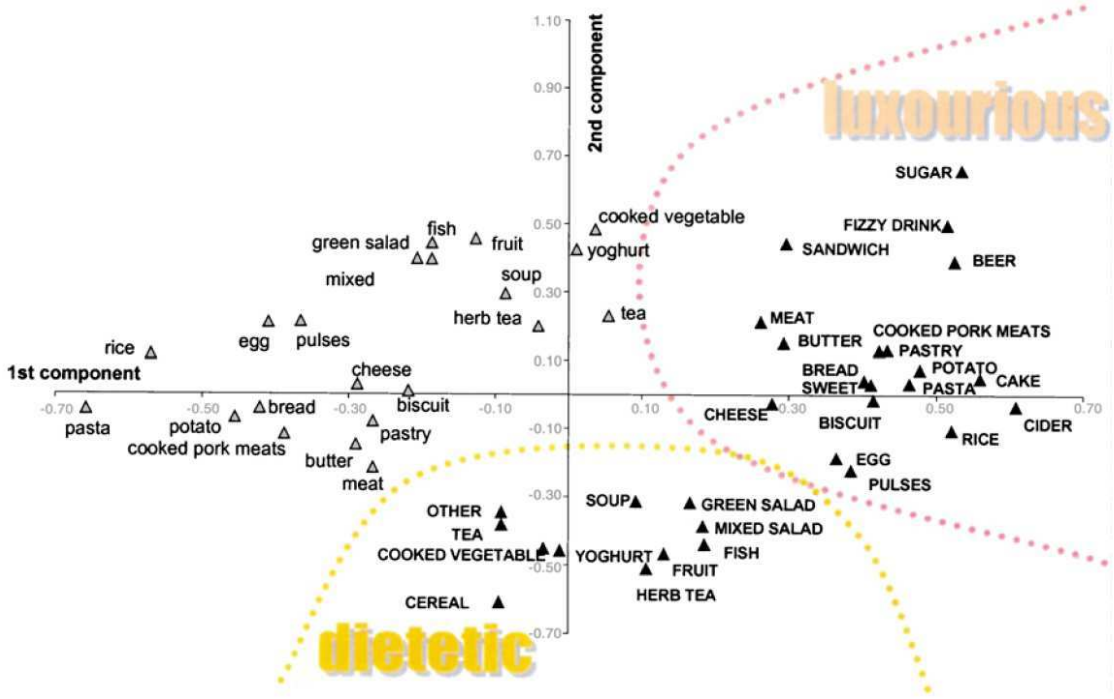

Figure 1. Map of food associations for women (first and second principal components); each food is visualised with a triangle: for 'over-consumption' categories, the name of the food is labelled in Capital letter, with a black triangle ▲; for 'non-consumption and under-consumption' categories, the name of the food is labelled in small letter, with a grey triangle △. For legibility, the most contributive categories were only plotted on each figure (categories with a contribution greater than the average contribution: >0.0114).

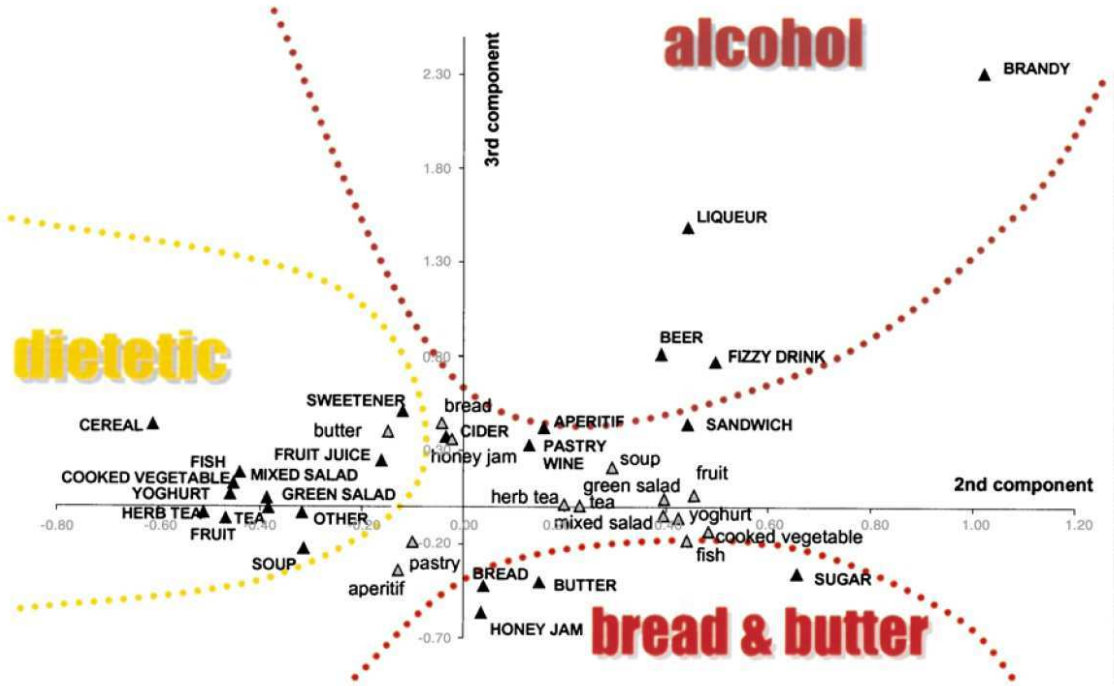

Figure 2. Map of food associations for women (second and third principal components); each food is visualised with a triangle: for 'over-consumption' categories, the name of the food is labelled in Capital letter, with a black triangle ▲; for 'non-consumption and under-consumption' categories, the name of the food is labelled in small letter, with a grey triangle △. For legibility, the most contributive categories were only plotted on each figure (categories with a contribution greater than the average contribution: >0.0114).

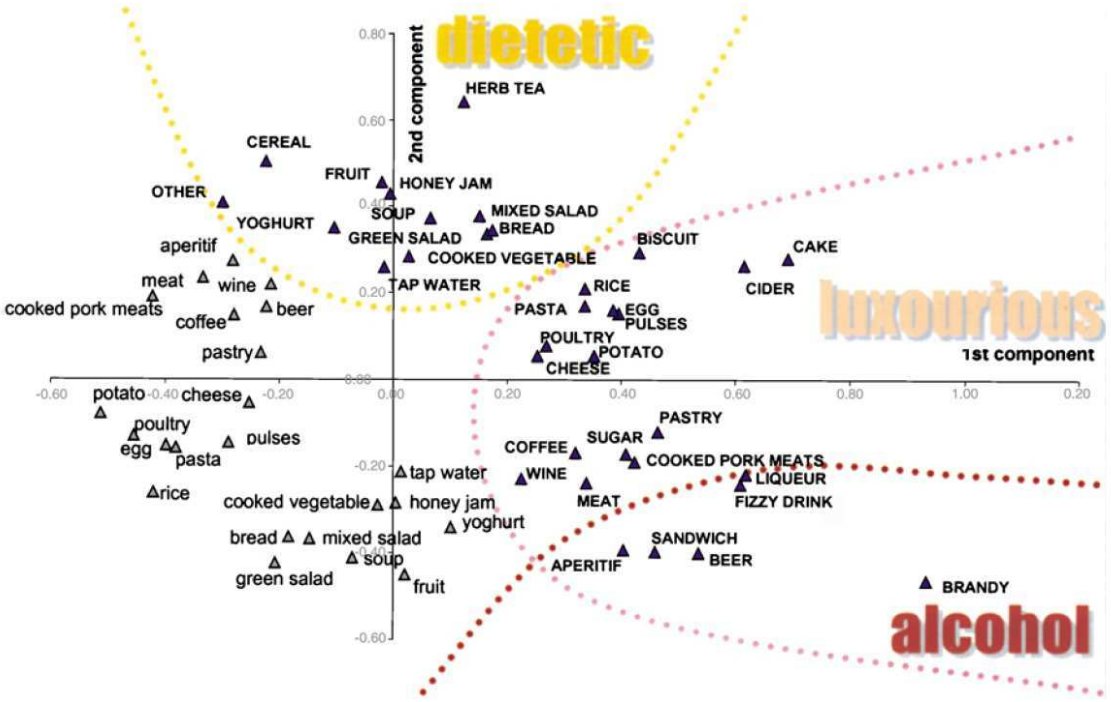

Figure 3. Map of food associations for men (first and second principal components); each food is visualised with a triangle: for 'over-consumption' categories, the name of the food is labelled in Capital letter, with a black triangle ▲; for 'non-consumption and under-consumption' categories, the name of the food is labelled in small letter, with a grey triangle △. For legibility, the most contributive categories were only plotted on each figure (categories with a contribution greater than the average contribution: >0.0114).

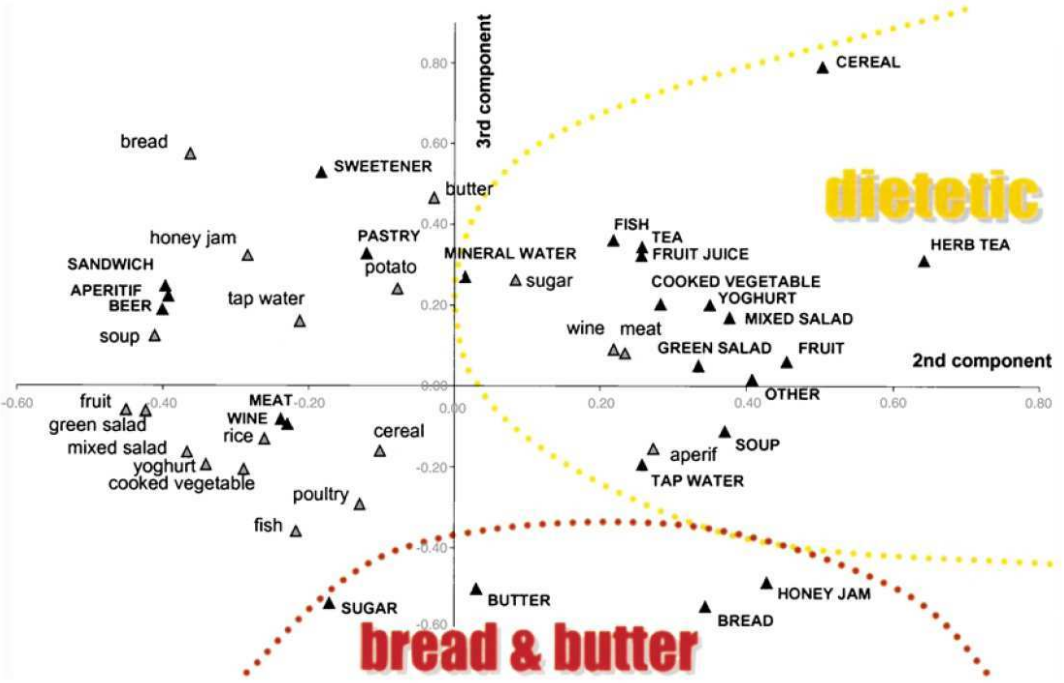

Figure 4. Map of food associations for men (second and third principal components); each food is visualised with a triangle: for 'over-consumption' categories, the name of the food is labelled in Capital letter, with a black triangle ▲; for 'non-consumption and under-consumption' categories, the name of the food is labelled in small letter, with a grey triangle △. For legibility, the most contributive categories were only plotted on each figure (categories with a contribution greater than the average contribution: >0.0114).

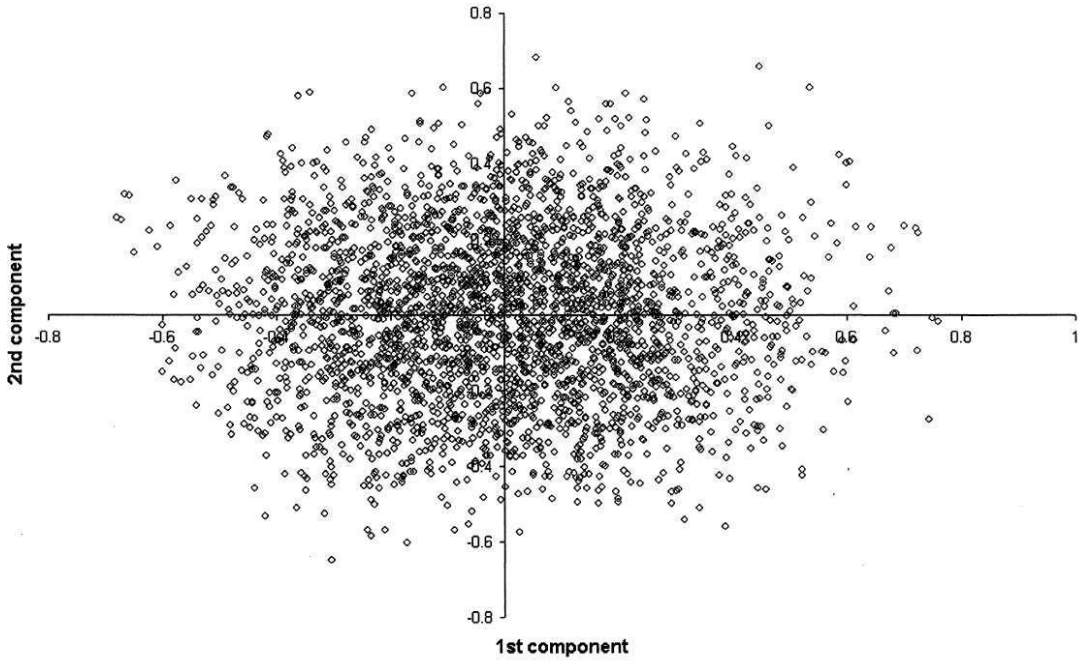

Figure 5. Map of association of individuals for women (first and second principal components); each individual value is visualised with a dot °.

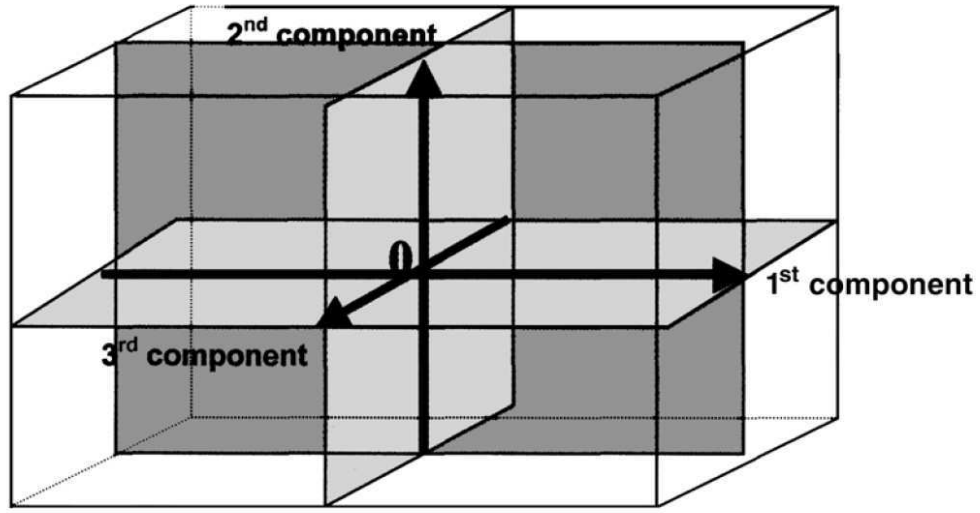

Figure 6. Partition of the plots of individuals into eight parts according to the sign of the principal components.

Group 1: 'modern', 'hedonistic' and 'greedy',
Group 2: 'hedonistic' avoiding alcohol, 'traditional' and 'greedy',
Group 3: 'modern', 'health conscious', and 'greedy',
Group 4: 'traditional' and 'greedy',
Group 5: 'modern',
Group 6: 'neutral',
Group 7: 'health conscious' and 'modern',
Group 8: 'health conscious' and 'traditional'.

Additional information on these dietary profiles is given in Table 3.

The description of the socio-demographic characteristics of the eight dietary profiles of men is given in Table 4. The eight men's dietary profiles have been described schematically, using the dietary patterns, in the following manner:

Group 1: 'hedonistic', 'health conscious' and 'modern',
Group 2: 'hedonistic' avoiding alcohol and 'traditional',
Group 3: 'hedonistic' and 'modern',
Group 4: 'hedonistic',

Table 2. Socio-demographic characteristics of the dietary profiles of women

Group	1	2	3	4	5	6	7	8
Number of subjects	332	399	344	397	361	381	374	335
Mean age, years (standard deviation)	48 (7)	47 (6)	49 (7)	49 (7)	47 (6)	49 (7)	49 (7)	51 (6)
Age class in years (%)								
35–39	22	22	17	14	21	18	15	9
40–44	26	25	22	25	24	20	22	19
45–49	27	29	27	26	32	24	26	27
50–54	13	13	20	19	12	20	17	24
55–60	12	11	14	16	10	18	20	21
Socio-professional categories (%)								
Farmers, self employed	4	4	3	3	4	6	5	3
Managerial staff, intermediate prof.	54	49	56	50	59	50	57	52
Employed, workers	21	21	21	20	19	20	20	14
Non-active subjects	21	26	20	26	17	23	18	31
Level of education (%)								
Elementary school	16	20	19	16	14	21	15	16
Secondary school	38	40	41	40	39	41	41	45
University or equivalent	47	40	40	43	48	38	45	39
Family situation (%)								
Living alone	19	15	17	13	23	22	21	18
Co-habitating	81	85	83	87	77	78	79	82
Tobacco habits (%)								
Non-smoker	48	63	60	63	43	62	48	67
Former smoker	31	24	26	27	35	27	40	26
Current smoker	21	13	13	11	22	11	12	7
BMI in kg/m^2 (%)								
<18.5	4	8	3	4	3	5	2	3
18.5–25	72	78	70	75	73	76	66	76
25–30	17	10	20	16	16	15	25	17
$\geqslant 30$	8	4	8	5	9	4	7	4
Geographical location (%)								
Paris and Ile de France	23	13	18	17	24	20	21	23
Centre-East	9	10	10	6	7	9	7	8
North-West	10	11	8	8	11	8	10	7
North-East	9	10	7	6	7	7	4	8
West	22	33	24	29	21	24	24	19
South-West	5	6	10	12	7	8	8	10
Rhône-Alpes-Auvergne	12	12	13	14	13	13	14	16
Mediterranean location	10	7	10	8	12	10	11	9

Group 5: 'health conscious' and 'modern',
Group 6: 'health conscious' and 'traditional',
Group 7: 'modern',
Group 8: 'neutral'.

Additional information on these dietary profiles is given in Table 5.

Discussion

Although the effects of individual foods or nutrients on the development of diseases and their risk factors have been investigated in many studies, little attention has been given to the effect of overall dietary patterns [25]. Using the data provided by the food frequency questionnaire completed by participants in the SU.VI.MAX. study, we identify dietary patterns, and looked for groups of subjects with similar dietary habits, in order to define 'dietary profiles'. Multiple correspondence analysis was performed on the food-frequency questionnaire collected from the SU.VI.MAX. cohort. The interpretable principal components were kept and graphical displays were constructed by projecting the data into a low dimensional space to allow the structure to be examined visually. The graphical displays representing the food items allowed visualisation of the food patterns, and the latter were used to characterise different dietary profiles [13–15]. Dissection of the cloud of points corresponding to individuals according to the orientation of the axes of the graphical display then

Table 3. Description of the eight women's dietary profiles

Group 1: 'modern', 'hedonistic' and 'greedy'
Women belonging to this group have a greater than average intake of coffee (1.3 times greater than the mean), fizzy drinks (3 times greater), fruit juice and alcoholic beverages, wine in particular (1.4 times greater), beer (3 times greater), cider (1.9 times greater), aperitifs (1.7 times greater), liqueurs (3.3 times greater) and brandies (5 times greater). Likewise, they have a greater than average consumption of sandwiches (1.8 times greater), biscuits, cakes, sweets, pastries (1.7 times greater), sugar (1.8 times greater), cooked pork meats (1.4 times greater) and of meat and pasta.

Group 2: 'hedonistic' avoiding alcohol, 'traditional' and 'greedy'
Women belonging to this group consume more chicory* and tap water and avoid alcoholic beverages. They consume more than the average amount of chocolate, biscuits, cakes, sweets, pastries and sugar (2.4 times greater than the mean), bread, butter (1.6 times greater), honey or jam, sandwiches, meat (1.3 times greater), cooked pork meats, pasta and potatoes.

Group 3: 'modern', 'health conscious' and 'greedy'
Women in this group are consumers of sweeteners (1.5 times greater than the mean). They are more than average consumers of tea, herbal teas (1.4 times greater), fruit juice (1.5 times greater), mineral water (1.4 times greater), fizzy drinks (1.5 times greater) and milk (1.2 times greater), and consume moderate amounts of alcoholic beverages. They consume more than the average amount of chocolate (1.2 times greater), cereals (1.7 times greater), yoghurts (1.3 times greater), fruit, cooked vegetables, mixed salad, green salad, pulses, (1.6 times greater), pasta, rice, fish (1.4 times greater), poultry, eggs, and cooked pork meats, as well as pastries, biscuits and cakes.

Group 4: 'traditional' and 'greedy'
Women in this group have a consumption greater than the mean of tea, herbal teas, chicory (1.4 times greater than the mean) and tap water (1.2 times greater), and they avoid fizzy drinks and alcoholic beverages. They eat biscuits, sweets, cakes, honey or jam (1.5 times greater), butter, bread and sugar, along with soup (1.3 times greater), fruit (1.3 times greater), mixed salad, cooked vegetables, green salad, pulses, potatoes, pasta (1.7 times greater), rice (1.4 times greater), eggs, poultry and fish.

Group 5: 'modern'
Women in this group are greater than the average consumers of coffee, fizzy drinks (1.7 times greater than the mean), aperitifs (1.4 times greater) and brandies (1.6 times greater). They consume sweeteners (1.5 times greater), sandwiches (1.7 times greater) and pastries, and avoid food such as soup, fruit, mixed salad, salad, pulses, potatoes, rice and bread.

Group 6: 'neutral'
This group does not reveal any particular supplementary food intake. Women in this group avoid alcoholic beverages, fizzy drinks, fruit juice and sweeteners. They take chicory (1.4 times greater than the average), bread, butter, honey or jam, and avoid cereals, biscuits, cakes, pastries, eggs and fruit.

Group 7: 'health conscious' and 'modern'
Women in this group consume greater than the average amount of tea (1.4 times greater than the mean), herbal teas, mineral waters, and avoid strong alcohol beverages. They consume sweeteners (1.7 times greater), cereals (2 times greater), yoghurts (1.3 times greater), cooked vegetables, mixed salad, green salad, fruit, poultry and fish. They avoid sugar, bread, butter, honey and jam, sweets, potatoes, pasta, rice, sandwiches, biscuits, cakes and pastries.

Group 8: 'health conscious' and 'traditional'
Women in this group consume more than the average amount of tea, herbal teas (1.5 times greater than the mean) and tap water, and they avoid coffee, fizzy drinks and all alcoholic beverages. They drink soup (1.3 times greater), cooked vegetables, fruit and yoghurts, and avoid sugar, pastries, cakes, biscuits and sandwiches.

* Chicory can be used as an additive put into coffee in France before filtering it, or as a substitute.

provided a readily interpretable typology of dietary behaviour.

A study of dietary patterns conducted on similar epidemiological data using multivariate approach was published recently. Schulze et al. [26] used dietary data collected by a food-frequency questionnaire completed by 8975 men aged 40–64 years and 13,379 women aged 35–64 years who were participating to the EPIC-Postdam survey. Dietary patterns were identified in this analysis using principal component analysis followed by Varimax rotation. The components were then used to investigate the relationship with nutrient intakes. These authors concluded that these components could be used as covariates when examining the role of a specific nutrient in order to establish whether this effect is independent of the overall dietary patterns.

In nutrition studies, the distributions of the food items are usually very highly positively skewed, therefore the linear Pearson correlation coefficient is inappropriate to measure the strength of links. So the use of principal component analysis is not relevant in this context as this method is based on linear correlations. Our alternative is to use multiple correspondence

Table 4. Socio-demographic characteristics of the dietary profiles of men

Group	1	2	3	4	5	6	7	8
Number of subjects	251	263	277	261	285	286	289	268
Mean age, years (standard deviation)	53 (5)	54 (5)	52 (4)	53 (4)	54 (5)	55 (5)	53 (5)	53 (4)
Age class in years (%)								
45–49	45	33	55	45	40	30	46	42
50–54	28	33	29	32	28	28	27	29
55–60	27	34	17	23	33	41	27	29
Socio-professional categories (%)								
Farmers, self employed	6	8	10	10	8	8	6	9
Managerial staff, intermediate prof.	63	60	70	59	62	60	65	64
Employed, workers	14	13	9	18	11	10	12	11
Non-active subjects	17	18	12	14	19	22	17	16
Level of education (%)								
Elementary school	23	28	19	31	20	21	18	23
Secondary school	35	29	36	40	35	43	38	39
University or equivalent	42	43	46	29	46	36	44	38
Family situation (%)								
Living alone	6	7	9	9	12	6	13	10
Co-habitating	94	93	91	91	88	94	87	90
Tobacco habits (%)								
Non-smoker	38	35	25	27	39	40	31	34
Former smoker	48	50	44	46	50	48	46	49
Current smoker	14	15	31	27	11	11	24	17
BMI in kg/m^2 (%)								
<18.5	0	0	0	1	0	1	0	1
18.5–25	43	60	37	45	51	57	39	53
25–30	49	36	51	47	40	37	50	39
$\geqslant 30$	8	4	12	8	8	4	11	7
Geographical location (%)								
Paris and Ile de France	14	13	24	19	17	14	22	22
Centre-East	7	11	8	8	6	10	6	8
North-West	12	5	10	15	8	9	6	13
North-East	9	9	8	8	5	5	8	9
West	29	34	22	27	23	23	16	23
South-West	12	9	8	8	9	12	9	6
Rhône-Alpes-Auvergne	8	11	13	10	15	18	19	12
Mediterranean location	9	8	7	5	16	9	14	8

analysis after having categorised the food items by quantiles. The major advantage of categorising food items is that it does not assume linear relationship and therefore decreases the influence of possible outliers data points [10], therefore in our approach all the dietary variables were dichotomised before the analysis.

Our results led to the definition of eight dietary profiles for each sex based on clusters of subjects according to their dietary patterns. These typologies may be used in analyses performed on the SU.VI.MAX. cohort and particularly to study the relationships between dietary behaviour and indirect risk factors such as abnormal blood lipid values, hyperglycaemia, hypertension, obesity or a precarious vitamin or mineral status or the occurrence of health-related events [9, 11, 25]. This methodology might be more appropriate in studies of diet-disease associations than the single food or nutrient approach that has dominated past epidemiological research. Our approach, which takes many aspects of dietary behaviour into account, may allow identification of the impact of certain behaviours (consumption of refined products, consumption of sugar, etc.) and may be used in other contexts as a contribution to the identification of factors in prevention campaigns.

Acknowledgements

The CE.R.I.E.S. is a research centre funded by CHANEL.

Table 5. Description of the eight men's dietary profiles

Group 1: 'hedonistic', 'health conscious' and 'modern'
Men in this group have a greater than average intake of tea (1.4 times greater than the mean), herbal teas (1.8 times greater), mineral waters, fizzy drinks (1.6 times greater), fruit juice (1.6 times greater) and a moderately greater intake of alcoholic beverages. They are greater consumers than the average of sweets (1.3 times greater), cakes (2.1 times greater), pastries (1.7 times greater), biscuits (1.9 times greater), sandwiches (1.3 times greater), yoghurts (1.3 times greater), cereals (1.6 times greater), fruit (1.3 times greater), eggs (1.5 times greater), soup, cooked vegetables (1.2 times greater), mixed salad (1.3 times greater), green salad (1.3 times greater), pulses (1.5 times greater), pasta (1.3 times greater), rice (1.5 times greater), fish (1.4 times greater) and poultry (1.4 times greater).

Group 2: 'hedonistic' avoiding alcohol and 'traditional'
Men in this group consume greater than the average amount of chicory, milk (1.3 times greater than the mean), cider (1.9 times greater) and tap water (1.2 times greater) and avoid fizzy drinks and aperitifs. They consume more chocolate (1.5 times greater), bread, butter (1.5 times greater), honey or jam (1.5 times greater), biscuits, cakes (1.5 times greater), sweets (1.3 times greater), sugar (1.5 times greater), soup (1.3 times greater), green salad, mixed salad, pulses (1.3 times greater), potatoes (1.3 times greater), pasta and eggs (1.3 times greater), and avoid sandwiches and cereals.

Group 3: 'hedonistic' and 'modern'
Men in this group are greater than the average consumers of coffee (1.2 times greater than the mean), fizzy drinks (2.1 times greater), mineral water, fruit juice and alcoholic beverages, especially wine (1.3 times greater), brandies (2.6 times greater) and liqueurs (1.9 times greater). They are greater consumers of sweeteners (1.6 times greater), meat (1.2 times greater), poultry (1.2 times greater), fish, cooked pork meats (1.4 times greater), sandwiches (1.9 times greater), pastries (1.6 times greater), and cakes, and they avoid chocolate, honey and jam.

Group 4: 'hedonistic'
Men in this group are greater than average consumers of coffee (1.2 times greater than the mean) and alcoholic beverages, wine in particular (1.4 times greater), and they avoid herbal and regular teas. They consume more meat (1.3 times greater), cooked pork meats (1.5 times greater), potatoes (1.3 times greater), pasta, bread, butter (1.7 times greater), sugar (1.9 times greater), sweets, pastries, cakes and sandwiches (1.5 times greater), and they avoid cereals and yoghurts.

Group 5: 'health conscious' and 'modern'
Men in this group consume more than the average amounts of tea (1.7 times greater than the mean), herbal teas (1.7 times greater), mineral water (1.2 times greater) and fruit juice (1.3 times greater), and they avoid all alcoholic beverages and fizzy drinks. They consume more sweeteners, cereals (2.5 times greater), fruit (1.3 times greater), yoghurts (1.5 times greater), cooked vegetables (1.3 times greater), mixed salad, green salad and fish and they avoid meat, cooked pork meats, potatoes, sugar, butter, cakes, pastries, sweets and sandwiches.

Group 6: 'health conscious' and 'traditional'
Men in this group consume greater than the average amounts of chicory (1.7 times greater than the mean), tap water (1.3 times greater), chocolate (1.8 times greater), bread (1.2 times greater), honey or jam (1.5 times greater), sweets, fruit, green salad, and soup (1.4 times greater) and avoid fizzy drinks and alcoholic beverages, sandwiches, cakes, pastries, and cooked pork meats.

Group 7: 'modern'
Men in this group consume greater amounts of sweeteners (1.8 times greater than the mean), more tea and mineral water and cereals (1.3 times greater). They avoid bread, honey and jam, chocolate, biscuits, cakes, sweets, but also eggs, fruit, mixed salad, cooked vegetables, soup and green salad.

Group 8: 'neutral'
Men in this group do not show any particular dietary preferences. They avoid all alcoholic drinks, fizzy drinks, fruit juice and sweeteners. They consume greater than the average amount of chicory (1.2 times greater than the mean), sugar and butter.

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Annexe 3. Article sur le comportement d'exposition au soleil

Sun exposure behaviour of a general adult population in France

Guinot C, Malvy D, Latreille J, Préziosi P, Galan P, Vaillant L, Tenenhaus M, Hercberg S, Tschachler E

Skin and Environment – Perception and Protection (J. Ring, S. Weidinger, U. Darsow, éditeurs), 10th EADV Congress, Munich, 2001. ISBN 88-323-1411-8, Monduzzi editore S.p.A., Bologne, 2001, p. 1099-106

Sun Exposure Behaviour of a General Adult Population in France

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Summary

To characterise sun exposure behaviour over the past year and during lifetime, a questionnaire was sent to the volunteers of the SU.VI.MAX epidemiological study. A clustering analysis was conducted on the first part of the questionnaire to select homogeneous groups of variables, then Principal Component Analyses (PCA) were performed on these groups to obtain scores. The same analysis was conducted on the lifetime data. Then to study the relationship between the data from both parts, a PCA was performed on all the scores. The three scores and the additional data from the first part of the questionnaire showed a good relationship with the nine scores and the additional data issued from the second part. The scores developed may be used in cohort studies to study the relationship between sun exposure, photoaging and the onset of skin cancers.

Keywords: Multiple logistic regression, Principal Component Analysis, self-administered questionnaire, sun exposure, sun protection.

Introduction

Ultraviolet radiation is known to play a major role in the development of skin cancers. Nevertheless, longer holidays, easier overseas travel and the fashion for tanning have led to an increase in sun exposure during the

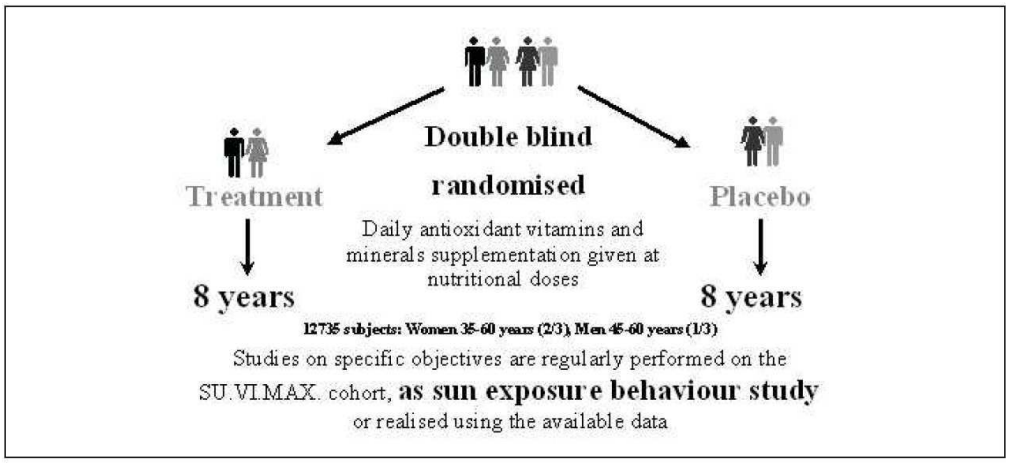

Figure 1: Experimental scheme of the SU.VI.MAX. study

last fifty years. The SU.VI.MAX. (SUpplémentation en Vitamines et Minéraux Anti-oXydants) ["Anti-oxidant vitamins and mineral supplementation"] study is an experimental epidemiological nutritional intervention study looking at the characteristic major chronic diseases of the industrialised nations [1-2] (Figure 1). A questionnaire on sun exposure was developed and distributed to the 12735 volunteers participating in the SU.VI.MAX. study with the aim of studying their sun exposure behaviour.

Materials and Methods

Eight dermatologists and epidemiologists contributed to the development of the questionnaire, which is presented in two parts, the first relating to sun exposure behaviour over the past year and the second to lifetime sun exposure. The questionnaire was mailed to the cohort of volunteers in February 1997. 70% of the questionnaires were returned in time for analysis and 63% were used.

A three step statistical analysis [3] was performed using SAS® version 6.12 [4]. 1- A descriptive analysis of sun exposure behaviour was conducted on male and female individuals in the different age group classes (UNIVARIATE procedure, and FREQ procedure, option CHISQ). Then to study the effect of gender, age and phototype on each of the questionnaire's main questions, a multiple logistic regression was performed (LOGISTIC procedure, option RL). 2- In order to characterise the sun exposure of men and women, various scores were produced. A typology of variables (VARCLUS procedure) was first used to obtain homogeneous clusters of variables. A Principal Component Analysis (PCA) was then performed on these groups of variables to produce scores (FACTOR and SCORE procedures). Cronbach's

coefficient alpha was then calculated for each of the scores to measure the internal consistency (CORR procedure, option ALPHA). 3- To examine the relationship between sun exposure over the past year and during lifetime, a Principal Component Analysis was performed on all the scores obtained from the two parts of the questionnaire and on some data which were not included in the scores (PRINCOMP and PLOT procedures). The number of compo-

Table 1. Distribution of the individuals

	Men			Women				
	Age groups			Age groups				
	45-49	50-54	55-60	35-39	40-44	45-49	50-54	55-60
1) Exposure over the past year								
Voluntary exposure	60%	54%	50%	77%	74%	66%	62%	52%
If voluntary exposure								
Tanning at the seaside	73%	67%	64%	71%	73%	65%	64%	68%
Tanning in the mountains	24%	24%	19%	30%	27%	26%	25%	16%
Tanning in the city	13%	12%	14%	22%	20%	19%	18%	16%
Tanning in the country	49%	51%	52%	50%	54%	53%	54%	47%
Tanning in a very sunny region	22%	25%	24%	22%	22%	23%	23%	21%
Tanning between 11 a.m. and 4 p.m.	49%	49%	47%	44%	48%	49%	44%	44%
Duration of exposure > 2h daily	33%	33%	31%	30%	28%	28%	25%	22%
Use of sun protection product	70%	63%	57%	90%	91%	90%	88%	85%
Gradual exposure	79%	79%	81%	80%	79%	82%	83%	83%
All the sample								
Sunburn during the year	48%	41%	32%	52%	46%	39%	35%	26%
Stay in a foreign country (> 1 month)	4%	3%	5%	2%	2%	2%	3%	4%
Use of other protections	72%	73%	76%	77%	78%	74%	78%	78%
Naturism (nudism)	8%	7%	7%	7%	7%	7%	6%	5%
UV tanning sessions	2%	1%	1%	6%	7%	5%	3%	1%
2) Lifetime sun exposure								
Voluntary exposure	54%	51%	51%	67%	68%	69%	70%	63%
If voluntary exposure								
Tanning between 11 a.m. and 4 p.m.	60%	63%	56%	59%	62%	60%	59%	55%
No use of sun protection product	13%	14%	15%	4%	3%	4%	3%	4%
Regular use of sun protection product	20%	18%	17%	43%	45%	42%	42%	44%
Gradual exposure	77%	76%	84%	76%	75%	76%	79%	83%
All the sample								
Use of other protections	76%	78%	79%	81%	79%	78%	82%	82%
Sunburn during childhood	90%	86%	84%	83%	79%	77%	74%	70%
Sunburn as an adult	92%	88%	87%	92%	90%	90%	88%	85%
Naturism (nudism)	11%	9%	9%	9%	11%	9%	10%	7%
UV tanning sessions	8%	8%	6%	26%	26%	23%	19%	17%
Stay in a sunny country (> 3 months)[$]	18%	26%	39%	11%	13%	12%	15%	15%
Outdoor occupation	19%	23%	24%	7%	7%	6%	9%	9%
Mountain sport in the sun	39%	38%	34%	39%	39%	36%	37%	30%
Nautical sport in the sun	24%	26%	26%	18%	18%	16%	18%	20%
Hobby in the sun	59%	60%	64%	40%	40%	45%	48%	50%

[$] The percentage of men of 50 to 60 years of age who stayed in a very sunny country for more than 3 months is very high, since many of them were in the Algerian war (1956-1962).

nents retained was determined using the scree test and the proportion of explained variance by each axis.

Results

1- Description of sun exposure behaviour

The distribution of the individuals according to the questionnaire's main questions was performed on 3259 men and 4825 women (Table 1). The effect of age, gender and phototype was tested on each of the main questions using multiple logistic regression. In order to study the effect of gender, only women aged between 45 and 60 years were included in the analysis so that age groups were comparable to those for men (Table 2). The darkest phototype (IV-VI) individuals were significantly more likely to report the voluntary exposure over the past year and during lifetime, as well as women and persons aged 45 to 54. Among the individuals who reported voluntary exposure over the

Table 2. Adjusted odds ratios for gender (Men: AOR=1), *age* (55-60 years: AOR=1), *and phototype* (phototype IV-VI: AOR=1); *AOR, Adjusted Odds Ratio; CI, Confidence Interval; * Wald test P-value <5%*

	Multivariate Model									
	Female		45-49 years		50-54 years		Phototype I-II		Phototype IIIa-IIIb	
	AOR	(95%CI)	AOR	(95%CI)	AOR	(95%CI)	AOR	(95%CI)	AOR	(95%CI)
1) Exposure over the past year										
Voluntary exposure	*1.4	1.2-1.6	*1.6	1.4-1.9	*1.3	1.1-1.5	*0.4	0.3-0.6	*0.6	0.6-0.7
If voluntary exposure										
Tanning at the seaside	0.9	0.8-1.0	1.1	0.9-1.3	0.9	0.7-1.2	0.6	0.4-1.1	0.9	0.7-1.0
Tanning in the mountains	1.0	0.9-1.2	*1.7	1.3-2.2	*1.7	1.3-2.1	1.3	0.7-2.3	1.0	0.8-1.2
Tanning in the city	*1.6	1.3-2.0	1.2	0.9-1.5	1.1	0.8-1.4	1.3	0.7-2.6	*0.8	0.6-1.0
Tanning in the country	1.0	0.9-1.2	1.0	0.9-1.2	1.1	0.9-1.4	1.4	0.8-2.4	1.0	0.8-1.1
Tanning in a very sunny region	1.0	0.8-1.2	1.0	0.8-1.2	0.9	0.7-1.2	0.7	0.4-1.4	0.8	0.7-1.0
Tanning between 11 a.m. and 4 p.m.	0.9	0.8-1.1	1.2	1.0-1.4	1.1	0.9-1.3	*0.5	0.3-0.8	*0.7	0.6-0.9
Duration of exposure > 2h daily	*0.7	0.6-0.8	1.2	1.0-1.5	1.1	0.9-1.5	*0.4	0.2-0.9	*0.8	0.7-0.9
Use of sun protection product	*3.7	3.1-4.6	*1.7	1.4-2.1	*1.3	1.1-1.7	1.8	0.9-3.9	*1.3	1.1-1.6
Gradual exposure	*1.1	0.9-1.3	0.8	0.6-1.0	0.8	0.6-1.1	2.0	0.9-4.8	*1.3	1.1-1.6
All the sample										
Sunburn during the year	*0.7	0.6-0.8	*1.9	1.7-2.2	*1.5	1.2-1.7	*1.7	1.2-2.5	*1.3	1.1-1.5
Stay in a foreign country (> 1month)	*0.6	0.4-0.9	*0.5	0.3-0.8	*0.5	0.3-0.8	0.8	0.2-3.4	0.8	0.5-1.2
Use of other protections	1.1	0.9-1.3	0.8	0.7-1.0	0.9	0.7-1.2	1.6	0.8-3.1	*1.3	1.1-1.6
Naturism (nudism)	0.9	0.7-1.1	*1.4	1.0-1.8	1.2	0.9-1.6	*0.4	0.2-1.0	*0.5	0.4-0.6
UV tanning sessions	*2.8	1.8-4.4	*3.0	1.7-5.4	1.4	0.7-2.7	1.6	0.6-4.7	1.2	0.8-1.9
2) Lifetime sun exposure										
Voluntary exposure	*2.0	1.8-2.3	*1.2	1.1-1.4	1.1	1.0-1.3	*0.4	0.3-0.5	*0.7	0.6-0.8
If voluntary exposure										
Tanning between 11a.m. and 4 p.m.	1.0	0.8-1.1	1.2	1.0-1.4	1.2	1.0-1.5	0.6	0.4-1.1	0.8	0.7-0.9
No use of sun protection product	*0.3	0.2-0.4	0.8	0.6-1.1	0.9	0.6-1.2	0.2	0.0-1.2	*0.6	0.4-0.8
Regular use of sun protection product	*3.2	2.7-3.8	1.0	0.8-1.2	0.9	0.8-1.2	*3.6	2.0-6.7	*1.2	1.0-1.4
Gradual exposure	1.0	0.8-1.1	*0.6	0.5-0.8	*0.7	0.5-0.9	*3.0	1.2-7.5	*1.2	1.0-1.5
All the sample										
Use of other protections	1.1	1.0-1.3	*0.8	0.7-1.0	0.9	0.8-1.1	*2.1	1.2-3.5	*1.4	1.2-1.6
Sunburn during childhood	*0.3	0.3-0.4	*1.6	1.3-1.9	1.2	1.0-1.4	*3.8	2.1-6.8	*2.2	1.8-2.5
Sunburn as an adult	*0.7	0.6-0.8	*1.7	1.3-2.1	1.2	0.9-1.5	*2.3	1.2-4.5	*2.0	1.7-2.4
Naturism (nudism)	0.9	0.7-1.1	1.3	1.0-1.6	1.2	1.0-1.6	0.7	0.3-1.3	*0.7	0.6-0.9
UV tanning sessions	*3.2	2.7-3.8	*1.5	1.2-1.8	1.2	1.0-1.5	0.9	0.5-1.5	1.0	0.8-1.2
Stay in a foreign country (> 3months)	*0.4	0.4-0.5	*0.5	0.4-0.6	*0.7	0.6-0.8	0.9	0.6-1.4	1.0	0.8-1.1
Outdoor occupation	*0.3	0.3-0.4	*0.7	0.6-0.9	0.9	0.8-1.1	0.7	0.4-1.2	*0.8	0.7-1.0
Mountain sport in the sun	*0.9	0.8-1.0	*1.2	1.1-1.4	*1.2	1.0-1.4	0.8	0.5-1.1	1.1	0.9-1.2
Nautical sport in the sun	*0.7	0.6-0.8	0.9	0.7-1.0	1.0	0.9-1.2	0.8	0.5-1.3	*0.9	0.8-1.0
Hobby in the sun	*0.6	0.5-0.6	*0.8	0.7-0.9	0.9	0.8-1.1	0.8	0.6-1.2	0.9	0.8-1.0

past year, women were about 4 times as likely to report using sunscreen than men, the use of sun protection products decreased as phototype darkened, and persons aged 55 to 60 were less likely to report the use of sun protection products. Among persons who reported voluntary exposure during lifetime, women were about 3 times as likely to report regularly using sunscreen than men, and the fairest phototype (I-II) were about 4 times as likely to report regularly using sunscreen. Men were significantly more likely to report sunburn during the year, during childhood, and as an adult, as well as youngest and fairest phototype individuals.

2- Characterisation of sun exposure

For the sun exposure over the past year, three scores were produced. Cronbach's coefficients indicated good internal consistency of these scores (Table 3). For lifetime sun exposure, nine indicators were produced. As the terminology of sun protection indices and the sun protection products themselves have changed over time, it was impossible to have a precise

Table 3. Scores for sun exposure over the past year (Cronbach's a)

Sunburn experienced over the past year (Cronbach's α=0.75)		
Score = -0.68	+0.75 If the individual suffered sunburn during the year	
	+1.08 If the number of sunburns is greater than 5 during the year	
	+0.96 If the most severe sunburn was more severe than simple redness	
	+0.93 If the skin peeled after a sunburn	
Sun protection over the past year (Cronbach's α=0.84)		
Score = -0.79	+0.49 If a protective product was used while getting a suntan	
	+0.55 If a protective product was used throughout the period of exposure	
	+0.54 If a protective product was applied regularly several times a day	
	+0.57 If a product with a SPF* rating of over 15 was used for the face	
	+0.59 If a product with a SPF rating of over 15 was used for the body	
	+0.52 If a protective product was used outside of the exposure period	
Intensity of sun exposure over the past year (Cronbach's α=0.69)		
Score = -1.13	+0.17 If tanning was done at the seaside	between 1 and 9 days
	+0.31 "	between 10 and 20 days
	+0.51 "	more than 20 days
	+0.28 If tanning was done in the city	between 1 and 9 days
	+0.43 "	between 10 and 20 days
	+0.56 "	more than 20 days
	+0.20 If tanning was done in the country	between 1 and 9 days
	+0.32 "	between 10 and 20 days
	+0.46 "	more than 20 days
	+0.32 If tanning was done in the mountains	between 1 and 9 days
	+0.40 "	between 10 and 20 days
	+0.46 "	more than 20 days
	+0.21 If sun exposure lasted	2 hours or less per day
	+0.50 "	more than 2 hours per day
	+0.49 If exposure	involved body and face
	+0.50 If exposure	was between 11 a.m. and 4 p.m.
	+0.54 If the subject feels that he or she	was moderately or greatly exposed
	+0.39 If sunbathing is	important or very important

* SPF: sun protection factor

estimation of lifetime sun protection, therefore a unique question was used to evaluate lifetime protection globally.

Here again, Cronbach's coefficients were satisfactory for all the scores (Table 4).

Table 4. Scores for lifetime sun exposure (Cronbach's a)

Intensity of lifetime sun exposure (Cronbach's α=0.80)	
Score = -1.34	+0.64 If in the habit of voluntary sun exposure +0.60 If the body and face were exposed +0.58 If the exposure was between 11 a.m. and 4 p.m. +0.48 If the subject feels that he or she was moderately or greatly exposed +0.52 If sunbathing is important or very important
Sunburn experienced during childhood (Cronbach's α=0.79)	
Score = -1.73	+0.86 If a sunburn occurred during childhood +0.46 If the sunburn occurred every summer time during childhood +0.69 If the most severe sunburn during childhood was more than a simple redness +0.78 If the skin peeled after a sunburn during childhood
Sunburn experienced as an adult (Cronbach's α=0.69)	
Score = -2.24	+1.17 If a sunburn occurred in adulthood +0.47 If the sunburn occurred every summertime in adulthood +0.80 If the most severe sunburn in adulthood was more than a simple redness +0.89 If the skin peeled after a sunburn in adulthood
Practice of naturism (Cronbach's α=0.63)	
Score = -0.30	+1.34 If naturism is practised +1.64 If naturism is practised several weeks per year +1.79 If the number of years that naturism has been practised is > 10 years *
UV tanning session (Cronbach's α=0.85)	
Score = -0.38	+1.29 If the subject engages in UV tanning sessions +2.89 If the subject engages in UV tanning sessions regularly +1.84 If the number of years of UV tanning is > 2 years *
Practice of mountain sport in which sun exposure is particularly great (Cronbach's α=0.84)	
Score = -0.65	+0.82 If the subject engages in a mountain sport where sun exposure is particularly great +1.00 If the number of days of sports activities is > 200 days * +0.94 If the subject still engages in that sport
Practice of nautical sport in which sun exposure is particularly great (Cronbach's α=0.83)	
Score = -0.43	+1.01 If the subject engages in a nautical sport where sun exposure is particularly great +1.31 If the number of days of sports activities is > 400 days * +1.29 If the subject still engages in that sport
Practice of hobby in which sun exposure is particularly great (Cronbach's α=0.86)	
Score = -0.88	+0.80 If the subject engages in a hobby where sun exposure is particularly great +0.79 If the number of days of hobby activities is > 900 days * +0.80 If the subject still engages in that hobby
Practice of an occupation where sun exposure is particularly great (Cronbach's α=0.83)	
Score = -0.29	+1.03 If an outdoor occupation is practised where sun exposure is particularly great +1.24 If the number of days that the 1st occupation was practised is >1000 * +2.09 If the number of days that the 2nd occupation was practised is >1078 * +1.07 If in the first occupation there was sun exposure between 11 a.m. and 4 p.m. +1.76 If in the second occupation there was sun exposure between 11 a.m. and 4 p.m.

* For the variables, estimating a duration of the distribution's median was used as a threshold for dichotomization

3- Relationship between sun exposure estimation over the past year and during lifetime

A Principal Component Analysis was performed on the 12 scores issued from the 2 parts of the questionnaire and on some additional data which were not used in the scores. The first six components accounted for 49% of total variance. A graphical display was produced by using the two first components as an axes system (Figure 2). The first component is a measure of the intensity of sun exposure, the use of sun protection products and the gradual sun exposure over the past year and during lifetime. The second component represents the use of other methods of sun protection over the past year and during lifetime, in relation with sun exposure avoidance.

The scores and the additional data from the first part of the questionnaire show a good relationship with the scores and the additional data from the second part of the questionnaire. For example, the score for "intensity of sun exposure over the past year" is linked to the score for "intensity of sun exposure during lifetime". Similarly, the gradual sun exposure over the past year is linked to the gradual sun exposure during lifetime.

The data which were not included in the scores, i.e. gradual sun exposure, other sun protection and use of sun protection products during lifetime, also contributed substantially to the components and hence to the characterisation of sun exposure behaviour.

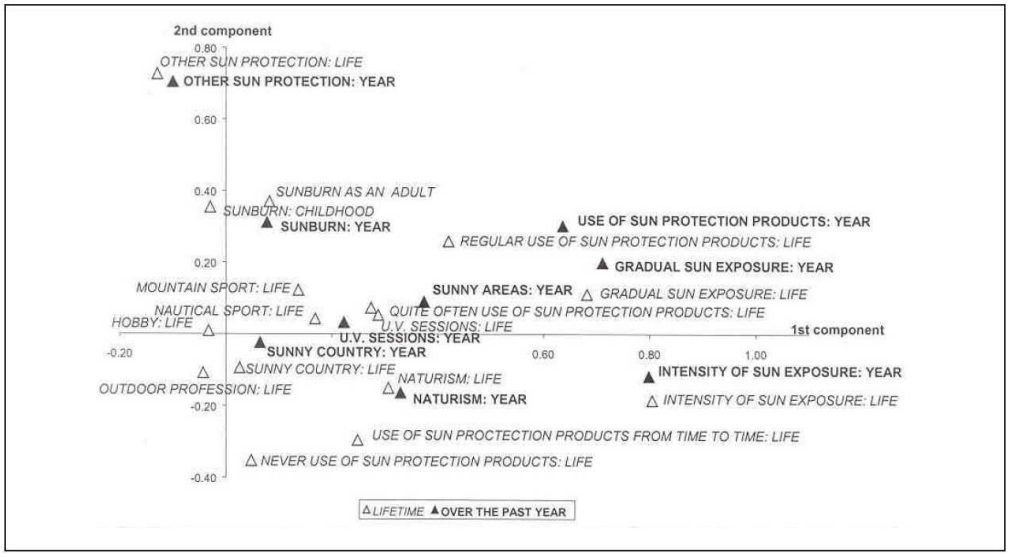

Figure 2: First factorial plane of the Principal Component Analysis

Conclusions

This study illustrates the various stages in the construction of scores based on qualitative data. Scores or indicators of this type are widely used in medical contexts to provide a quantitative measure of the phenomenon under study before proceeding to a more detailed analysis. The data also enabled the description of the sun exposure and protection behaviour of individuals participating in the SU.VI.MAX. cohort as well as the identification of differences in behaviour between men and women, and between younger and less young subjects with respect to the degree of sun exposure and the use of sun protection. The relationship identified between the use of protective products and the susceptibility to sunburn provides a good illustration of the difficulty of interpreting the link between photo-protection and the incidence of photo-induced dermatological pathologies. This study enabled the construction of quantitative indicators of sun exposure and protection behaviour. The results of this analysis will be used in the study of the relationship between sun exposure, photo-ageing and the incidence of skin cancers in the SU.VI.MAX. cohort.

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Annexe 4. Article sur la sensibilité naturelle de la peau au soleil

Sun-reactive skin Type in 4912 French Adults participating in the SU.VI.MAX Study

Guinot C, Malvy D, Latreille J, Ezzedine K, Galan P, Tenenhaus M, Ambroisine L, Hercberg S, Tschachler E

Photochem Photobiol, 2005, 81, 934-40

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Sun-reactive Skin Type in 4912 French Adults Participating in the SU.VI.MAX Study¶

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ABSTRACT

Phototype classifications were initially developed in an attempt to predict the skin reactions of patients to phototherapy and are now widely used to advise individuals with regard to sun protection. A transversal study was conducted on the SU.VI.MAX cohort to estimate the frequency of sun-reactive skin features in a large, general adult population-based sample, and to describe the associations between these features. The data were collected 3 years after the beginning of the SU.VI.MAX nutritional intervention study on 4912 volunteers (2868 women aged 35–60 years and 2044 men aged 45–60 years). A multiple correspondence analysis was performed to study the associations between the features. The results showed that these features correspond to a one-dimensional phenomenon, which allowed us to establish a score to summarize skin sensitivity to sun exposure. Furthermore, we found a link between gender and phototype using the Césarini classification (phototype ≥ IV: 37% of women, 47% of men). The analysis of the relationship with sun-reactive skin features and the score revealed the same trend. Phenotypic evaluation appears to be a good estimator of skin sensitivity to sun exposure for clinical screening or for use in research, and is easy to collect at a lower cost. Moreover, the sun sensitivity difference between gender should be considered in education about photoprotection.

INTRODUCTION

The concept of a sun-reactive skin type has been widely used among dermatologists. Recently, this concept has generated controversy in large part because of its subjectivity and ambiguities in its definition (1,2). Propensity to redden after sun exposure, constitutive and facultative skin pigmentation, and pigmentation of hair reflect polygenetic inheritance (3,4). These features are commonly used in epidemiological studies to assess sun sensitivity and to estimate an individual's ability to resist UV damage (5). However, repeated sun exposure may modify transitorily sun sensitivity, as adapted subjects can respond more like higher phototypes than nonadapted subjects of the same original phototype (6). Moreover, freckles and history of sunburn are linked to an individuals' sun sensitivity and behavior (7).

The phototype classification, which is indicative of the skin's natural protection against the sun, was proposed in the mid-1970s by Fitzpatrick (5) in order to produce a simple tool to help with the dosing of UV therapy for certain skin diseases. Its use was subsequently extended to assess the individual risk of sunburn and to define the principles of suitable protection (5). The phototype classification used in the present study was initially proposed by Césarini in 1977 (8). It is based on two dynamic skin reactions after sun exposure that are also used in Fitzpatrick's classification (*i.e.* the frequency of sunburn and the degree of tanning) and on three phenotypic features (*i.e.* the skin color in winter, the natural hair color at the age of 20 and the number of freckles). Combinations of these features led to an eight-class typology. However, individuals rarely have all of the features defining a class, and an expert's decision to place a subject into one class and not into another is to some extent a subjective one (9,10).

The aim of the present study was therefore to estimate the frequency of sun-reactive skin features used to determine Césarini phototype from a large French adult population sample, and to investigate the links between these features. Furthermore, we studied their relationships with gender and geographic location.

MATERIALS AND METHODS

Study design. The SU.VI.MAX study (SUppléments en VItamines et Minéraux Anti-oXydants—antioxidant vitamin and mineral supplements) is an experimental epidemiological study of nutritional intervention conducted

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†CE.R.I.E.S. is the research center on human skin, founded by Chanel.

Abbreviations: a* and b*, chromaticity coordinates; CCPPRB, Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale; CIEL*a*b*, Commission Internationale de l'Eclairage colour space; CNIL, Comité National Informatique et Liberté; ITA, individual typological angle (expressed in degrees); L*, measurement of brightness; MCA, multiple correspondence analysis; *r*, Pearson correlation coefficient; SU.VI.MAX study, study on antioxidant vitamin and mineral supplements (in French, SUppléments en VItamines et Minéraux Anti-oXydant)

Table 1. Sun-reactive skin type classification (Césarini classification)

Sun-reactive skin features					
Natural hair color at age 20	Skin color in winter	Freckles	Sunburn event frequency	Sun tan intensity	Phototype
White	Albino	No	Always	No	0
Red	Whitish	Frequent	Always	No	I
Blond	Light	Frequent	Always	Slight	II
Blond	Light	Some	Frequent	Light/dark	IIIa
Chestnut	Darkish	Some	Frequent	Light/dark	IIIb
Brown	Darkish	No	Rare	Dark	IV
Brown	Darkish	No	Exceptional	Very dark	V
Black	Black	No	No	Black	VI

in France that studies the main chronic disorders prevalent in industrialized countries and involves a large sample of men and women across the country (11). The study objectives, design, and population characteristics have been described elsewhere (12). Briefly, the SU.VI.MAX study is a randomized, double-blind, placebo-controlled primary prevention trial designed to test the efficacy of a daily supplement with antioxidant vitamins and minerals at nutritional doses (6 mg beta-carotene, 120 mg vitamin C, 30 mg vitamin E, 20 mg zinc and 100 µg selenium/day) in reducing the main causes of premature death conducted in a free-living adult population sample.

After a national multimedia campaign in 1994, about 80 000 subjects who sought to participate were requested to complete a questionnaire on demographic, lifestyle, nutritional and present and past health information. To be eligible, the subjects had to (1) provide complete data; (2) be in the selected age range; (3) declare themselves free of any severe pathology; (4) not be taking supplements containing any of the studied vitamins or minerals; (5) manifest no qualms about complying with the protocol constraints and (6) express no ambiguous motivation or obsessional behavior concerning diet and health. The recruitment began in October 1994 and ended in April 1995. Eligible subjects (12 741; women aged 35–60 years, men aged 45–60 years) were recruited according to gender, age group, smoking habits and geographic location. They were randomly included and were followed-up for 8 years (13). The framework relating to markers applicable to the SU.VI.MAX population offers the opportunity to study the sensitivity of human skin to sun exposure.

The SU.VI.MAX study was approved by the ethical committee for studies with human subjects (CCPPRB No. 706) of Paris-Cochin, and the "Comité National Informatique et Liberté" (CNIL No. 334641), which advocates that all medical information be confidential and anonymous. All participants provided an informed consent.

Study sample. During the first medical examination of volunteers, performed in 1996, investigators trained by a senior dermatologist determined phototype using an attribution rule proposed by Césarini in 1977 (Table 1). Unfortunately, the individual sun-reactive skin features were not collected at this time; only the resulting classifications were collected. Therefore, these features were collected by the same investigators during the second clinical examination in 1998. However, due to budget constraints, this research could be conducted only on a subsample of the cohort. To have a satisfactory profile of the distribution of sun-reactive skin features in the French population, 4922 individuals were sampled according to French regions among the volunteers of the cohort. Furthermore, in order to investigate an eventual regional effect, the regions were arbitrary grouped into four major geographic locations: (1) west and northwest; (2) northeast, east-central, and Ile-de-France; (3) southwest; and (4) Rhône-Alpes-Auvergne and Mediterranean.

In the classification proposed by Césarini, the sunburn event frequency feature is quantified into five categories (Table 1). During their training, the investigators declared they were unable to make a clear distinction between "exceptional" and "rare." Therefore, it was decided to consider these two levels as just one category. On the other hand, a technical problem occurred during the data computerization process for the variable that described the freckles of 890 volunteers; consequently, this information was definitively lost for these individuals. Moreover, due to their extreme rareness in our sample, seven black-skinned individuals and three premature white-haired individuals at age 20 (premature graying reported to be inherited in an autosomal dominant pattern) (14) were excluded from the analysis.

Table 2. Distribution of sun-reactive skin features and phototype according to gender

Sun-reactive skin features and phototype (%)	Gender*		
	Women (n = 2868)	Men (n = 2044)	Total sample (n = 4912)
Hair color			
Red	1§	1	1
Blond	9	7	8
Chestnut	67	60	64
Brown	22	30	25
Black	1	2	2
Skin color			
Whitish	2§	1	2
Light	60	50	56
Darkish	38	49	43
Freckles†			
No	72§	78	75
Some	25	19	22
Frequent	3	3	3
Sunburn event frequency			
No	5§	4	5
Exceptional/rare	46	54	50
Frequent	37	33	35
Always	12	9	10
Sun tan intensity			
No	1§	0	1
Slight	33	21	28
Light	31	28	29
Dark	35	49	41
Very dark	0	1	1
Césarini phototype‡			
I	0§	0	0
II	3	2	2
IIIa	13	6	10
IIIb	48	45	47
IV	31	39	34
V	6	8	7

*Due to missing data, values do not always add up to 2044 for men or to 2868 for women.

†Due to missing data, the skin feature "freckles" was available for only 4022 volunteers.

‡Phototype was determined in 1996 for only 4274 of the 4912 volunteers.

§Significant association between each feature and gender (chi-square test: $P < 0.0001$).

The skin color of an additional subsample of 545 women was measured using a Minolta CM2600d spectrocolorimeter (Osaka, Japan) in standardized environmental conditions: room temperature (mean ± SD) 21°C ± 3°, relative humidity 37% ± 5%. The skin color was expressed in the L*a*b* color space (15) (L* corresponding to the brightness, a* to the red intensity and b* to the yellow intensity). Moreover, individual typological angle (ITA°), which combined L* and b* measurements, was calculated for each individual (16).

Statistical analyses. All statistical analyses were performed using SAS® software release 8.2. (17). A descriptive analysis of sun-reactive skin features and Césarini phototypes determined in 1996 was first performed for both genders (FREQ procedure, option CHISQ). Moreover, to test a possible effect of geographic location, the frequency of sun-reactive skin features and phototypes determined using the Césarini classification in 1996 was described as follows: north versus south, and west versus east (FREQ procedure, option CHISQ). Mean values were compared using analysis of variance and the Tukey test (UNIVARIATE procedure, and GLM procedure with the Tukey option), and associations between quantitative variables were assessed by Pearson correlation coefficient (CORR pro-

Table 3. Distribution of sun-reactive skin features and phototype according to geographic location

Sun-reactive skin features and phototype (%)	Geographic location*			
	West (n = 2254)	East (n = 2639)	North (n = 2751)	South (n = 2142)
Hair color				
Red	1§	1	1§	1
Blond	8	9	8	9
Chestnut	68	61	66	61
Brown	22	28	23	28
Black	2	1	2	1
Skin color				
Whitish	1§	2	2§	0
Light	63	50	61	49
Darkish	35	49	36	51
Freckles†				
No	63§	83	70§	79
Some	30	17	25	20
Frequent	7	0	5	1
Sunburn event frequency				
No	2§	7	3§	7
Exceptional/rare	55	45	53	46
Frequent	34	36	35	35
Always	9	11	9	12
Sun tan intensity				
No	0§	1	1§	0
Slight	29	27	30	26
Light	24	34	26	34
Dark	46	37	42	40
Very dark	1	0	1	0
Césarini phototype‡				
I	0§	0	0§	0
II	1	3	2	3
IIIa	7	13	8	12
IIIb	49	44	47	46
IV	35	34	38	30
V	7	6	5	9

*Due to missing data, values do not always add up to 2254 for the west, 2639 for the east, 2751 for the north and 2142 for the south of France.

†Due to missing data, the skin feature "freckles" was available for only 4003 volunteers.

‡Phototype was available for only 4269 volunteers.

§Significant association between each feature, prototype and geographic location (west versus east, and north versus south) (chi-square test: $P < 0.0001$).

Table 4. Distribution of sun-reactive skin features according to Césarini phototype

Sun-reactive skin features (%)	Césarini phototype					
	I (0.3%)	II (2.2%)	IIIa (10.8%)	IIIb (46.4%)	IV (34.2%)	V (6.1%)
Hair color						
Red	64*	4	2	1	0	0
Blond	0	41	29	7	2	0
Chestnut	36	53	59	72	59	44
Brown	0	1	10	19	35	52
Black	0	0	0	1	3	3
Skin color						
Whitish	9*	3	3	1	1	1
Light	82	89	75	67	38	13
Darkish	9	7	22	31	61	86
Freckles						
Absent	20*	49	61	71	85	89
Some	80	50	35	26	13	10
Frequent	0	1	4	3	2	1
Sunburn event frequency						
No	0*	1	4	3	7	16
Exceptional/rare	18	9	22	39	59	75
Frequent	18	47	51	45	22	8
Always	64	43	22	13	3	2
Sun tan intensity						
No	0*	8	1	1	0	0
Slight	45	63	47	34	16	6
Light	45	28	33	24	24	12
Dark	9	1	19	30	58	82
Very dark	0	0	0	1	1	1

*The underlying hypotheses of the chi-square test are not valid, therefore P values from the Fisher exact test were estimated using a Monte Carlo method ($P < 0.001$).

cedure). The relationships between sun-reactive skin features were studied using univariate methods (FREQ procedure, option CHISQ; or, when underlying hypotheses were not valid, P values from the Fisher exact test were estimated using a Monte Carlo method (18)) and also with multiple correspondence analysis (MCA) (CORRESP procedure).

MCA is a factor analysis method. The aim of factor analysis methods is to reduce the multidimensionality of the data by summarizing a set of categorical variables into a small number of variables called principal components. The principal components are defined as linear functions of dummy variables that describe the various categories of the features, weighted according to their relative importance (19–21). These weights associated with the various categories are called the loadings. The loading of a particular category is equal to the average of the principal component for the individuals who present this category, up to a multiplicative constant. To avoid the relationships between frequent categories of the variables to be hidden by rare categories, an essential thing that must be performed before carrying out an MCA is, for each variable, to group a rare category with another category. Therefore, red and blond hair, brown and black hair, light and creamy skin, and dark and very dark tan were grouped together before performing the MCA.

Finally, a loading plot was obtained using the loadings related with the first two principal components from the MCA. This two-dimensional graphical display shows the associations of the categories of the features. Moreover, the relationships between these features and several supplementary variables—such as gender and phototype determined using the Césarini classification—were illustrated by locating the centroid of each category for each supplementary variable on the same graphical display. To describe the uncertainty of the centroid of each category of the features and of the supplementary variables, 95% confidence ellipses were added on the figure, which shows the associations between the features (22).

With regard to the age differences at inclusion in the female and male groups, we checked that comparable results were obtained using the same analysis applied on restricted subsamples for similar age ranges (data available on request).

RESULTS

Significant associations were found between each sun-reactive skin feature and gender (Table 2): 23% of women have brown or black hair at 20 years of age compared with 32% of men, 38% of women have darkish skin compared with 49% of men, 12% of women reported they always burn after sun exposure compared with 9% of men and 35% of women reported they achieve a dark or even very dark tan compared with 50% of men. Moreover, 37% of women had phototype ≥IV according to the Césarini classification compared with 47% of men. Significant associations were found between each sun-reactive skin feature and geographic location

Table 5. Association of sun-reactive skin features (percentage)

Sun-reactive skin features (%)	Skin color			Freckles			Sunburn event frequency				Sun tan intensity				
	Whitish	Light	Darkish	Absent	Some	Frequent	No	Exceptional or rare	Frequent	Always	No	Slight	Light	Dark	Very dark
Hair color															
Red	14*	69	17	20*	66	14	0*	11	39	50	3†	47	42	8	0
Blond	3	75	22	64	32	4	4	33	45	18	1	38	39	22	0
Chestnut	1	61	37	74	23	3	4	48	37	10	0	31	31	37	0
Brown	1	37	62	82	17	1	8	58	27	7	1	18	24	56	1
Black	0	20	82	80	16	5	12	71	15	3	0	16	4	76	4
Skin color															
Whitish				64*	35	1	0*	23	46	31	4*	51	28	17	0
Light				65	30	5	3	36	46	15	1	43	35	21	0
Darkish				87	12	1	8	69	21	3	0	8	23	68	1
Freckles															
Absent							7*	55	30	7	1*	26	27	46	1
Some							3	36	47	18	1	42	31	26	0
Frequent							1	53	42	3	2	48	1	49	0
Sunburn event frequency															
No											1†	17	20	62	0
Exceptional/rare											0	16	23	60	1
Frequent											1	40	38	22	0
Always											3	53	35	9	0

*Significant association between each pair of features (chi-square test: $P < 0.001$).
†For that pair of features the underlying hypotheses of the chi-square test are not valid, therefore the P value for the Fisher exact test was estimated using a Monte Carlo method ($P < 0.001$).

(Table 3): for example, darkish skin color seems more frequent in eastern France (49 %) compared with that in western France (35%), and more frequent in southern France (51%) compared with northern France (36%); and freckles are more frequent in the west (37%) compared with the east (17%), and more frequent in the north (30%) compared with the south (21%). The distribution of individuals among the Césarini phototype was as follows (Table 4): 0.3% in phototype I, 2.2% in phototype II, 10.8% in phototype IIIa, 46.4% in phototype IIIb, 34.2% in phototype IV, and 6.1% in phototype V. As expected, significant associations between each sun-reactive skin feature and Césarini phototype, and also between each pair of features were found (Table 4 and Table 5, respectively).

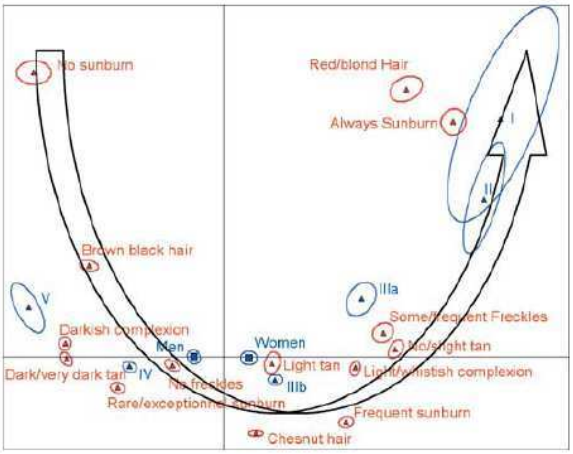

Figure 1. Loading plot of the associations between sun-reactive skin features (red ▲) with Césarini phototypes determined during the 1996 survey (dark blue ▲) and genders (dark blue ■) used as illustrative variables. This map shows a U-shaped configuration centered approximately at the origin. This shape, called a Guttman effect, which is depicted by an arrow, suggests a primarily unidimensional phenomenon. The features are ranked from the left to the right parts of the map according to their increasing implication in skin sensitivity to sun exposure. Ninety-five percent confidence ellipses describe the uncertainty of the categories of the features (red ellipses) and of the illustrative variables (blue ellipses).

MCA allowed the construction of two principal components that best summarized sun-reactive skin features; the first component accounting for 23.7% of the total inertia, and the second for 12.1%. The loading plot, which shows the associations between the features reveals a U-shaped configuration centered approximately at the origin (Fig. 1). This shape, called a "Guttman effect," which is depicted by an arrow on the figure, suggests primarily a one-dimensional phenomenon. The features are ranked from the left to the right part of the map according to their increasing implication in skin sensitivity to sun exposure. The range of sun protection extends from fair-skinned individuals with blond hair who systematically burn to achieve a light or even only a slight tan, to dark-skinned individuals with brown or black hair who never burn, resulting in a dark or even a very dark tan.

In Fig. 1, the centroid of each Césarini phototype is surrounded by the features that characterize each phototype. Notice that the centroids for Césarini phototypes are also ordered according to a U-shaped configuration. Furthermore, the centroid for men is placed on the left of the map and for women on the right according to their respective link with skin sensitivity to sun exposure. Ninety-five percent confidence ellipses show that the uncertainty of the centroids of the categories of the skin features and of the gender is small, whereas the uncertainty of the phototypes is uneven and remarkably large for phototypes I and II.

Table 6. Formula for calculating the skin sensitivity to sun exposure score*

Sun-reactive skin features	Categories	Coefficient	Score calculation†
Hair color	Red/blond	+1.16	
	Chestnut	+0.20	
	Brown/black	−0.87	
Skin color	Whitish/light	+0.83	
	Darkish	−1.02	
Freckles	Absent	−0.34	
	Some/frequent	+1.01	
Sunburn event frequency	No	−1.23	
	Exceptional/rare	−0.67	
	Frequent	+0.78	
	Always	+1.45	
Sun tan intensity	No/slight	+1.09	
	Light	+0.30	
	Dark/very dark	−1.00	
	Constant	+4.46	+4.46
Skin sensitivity to sun exposure score		=	

*The minimal score value, which corresponds to the lowest skin sensitivity, is equal to 0, and the maximal score value, which corresponds to the highest skin sensitivity, is equal to 10.

†For a particular individual, the value of the corresponding category must be reported in the last column. Then the score is obtained by adding to the constant the reported coefficient for each feature. For example, an individual with blond hair, light skin color, some freckles, frequent sunburns and light sun tan (1.16 + 0.83 + 1.01 + 0.78 + 0.30 + 4.46), obtains a score equal to 8.54.

Table 7. Descriptive analysis of the skin sensitivity to sun exposure score according to Césarini phototype, gender and geographic location

	Skin sensitivity to sun exposure score		
	No. of individuals	Mean (± standard deviation)	[95% Confidence interval]
Césarini phototype			
I	10	8.3 (±1.8)*[a]	[7.0–9.5]
II	76	8.0 (±1.4)[a]	[7.7–8.2]
IIIa	373	6.3 (±2.0)[b]	[6.1–6.5]
IIIb	1598	5.1 (±2.3)[b]	[5.0–5.2]
IV	1179	3.1 (±2.1)[c]	[3.0–3.3]
V	210	1.8 (±1.6)[d]	[1.6–2.0]
Gender			
Male	1485	4.0 (±2.5)†	[3.9–4.1]
Female	1961	4.8 (±2.5)	[4.7–4.9]
Geographic location			
West	1334	4.8 (±2.6)†	[4.6–4.9]
East	2107	4.3 (±2.5)	[4.2–4.4]
North	1587	4.6 (±2.6)†	[4.5–4.8]
South	1854	4.3 (±2.5)	[4.2–4.4]

*Significant difference between the mean values (Fisher test: $P < 0.001$), and multiple comparison with the Tukey test (a same letter indicates a nonsignificant difference; different letters indicate significant differences).

†Significant difference between the mean values (Fisher test: $P < 0.001$).

The Guttman effect suggests that the first principal component is a good summary of sun-reactive skin features. Therefore, the first principal component was used to synthesize these features into a single factor, called skin sensitivity to sun exposure score. The calculation of this score is given by a formula based on the first principal component loadings transformed to obtain a minimal score value equal to zero and a maximal score value equal to 10 (Table 6). A descriptive analysis of this new indicator is given according to Césarini phototype, gender and geographic location (Table 7). A significant link was found with Césarini phototype: the higher the mean score (8.3 ± 1.8), the fairer the phototype (I). Moreover, a significant smaller mean score was found for women (4.8 ± 2.5) compared with that for men (4.0 ± 2.5), and significant links were also found for geographic location, with a higher mean score for the north of France (4.6 ± 2.6) compared with the south (4.3 ± 2.5), and for the west (4.8 ± 2.6) compared with the east (4.3 ± 2.5).

This score was calculated for an additional subsample of 545 women participating in the cohort. It was positively correlated with the brightness of the skin (L*, $r = 0.28$, $P < 0.001$), the yellow intensity (b*, $r = 0.27$, $P < 0.001$), and the individual typological angle (ITA°, $r = 0.34$, $P < 0.001$) (data available on request).

Concerning potential effects of antioxidant supplements on the skin features, we performed additional analyses that show that the distributions of the features and phototypes were comparable in placebo and antioxidant groups (data not shown) and that the mean values of the skin sensitivity to sun exposure score were not found to be significantly different in both groups (4.5 ± 2.6 in the placebo group versus 4.4 ± 2.5 in the antioxidant group).

DISCUSSION

Here we have studied the relationship between each pair of sun-reactive skin features used in the Césarini classification (8), then we investigated the association between all features by MCA. This method is commonly used to search for an optimal scaling of items (in our case, optimal scaling of the categories of the features) for a given set of variables (the sun-reactive skin features) by producing optimal principal components. When the information contained in the data addresses a one-dimensional phenomenon, the loadings related to the first principal component summarize this phenomenon perfectly well, and the loadings related to the next components are polynomial functions of increasing orders of the loadings related to the first component. Consequently, the graphical display, which shows the relationships between the features, dramatically yields a curvilinear line (23). This curve is called the Guttman effect, referring to the Guttman scale: the first axis opposes extreme values, and the second axis opposes intermediate values to extreme values (24,25). This finding shows that the first principal component is actually a good summary of the skin sensitivity to sun exposure, and could enable us to synthesize variables into a single factor, which could be labeled as skin sensitivity to sun exposure score. The potential of this score was tested by studying the correlation between the skin color measurements assessed by the CIEL*a*b* system and the score; on an additional subsample of women participating to the cohort, as L* and b* measurements are known to be linearly correlated with the minimal erythema dose (26). The significant correlations found for the brightness of the skin (L*) and the yellow intensity (b*) confirm that our score could be an easy-to-collect tool that could serve for epidemiological studies to summarize the skin sensitivity to sun exposure.

No significant effect of antioxidant supplements was found, either for the skin features or for the skin sensitivity to sun exposure score. Protective effects of antioxidant supplements against UV-induced damage have already been demonstrated in few strictly controlled clinical studies: a significant decrease of erythema after artificial UV exposure measured using chromameter devices and the CIEL*a*b* system (27,28), and based on human skin biopsies, significant reductions of the UV-induced p53 expression, the number of apoptotic sunburn cells and the levels of superficial lipoperoxide, as well as a significant increase in UV-induced neomelanogenesis (28). However, to our knowledge, no significant effect was reported on phenotypic data in the context of an intervention epidemiological study concerning nutritional antioxidant supplements. Therefore, we can conclude that the features assessed in our study were not altered by the antioxidant supplements tested.

Hence, we found a link between gender, sun-reactive skin features and phototype determined using the Césarini classification (*i.e.* women who have a greater sun sensitivity and a fairer phototype than men). Concerning the color of the skin, the most determinant factor is the quantity and quality of melanin (3). The capacity of epidermal melanocytes to synthesize melanin, both in a resting state (constitutive color) and after activation by sunlight (facultative color), is known to strongly vary (6). Both anthropological and genetic studies suggest that the constitutive color of the skin—as well as hair and eyes—is determined by different sets of genes (29,30). In addition, nonmelanin pigments such as oxygenated and reduced hemoglobin, and exogenous pigments such as carotenoids from food intake as well as differences in sun exposure history, can influence the skin color (3).

Hormonal influences, differences in melanin, and the above-cited components such as hemoglobin or carotenoids, are likely to be responsible for the findings that the skin of adult women is slightly lighter than that of men (29,31). This observation is also in line with the tendency of artists to portray the female body with lighter color than that of the male body (32). These gender differences have also been corroborated using spectrophotometric measurements in the visible spectrum in various ethnic populations (33–38), and in a recent exploratory spectroscopic study (39). Simple hormonal effects cannot explain, per se, such differences in constitutive skin pigmentation. Indeed, both testosterone and estrogen may lead to pigmentation allowing a different response to sun exposure (3,4,29). Even if pigmentation is stronger on exposed than nonexposed sites, marginal gender differences have been found in facultative pigmentation (40). In addition, women are considered to be more homogenous in skin color than men, with smaller topological variations (41), and with questionable and intricate changes related to age (37,42,43). Indeed, freckles seem less prevalent with age, becoming equally distributed on different facial and body sites and more frequent in women (44). Wagner *et al.* (4) reported that the constitutive pigmentation was slightly lower in women than in men, but they found a higher propensity to sunburn in men than in women. However, in our study, self-reported sunburns appear less frequently in men.

In the present study, we have been able to identify distribution differences between men and women according to sun-reactive skin features. A link between skin color and gender has already been previously reported in published papers (3), possibly due to a hormonal influence, probable genetic factors, as well as the subjectiveness of self-estimation of features of skin sensitivity to sun exposure (39,40). Moreover, owing to the differences reported among gender and age groups, future investigations on human skin color should definitely be conducted by balancing individuals according to age group and gender in order to obtain more accurate information. Despite obvious unsatisfactory weaknesses, phototype classification is often used in clinical and epidemiological studies, because it can be easily assessed without any equipment. Therefore, phenotypic evaluation appears to be a good estimator of skin sensitivity to sun exposure for clinical screening or for use in research, and is easy to collect at a relatively low cost.

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Annexe 5. Article sur la sensibilité de la peau auto-déclarée

Self-reported skin sensitivity in a general adult population in France: data of the SU.VI.MAX cohort

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ORIGINAL ARTICLE

Self-reported skin sensitivity in a general adult population in France: data of the SU.VI.MAX cohort

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Keywords
epidemiological study, factor analysis method, self-administered questionnaire, self-reported skin sensitivity, sun sensitivity

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Abstract

Objective This study aimed to examine the frequency of self-assessed facial skin sensitivity and its different patterns, and the relationship with gender and sun sensitivity in a general adult population.

Methods A standardized 11-item questionnaire investigating reactions experienced during the past year was developed. The questions explored different patterns of skin sensitivity: pattern I (blushing related to vascular reactivity), pattern II (skin reactions to certain environmental conditions), pattern III (skin reactions after substance contact), and for women pattern IV ('breakout of spots' related to menstrual cycle). Additional items were addressed for women and men, including sun sensitivity. The questionnaire was administered to a large middle-aged population involved in the 'Supplément en Vitamines et Minéraux Antioxydants' (SU.VI.MAX) cohort.

Results Sensitive facial skin was reported by 61% of the women ($n = 5074$) and 32% of the men ($n = 3448$), and the frequency decreased with age. The frequency of patterns I, II and III was greater for women (78, 72 and 58%, respectively) than for men (56, 48 and 28%) of comparable classes of age. The frequency of pattern IV was reported by 49% of premenopausal women, and skin reactions after shaving by 41% of the men. Sun sensitivity was found to be a major component of skin sensitivity. Factor analysis showed that individuals with fair phototype frequently evoked reactions associated with pattern I, and skin redness and burning sensations were related to certain environmental conditions (pattern II).

Conclusion Skin sensitivity is a common concern that declines with age and is relevant for men as well as for women.

Introduction

The concept of sensitive skin stems from observations of subjective adverse reactions occurring in response to topically applied agents.[1–3] It has been defined as the potential to experience cutaneous discomfort in the absence of any clinical or histological evidence of skin lesions.[1] Individuals report complaints such as reddening, burning, stinging and itching or tightness sensations, on contact with certain cosmetic products as well as under certain environmental conditions.[4] The suggestion by Simion and Rau[5] that complaints of sensitive skin are invariably associated with a response to external factors was a valuable initial observation. Johnson and Page[6] later widened the list of possible causes to include factors that they described as intrinsic, lifestyle and environmental, as well as the use of cosmetics.

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Sensitive skin is a phenomenon with an apparently widespread distribution. About half of all skin reactions to cosmetics and toiletries are reported to be sensory phenomena with no visible effects,[7] thus falling within the definition. However, the subjective nature of this complex phenomenon and the lack of a consistent definition have made it difficult to study. Its frequency remains uncertain and its determinants and underlying mechanisms have been the subject of controversy.[2] Until now, any assessment of the different patterns of sensitive skin and their occurrence is clearly hampered by the lack of a suitable approach.

Patients with sensitive skin rely on diagnostic skills and patience, as it is impossible to delineate responsible aetiological factors based on morphological characteristics and objective cutaneous signs.[8–10] Furthermore, the features and complaints, which are very similar among these individuals, do not necessarily reflect the severity of the objective findings. As a result, the management of such patients may pose a challenge for dermatologists.

Recently, we performed two studies on women in the Ile-de-France region.[4] In the first study a sample of 319 healthy female volunteers were asked to rate the degree of perceived sensitivity of their facial skin on a four-point scale from 1 = not sensitive to 4 = very sensitive. Those who reported skin sensitivity (self-ratings 2–4) were asked to describe their skin reactions together with the causes to which they attributed them. This study provided a list of skin reactions based on unprompted statements. In a second study, conducted on a sample of 255 healthy female volunteers with self-declared skin sensitivity, unprompted statements were recorded by a physician according to a pre-established list based on the results of the first study. A factor analysis method was consecutively applied on the data of the second study. The principle of factor analysis methods is to summarize the data by obtaining a small set of new variables that can be used to approximate the initial set of variables. These new variables, called principal components, are designed to retain salient features of the data, to discard the background noise, and to feed back understandable summaries of the information.[11–13] From these components, four groups of skin reactions related to specific causes were defined, suggesting four different non-exclusive patterns of skin sensitivity. The first association of skin reactions (pattern I) grouped blushing following emotional events, consumption of alcohol or spices or sudden changes of temperature. The second association (pattern II) grouped together different skin reactions (redness, tightness, itching, burning, stinging) following exposure to certain environmental conditions (i.e. cold, wind), and the third association (pattern III) grouped similar skin reactions after substance contact (soap, hard water, chloride, cosmetic products). For women, pattern IV was defined as 'breakout of spots' associated with the menstrual cycle.[4]

Here we develop a simple, self-administered questionnaire that uses clearly defined content items to characterize those individuals who report themselves experiencing sensitive skin. Using this questionnaire we conducted a survey to study the occurrence of skin sensitivity and associated reactions in a healthy adult population living in France, recruited in the 'Supplément en Vitamines et Minéraux Antioxydants' (SU.VI.MAX) cohort.[14]

Population, material and methods

Study setting

This study was conducted within the framework of the French SU.VI.MAX study. The study objectives, design and population characteristics have been described elsewhere.[15] In brief, the SU.VI.MAX is a randomized double-blind, placebo-controlled study. This primary-prevention trial was designed to test the efficacy of a daily supplement with antioxidant vitamins and minerals, at nutritional doses, in reducing the main causes of premature death conducted in a 'free-living' adult population sample. Among the eligible subjects (14 412), 12 741 individuals were finally recruited in 1994 according to gender and age group (women aged 35–60 years and men aged 45–60 years), smoking habits and geographic location, and were followed up for 8 years. The SU.VI.MAX study has been approved by the ethical committee for studies with human subjects (CCPPRB no. 706) of Paris-Cochin, and the 'Comité National Informatique et Liberté' (CNIL no. 334641), which advocates that all medical information be confidential and anonymous. All participants gave their informed consent to participate in the study.

Questionnaire

A standardized 11-item questionnaire for women was constructed for self-completion, and specific items were added for men, including questions related to shaving. This questionnaire is shown in the Appendix. It includes six main questions investigating each of the four patterns of skin sensitivity that may have been experienced during the past year (blushing related to vascular reactivity, skin reactions to certain environmental conditions, skin reactions after substance contact, and for women 'breakout of spots' related to the menstrual cycle). Moreover, three questions investigating sun sensitivity were added.[16]

Following an initial question about their perception of their facial skin sensitivity, all individuals were asked whether they experienced blushing of the skin in

response to stress, spices or alcohol consumption or rapid changes of environmental temperature (questions 2–4), corresponding to pattern I. Then, a detailed list of skin reactions related to certain environmental conditions is proposed in question 5, which investigates pattern II. Similarly, question 6 provides the same list of skin reactions related to the application of cosmetic products adapted for women and for men, corresponding to pattern III. Question 7 investigates dependence on the use of cosmetics for women, and skin reactions related to shaving for men. Self-reported sun sensitivity is examined in questions 8 and 9, including the fourfold phototype classification developed by Pathak and Fitzpatrick.[16] Question 10 enquires about the occurrence of 'breakout of spots' associated with sun exposure. Finally, question 11 asks women about the occurrence of 'breakout of spots' declared to be in relation to the menstrual cycle, corresponding to pattern IV.

Study sample

In 1998, these questionnaires were sent to all the volunteers participating in the SU.VI.MAX cohort. During the medical examination performed at inclusion, the individuals were also asked by a physician to supply a history of their sunburn susceptibility, tanning ability and phenotypic information: complexion during winter, hair colour at age 20 and freckles.[17] The investigator then determined their sun sensitivity using an attribution rule, derived from the phototype classification proposed by Césarini in 1977.[18] This classification is based, on the one hand, on two dynamic parameters, sunburn susceptibility and tanning ability, and, on the other hand, on phenotypic information. This information, as well as age, gender, and self-reported cosmetic skin type (normal, dry or oily) throughout spring/summer and autumn/winter, were used in the analysis.

Statistical analysis

Statistical analyses were performed using the SAS® software release 8.02.[19] Skin reactions were initially grouped according to the different patterns of skin sensitivity. Frequencies were described according to age and gender (MEANS and FREQ procedures). For each gender, relationships between the skin reactions were explored using multiple correspondence analysis (MCA) (CORRESP procedure), which is a factor analysis method. The aim of this type of method is to reduce the multidimensionality of the data by summarizing a set of categorical variables into a small number of variables called principal components. The principal components are defined as linear functions of the original variables, weighted according to their relative importance.[11–13] The percentage of explained variance of each component was estimated using the Benzecri formula (BENZECRI option).[20] Moreover, each principal component has been interpreted by examining the partial contribution of each skin reaction to the variance. Two-dimensional graphical displays were obtained using principal components as axes systems to show the proximities between the individuals and also the associations between the various skin reactions; individuals with similar skin reactions are close on the first graphical display and skin reactions strongly associated are close on the second graphical display. These representations have the very useful property of being strongly linked together: one graph can be used to interpret the other, and vice versa. Therefore, groups of individuals having similar features can be identified. Moreover, supplementary variables (such as self-reported skin sensitivity and self-assessed sun sensitivity according to Fitzpatrick's classification) were added on these graphs to show their relationship with the skin reactions. The links between the additional information for each pattern of skin sensitivity (and for shaving in men) were also examined using similar data analyses.

Results

Completed questionnaires were returned by 72% of individuals, of which 93% could be evaluated: 5074 from women and 3448 from men. The percentage of non-respondents to the questionnaire decreased significantly with age. Moreover, this percentage was significantly higher for women, single people, people living in the Ile-de-France area, and for the highest socio-professional categories. Similar non-respondents' characteristics were found in studies on sun exposure habits and on dietary behaviour that we performed previously on the SU.VI.MAX cohort.[21,22]

The response to key items and for each skin reaction expressed by gender and self-reported skin sensitivity is given in Table 1. For comparable classes of age (i.e. 45–60 years of age), 90% of the women who stated that they had sensitive facial skin (76% of the men) reported having experienced pattern I skin reactions; nevertheless, 62% of the women who stated that they did not have sensitive facial skin also reported having experienced pattern I skin reactions (47% of the men). Similar trends were found for the other skin sensitivity patterns. Moreover, 45% of the women who stated that they had sensitive facial skin reported being phototype I or II according to Fitzpatrick's classification (9% phototype I plus 36% phototype II) (34% of the men, 4% plus 30%). However, only 17% of the women who stated that they did not have

Table 1 Percentage response by gender and self-reported skin sensitivity to the 10 main questions of the sensitive skin questionnaire

	Men (3448)*			Women (5074)*					
	45–60 years Sensitive skin			35–60 years (5074) Sensitive skin			45–60 years (3413) Sensitive skin		
	No (2299)	Yes (1082)	Total	No (1969)	Yes (3010)	Total	No (1395)	Yes (1946)	Total
Pattern I: Redness related to vascular reactivity	47	76	56	63	89	78	62	90	78
Redness associated with emotion or stress (Q2)	30	60	39	44	75	63	43	76	62
Redness associated with alcoholic drinks or spicy food (Q3)	26	50	33	36	63	52	37	65	53
Redness associated with rapid changes of temperature (Q4)	24	47	31	33	68	54	33	69	54
Pattern II: Skin reactions to certain environmental conditions (Q5)	37	39	48	54	87	74	52	87	72
Pattern III: Skin reactions after substance contact (Q6)	19	49	28	35	75	60	34	75	58
Pattern IV: 'Breakout of spots' related to menstrual cycle (Q11)†	–	–	–	42	54	49	–	–	–
Skin reactions when not using usual cosmetic products (Q7)	–	–	–	48	72	60	–	–	–
Skin reactions after shaving (Q7)	32	60	41	–	–	–	–	–	–
Considering facial skin sun-sensitive (Q8)	16	69	33	25	76	56	26	77	56
Sun sensitivity‡ (Q9) I: Always burns, never tans	1	4	2	2	9	6	3	9	6
II: Always burns, sometimes tans	9	30	16	15	36	28	14	36	27
III: Sometimes burns, always tans	65	55	62	57	47	51	56	47	51
IV: Never burns, always tans	25	10	20	24	7	14	26	8	15
'Breakout of spots' after sun exposure (Q10)	3	13	6	8	24	18	7	23	16

*Values do not always add up to 3448 for men or to 5074 for women because of missing data.
†Non-menopausal women only.
‡Fitzpatrick's classification.

sensitive facial skin reported being phototype I or II (3% plus 4%) (10% of the men, 1% plus 9%).

Furthermore, for comparable classes of age, 81% of the 1395 women who stated that they did not have sensitive facial skin, reported having experienced at least one of the skin reactions listed in the questionnaire during the past year (68% of the 2299 men). Nevertheless, for both genders, individuals who stated that they had sensitive facial skin reported a significantly greater number of skin reactions than individuals who stated that they did not have sensitive skin; for example (for comparable classes of age) 8.2 ± 3.6 (mean ± standard error) vs. 3.6 ± 2.8 for women, and 6.1 ± 3.7 vs. 2.4 ± 2.6 for men. However, 9% of women who stated that they did not have sensitive facial skin (11% of men) reported a greater number of sensitive skin reactions than the mean value of the number of sensitive skin reactions reported by people who declared that they had sensitive facial skin (i.e. greater than 8.2 for women and 6.1 for men).

The response to key items and for each skin reaction expressed by age group and gender whatever the self-reported skin sensitivity is given in Table 2. Overall, some 61% of the women and 32% of the men stated that they had sensitive facial skin (58% of the women for comparable classes of age 45–60 years). For comparable classes of age, 78% of the women and 56% of the men stated having experienced pattern I skin reactions. Similarly, 72% of the women stated having experienced pattern II skin reactions (48% of the men). Fifty-eight per cent of the women stated having experienced pattern III skin reactions (28% of the men). Regarding pattern IV skin reaction, 49% of the non-menopausal women stated having experienced 'breakout of spots' associated with menstrual cycle. Forty-one per cent of the men stated having experienced skin reactions after shaving.

For both genders, the frequencies of self-reported skin sensitivity, patterns I, II and III skin reactions, pattern IV skin reaction for premenopausal women, as well as skin reactions after shaving for men, were found to be significantly greater among the youngest subjects.

Fifty-six per cent of the women and 33% of the men claimed to have sun sensitivity: for comparable classes of age according to Fitzpatrick's classification, 6% of the women declared experiencing reactions to sun exposure compatible with phototype I (2% for men), 27% with phototype II (16%), 51% with phototype III (62%) and 16% with phototype IV (20%).

For both genders the frequency of patterns I, II and III skin reactions decreased when sun sensitivity increased, with phototype determined during the medical evaluation using Césarini's classification (Table 3), as well as with self-assessed sun sensitivity using Fitzpatrick's classification recorded in the questionnaire (data not shown). The frequency of skin reactions after shaving also declined

Table 2 Percentage response by gender and age groups (years) to the 11 main questions of the sensitive skin questionnaire

	Men (3448)*				Women (5074)*						
	45–49 (1192)	50–54 (1033)	55–60 (1202)	Total 45–60	35–39 (579)	40–44 (998)	45–49 (1446)	50–54 (985)	55–60 (982)	Total 35–60	Total 45–60
Considering facial skin sensitive (Q1)	35	32	29	32‡	67	64	60	59	55	61‡	58¶
Pattern I: Redness related to vascular reactivity	58	56	54	56	80	79	80	77	76	78	78¶
Redness associated with emotion or stress (Q2)	41	40	36	39	67	64	64	63	58	63‡	62¶
Redness associated with alcoholic drinks or spicy food (Q3)	34	32	34	33	49	50	54	52	54	52	53¶
Redness associated with rapid changes of temperature (Q4)	32	33	29	31	53	56	55	52	53	54	54¶
Pattern II: Skin reactions to certain environmental conditions (Q5)	52	47	43	48‡	76	79	74	72	69	74‡	72¶
Redness	27	24	23	25	48	51	48	46	45	48	47¶
Stinging not combined with redness	14	12	13	13	20	18	18	17	16	17	17¶
Itching not combined with redness	5	6	6	6	8	7	5	6	8	7	6
Burning sensation	12	13	11	12	26	26	26	23	21	25‡	24¶
Tightness	15	11	9	12‡	45	42	37	35	31	37‡	34¶
Scurves or localized desquamation	13	12	8	11‡	21	19	16	16	15	17‡	15¶
Pattern III: Skin reactions after substance contact (Q6)	32	28	25	28‡	66	63	58	59	56	60‡	58¶
Redness	16	13	12	14‡	36	35	35	35	32	34	34¶
Stinging not combined with redness	9	8	9	8	21	24	20	19	21	21	20¶
Itching not combined with redness	7	8	7	7	15	16	14	13	14	14	14¶
Burning sensation	11	7	7	9‡	22	20	19	17	15	18‡	17¶
Tightness	5	4	3	4‡	29	27	24	26	23	25	24¶
Scurves or localized desquamation	7	6	4	6‡	14	15	14	13	11	14	13¶
'Spots' after product application	7	6	3	5‡	27	20	17	15	13	18‡	15¶
Pattern IV: 'Breakout of spots' related to menstrual cycle (Q11)†	–	–	–	–	67	57	45	31	11	49‡	–
Skin reactions when not use usual cosmetic products (Q7)	–	–	–	–	66	64	58	59	56	60‡	–
Skin reactions after shaving (Q7)	48	39	36	41‡	–	–	–	–	–	–	–
Redness	27	21	18	22‡	–	–	–	–	–	–	–
Stinging not combined with redness	12	9	8	10‡	–	–	–	–	–	–	–
Itching not combined with redness	6	6	4	5	–	–	–	–	–	–	–
Burning sensation	24	18	16	19‡	–	–	–	–	–	–	–
Tightness	9	7	5	7‡	–	–	–	–	–	–	–
Scurves or localized desquamation	5	2	3	3‡	–	–	–	–	–	–	–
'Spots' after shaving	10	7	6	7‡	–	–	–	–	–	–	–
Considering facial skin sun-sensitive (Q8)	36	34	29	33‡	57	57	55	56	55	56	56¶
Sun sensitivity†† (Q9) I: Always burns, never tans	3	2	2	2	7	6	6	5	8	6	6¶
II: Always burns, sometimes tans	17	15	15	16	30	30	27	26	26	28	27¶
III: Sometimes burns, always tans	63	63	59	62‡	52	53	53	52	46	51‡	51¶
IV: Never burns, always tans	16	19	24	20§	11	11	14	16	18	14§	15¶
'Breakout of spots' after sun exposure (Q10)	7	7	5	6	23	20	19	14	15	18‡	16¶

*Values do not always add up to 3448 for men or to 5074 for women because of missing data.
†Non-menopausal women only.
‡Significantly greater in the youngest subjects (χ^2-test, $P < 0.05$).
§Significantly greater in the oldest subjects (χ^2-test, $P < 0.05$).
¶In similar class of age, significantly greater in women than in men (χ^2-test, $P < 0.05$).
††Fitzpatrick's classification.

significantly with sun sensitivity in men. By contrast, the frequency of 'breakout of spots' in non-menopausal women remained stable whatever the sun sensitivity, at about 50%.

Self-reported facial skin sensitivity appeared to be strongly linked to self-reported cosmetic skin type: 76% of the women who declared having dry skin in autumn/winter (76% in spring/summer) stated that they had sensitive skin. By contrast, 34% of the women who declared having normal skin in autumn/winter (42% in spring/summer) stated that they had sensitive skin. Moreover, 72% of the women who declared having an oily skin in autumn/winter (68% in spring/summer) and 42% of the women who declared having normal skin in autumn/winter (42% in spring/summer) stated that they experienced pattern IV skin reaction (data available upon request).

Table 3 Relationship between the different patterns of skin sensitivity and sun sensitivity by gender. Data are expressed in percentage (number of subjects)

	Men (3448)*				Women (5074)*			
Phototypes§	Pattern I (2783)	Pattern II (2709)	Pattern III (2643)	Reactions after shaving (2714)	Pattern I (3830)	Pattern II (3717)	Pattern III (3681)	Pattern IV (2404)‡
I and II	71†	69†	42†	57†	89†	81†	73†	52
IIIa	64	49	32	45	84	80	63	46
IIIb	61	50	30	42	81	76	62	49
IV	52	45	28	40	75	71	58	50
V and VI	40	34	19	36	61	64	47	51
Total	56	47	29	41	79	75	60	49

*Values do not always add up to 3448 for men or to 5074 for women because of missing data.
†Significantly greater in individuals with fair phototype (χ^2 test, $P < 0.05$).
‡Non-menopausal women only.
§Césarini's classification.

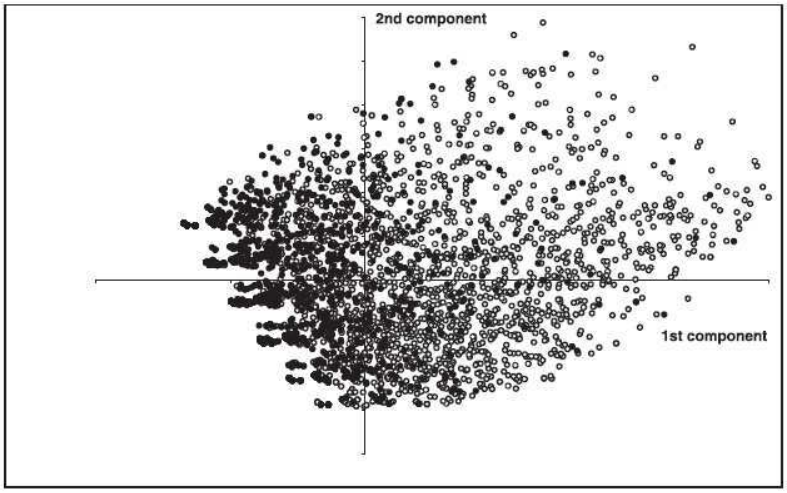

fig. 1 Map of proximities between women. The first and second principal components of the multiple correspondence analysis (MCA) were used as the axes. The first component explains 89% of the total variance, and the second component 8%. Each women is indicated by a symbol according to the self-reported facial skin sensitivity: an open circle (○) for women who declared that they had sensitive facial skin and a filled circle (●) for women who stated that they did not have sensitive facial skin. Some individuals are hidden by others.

The proximities between the individuals as well as the associations between the skin reactions were analysed using MCA. Figure 1 shows the proximities between women. To facilitate the interpretation of the proximities between women, their self-reported facial skin sensitivity was indicated using two types of symbols: open circles (sensitive) and filled circles (not sensitive). Women who stated that they did not have sensitive facial skin are in the majority located in the left part of the figure, and are opposed by women who stated that they had sensitive facial skin and who are located in the majority on the right part. Similar results were obtained for men (data not shown).

Figure 2a shows the associations between the skin reactions reported by women. Globally, the first component, which accounts for 89% of the total inertia (explained variance), opposes the absence of reported skin reaction on the left, to reported skin reactions on the right (indicated on the graph by a schematic blue broken line). The skin reactions contributing most to the component are the following: redness after substance contact (partial contribution to the first component of inertia 8%), scurves after substance contact (7%), stinging after substance contact (7%), scurves to certain environmental conditions (7%), and burning sensation after substance contact (7%). The second component (8% of the total inertia) groups pattern I skin reactions (blushing) and redness and burning sensations related to certain environmental conditions at the bottom of the graph, and opposes them to the other skin sensitivity reactions. The skin reactions contributing most to this component are the following: redness associated with alcoholic drinks or spicy food (partial contribution to the second component inertia 16%), redness associated with rapid changes of temperature (16%), redness associated with certain environmental condition (15%), and redness associated with emotion or stress (14%). The phototype determined using Fitzpatrick's classification

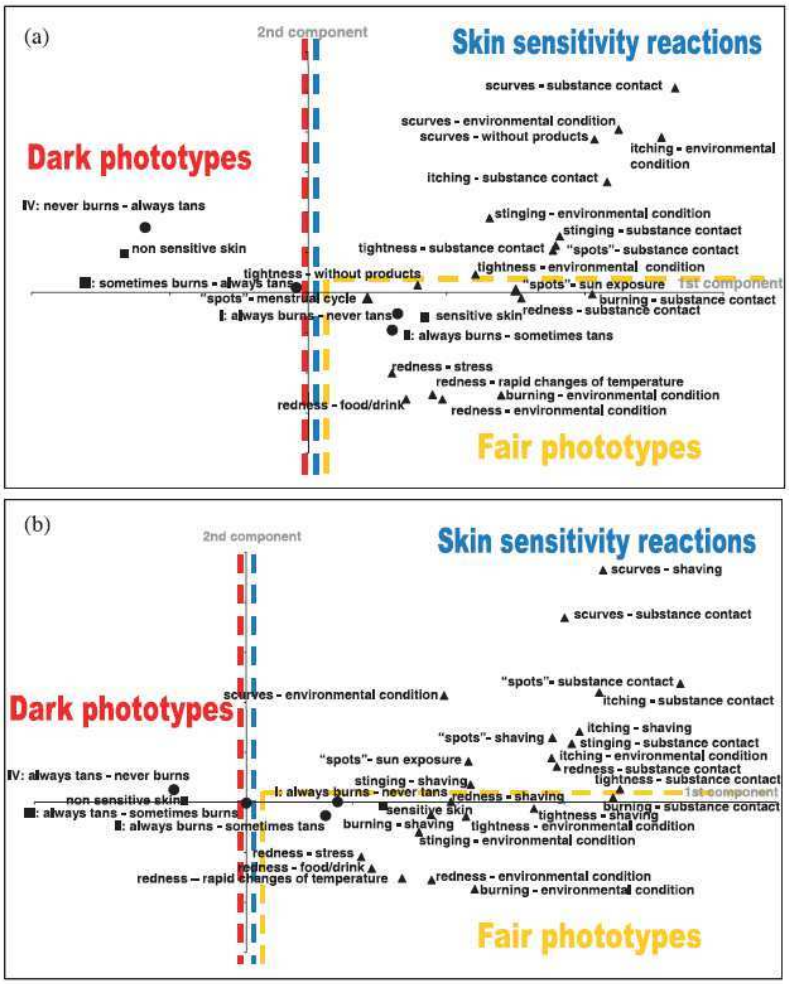

fig. 2 Map of relationships between skin sensitivity reactions (▲) for (a) women and (b) men. The first and second principal components of the multiple correspondence analysis (MCA) were used as the axes. For women, the first component explains 89% of the total variance, and the second component 8%. For men, the first component explains 87% of the total variance, and the second component 6%. Self-reported skin sensitivity indicated by filled squares (■) and self-assessed sun sensitivity (Fitzpatrick's classification) indicated by filled circles (●) were added on the graph as supplementary variables to show their association with skin reactions. Broken lines were added to underline the association with the map showing the proximities between the individuals; the blue line indicates the area where individuals who declared having skin reactions are situated, and the yellow and red lines show the areas where individuals with fair and dark phototypes are placed, respectively.

was used as a supplementary variable. This figure suggests that individuals with a dark phototype are located in the majority on the left part (indicated on the graph by a schematic red broken line), and are associated with self-declared non-sensitive skin (also used as a supplementary variable) and with the absence of a reported skin reaction. On the contrary, individuals with a fair phototype, which are located on the lower part on the right (indicated on the graph by a schematic yellow broken line), are associated with a self-declared sensitive skin and with the presence of reported skin reactions related to pattern I. Figure 2b shows similar associations between various skin reactions reported by men.

Finally, the relationships between the complementary questions for each pattern of skin sensitivity were analysed in the same way for both genders. For pattern I skin reactions, 'localized' blushing is associated with 'stinging' sensations; and for patterns II and III, as well as for skin reactions related to shaving in men, 'persistent' skin reactions whatever the type are systematically opposed to 'transient' skin reactions (graphical displays not shown).

Discussion

Skin sensitivity is a widespread condition, whose boundaries have been neither well defined nor intensively investigated. Moreover, contradictory findings have been reported: 'sensitive skin' has been related either to constitutional anomalies or to skin disorders or occupational exposure to irritants.[1–9] Nevertheless, 40–70% of the population of women living in developed countries report that they have sensitive skin.[4,6,23–26]

The use of the questionnaire presented here allows rapid and relatively easy identification of individuals presenting different and non-exclusive features of skin sensitivity. Such a device should be of value in further

describing complaints associated with facial skin sensitivity in different geographical regions or among different populations living in the same region. In addition, this tool may help to assess skin sensitivity reactions to the application of dermatological and cosmetic products.[1] This questionnaire may therefore be used in the selection of homogeneous groups of subjects to further investigate the phenomenon of skin sensitivity in specific contexts.

Sensitive skin reactions are significantly less frequently reported among older individuals for both genders. This finding is probably linked to age-related behaviour and to a tendency to avoid environmental conditions and contact with certain substances whenever skin reactions have been previously experienced. Moreover, significant decreases in the performance of sensory skin properties have been documented as a part of chronological ageing.[27]

In other respects, women more frequently reported a sensitive skin than men. Among the determinants known to play a role in expression of skin sensitivity, individual genetic predispositions and sex-related physiological characteristics may at least in part contribute to this difference.[28,29]

However, this questionnaire was not constructed with the aim of identifying individuals' facial skin sensitivity according to the number of skin reactions reported; although some individuals stated that they did not have facial skin sensitivity, they did report skin reactions. Furthermore, some individuals who declared that they had sensitive facial skin reported less skin reactions than some individuals who stated that they did not have sensitive facial skin.

Several studies on skin sensitivity have been published. Ayala *et al.* developed a 12-item questionnaire to investigate the causes and reactions of sensitive skin in 500 individuals.[23] Thirty-seven per cent declared having sensitive skin, the majority reporting erythema, dryness and pruritus. Environmental stimuli, such as sunlight and cold climate, and substance contact (detergent and/or cosmetics) were considered to be the main causative agents. Another study was conducted among 1000 Caucasian women recruited from five geographical locations in the USA.[30] The study compared the responses of two groups who perceived themselves to have sensitive or non-sensitive skin. The findings did not allow for the demonstration of a sustainable relationship between sensitive skin perception and skin reactions following application of chemical products. A third study investigated skin sensitivity and reactions to cosmetic products in 3300 women and 500 men living in the UK.[24] The frequency of skin sensitivity and the frequency of skin reactions to cosmetic products were 51% and 58% for women, and 38% and 31% for men, respectively. Associations between self-reported skin sensitivity and both dry skin and blushing/flushing reactions were also reported. Unfortunately, neither age distribution nor results according to age groups were provided in these studies, so a comparison of frequency with our own results is impossible. A fourth study examined possible ethnic variations in the perception of sensitive skin.[26] Sensitive facial skin assessment and general skin condition data were collected by telephone interviews among four different ethnic groups in the San Francisco area (Afro-Americans, Asians, Euro-Americans, Hispanics; approximately 200 women per group). The authors found that the frequency of self-reported skin sensitivity was equivalent according to the ethnic background, but observed slight differences concerning the frequency of skin symptoms evoked.

The SU.VI.MAX cohort gave us the opportunity to describe the frequency of different patterns of facial skin sensitivity on a large, general adult population-based sample. In comparable age groups, the frequency of self-assessed skin sensitivity was roughly double in women as compared to men. In both genders, this frequency decreased with age. For patterns I, II and III skin reactions, the frequency was greater in women in comparable age groups, and in both genders the frequency also appeared greater among the youngest individuals. As expected, pattern IV skin reaction was also more frequent in younger women. Similarly, skin reactions after shaving were more frequent in younger men.

For pattern I skin reactions, the opposition of 'localized' to 'diffuse' blushing, and also for pattern II and III skin reactions, the systematic opposition of 'persistent' to 'transient' skin reactions indicate groups of individuals with different features of skin sensitivity, which might reflect different underlying mechanisms. In a given population, different individuals may therefore display a wide range of features relating to skin sensitivity. These different patterns may be due to different underlying mechanisms that need further investigation. Nevertheless, some suggestions can be formulated concerning their links with subclinical skin conditions. For example, skin reactions related to pattern I may be consistent with Rosacea stage I as defined by Plewig and Kligman.[31] Skin reactions related to pattern II may correspond to subclinical seborrhoeic dermatitis or eczema features, as the patients who experience seborrhoeic dermatitis do not necessarily show an oilier skin than non-affected individuals.[32] Pattern III conditions may be matched with subclinical allergic contact dermatitis or atopic dermatitis.[25] In fact, subclinical allergic contact dermatitis may influence the expression of skin sensitivity, as well as those of certain clinical signs reported in the context of inflammatory skin pathways.[30] Finally, skin reactions linked to the menstrual cycle in women (pattern IV) are linked with acne flares: indeed, an increase in the occurrence of inflammatory acne lesions during the premenstrual period has been reported previously.[33]

For both genders, the number of reported skin reactions was significantly greater in individuals who stated that they had sensitive facial skin than among individuals who stated that they did not experience sensitive skin. These results are consistent with those reported by Willis *et al.*[25] as well as with results from recent studies conducted to test skin reactions after application of chemical substances. Individuals with self-reported skin sensitivity were more susceptible to skin reactions in response to irritant products.[10,34]

The assessment of the associations between the various skin reactions illustrates that sun sensitivity is a major determinant of skin sensitivity, and also demonstrates the relationship between the fair-phototype individuals and the occurrence of blushing following emotional events, consumption of alcohol or spices, and sudden changes in external temperature, all reactions that are thought to be related to vascular reactivity.

In conclusion, our study confirms the multidimensional nature of facial skin sensitivity and demonstrates that sun sensitivity represents a major determinant of skin sensitivity. Whereas the same associations of skin reactions are found in both genders, skin sensitivity and its various skin reactions appear approximately half as frequently among men, and finally skin reactions decrease with age.

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APPENDIX 1 Self-assessed sensitive skin questionnaire (women and men)

This questionnaire is designed to assess the frequency of skin reactions that may appear or be felt on the face. These may be visible reactions or simply sensations perceived as unusual. They may appear during exposure to certain climatic conditions, under certain conditions of daily life, or following the application of certain substances to the face.

We would like to know if during the past year, your face has been affected by any of the skin reactions listed in this questionnaire.

1. Do you consider the skin on your face to be sensitive? **Y/N**
Whether you answered yes or no, it is important that you answer all the questions below.

2. When you are affected by emotion or stress, does your face ever show signs of REDNESS that then quickly disappear in the next few minutes? **Y/N**

3. When you drink certain alcoholic drinks or eat spicy food, are you sometimes affected by REDNESS on the face that quickly disappears in the next few minutes? **Y/N**

4. When you are exposed to rapid changes of temperature, for example passing rapidly from a hot atmosphere to a cold one, or vice versa, is your face affected by REDNESS that disappears again in the next few minutes? **Y/N**

5. When you are exposed to cold weather, wind or dry air (air conditioning, for example), is your face ever affected by one or more of the conditions described below? **Y/N**
(a) REDNESS Y/N
(b) STINGING not combined with redness (repeated light stinging sensation) Y/N
(c) ITCHING not combined with redness (sensation giving rise to a desire to scratch) Y/N
(d) BURNING SENSATION (localized warmth) Y/N
(e) TIGHTNESS (brief stretching sensations in different directions) Y/N
(f) SCURVES or localized DESQUAMATION (skin that peels without sunburn) Y/N

6. Women: When applying certain cosmetic products that you are not used to (cleansers, whether rinsed off with water or not, skin-care creams or make-up), is your face ever affected by one or more of the conditions described below? **Y/N**
OR
Men: When applying certain cosmetic products that you are not used to (cleansers, whether rinsed off with water or not, skin-care creams or shaving creams), is your face ever affected by one or more of the conditions described below? **Y/N**
(a) REDNESS Y/N
(b) STINGING not combined with redness (repeated light stinging sensation) Y/N

(c) ITCHING not combined with redness (sensation giving rise to a desire to scratch) Y/N
(d) BURNING SENSATION (localized warmth) Y/N
(e) TIGHTNESS (brief stretching sensations in different directions) Y/N
(f) SCURVES or localized DESQUAMATION (skin that peels without sunburn) Y/N
(g) 'SPOTS' appeared after product application Y/N

7. **Women: When you do not use a cosmetic product, for example your usual skin cream, is your face ever affected by one or more of the conditions described below? Y/N**
(a) TIGHTNESS (brief stretching sensations in different directions) Y/N
(b) SCURVES or localized DESQUAMATION (skin that peels without sunburn) Y/N

OR

Men: When you shave, or immediately after shaving, is your face ever affected by one or more of the conditions described below? Y/N
(a) REDNESS Y/N
(b) STINGING not combined with redness (repeated light stinging sensation) Y/N
(c) ITCHING not combined with redness (sensation giving rise to a desire to scratch) Y/N
(d) BURNING SENSATION (localized warmth) Y/N
(e) TIGHTNESS (brief stretching sensations in different directions) Y/N
(f) SCURVES or localized DESQUAMATION (skin that peels without sunburn) Y/N
(g) 'SPOTS' appeared after shaving Y/N

8. **Do you consider the skin of your face to be sun-sensitive? Y/N**
9. **Given that sunburn is a burn that may take the form of simple redness, painful redness or redness with blistering, how sensitive is your skin after the first exposure to the sun without applying any sun protection product?**
1 = Always burns, never tans *2 = Always burns, sometimes tans*
3 = Sometimes burns, always tans *4 = Never burns, always tans* ☐
10. **After a period of exposure to the sun, is your face ever affected by one or more of the conditions described below?**
(a) Breakout of 'SPOTS' Y/N
(b) Other conditions Y/N
11. **Women: Do you ever suffer from 'SPOTS' at certain times during your menstrual cycle? Y/N**

Annexe 6. Article sur le lien entre phototype, statut en vit D et DMO

Phototype, statut en vitamine D et densité minérale osseuse chez des femmes à risque d'ostéoporose [Phototype, vitamin D status and bone mineral density among women at risk of osteoporosis]

Guinot C, Ezzedine K, Mauger E, Ambroisine L, Latreille J, Bertrais S, Preziosi P, Galan P, Chapuy MC, Arnaud S, Meunier PJ, Tschachler E, Hercberg S, Malvy S

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Article original

Phototype, statut en vitamine D et densité minérale osseuse chez des femmes à risque d'ostéoporose

Phototype, vitamin D status and bone mineral density among women at risk of osteoporosis

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Résumé

Propos. – Le but de cette étude était de déterminer les facteurs associés à la valeur de la densité minérale osseuse (DMO) mesurée au niveau du col du fémur dans un groupe de femmes d'âge moyen considérées à risque d'ostéoporose (statut précaire en vitamine D [25(OH)D3 < 78 nmol/L] et hyperparathyroïdisme [parathormone circulante > 36 pg/mL]).

Méthodes. – Cette étude a été conduite en deux temps chez 122 femmes de la cohorte SUVIMAX (supplémentation en vitamines et minéraux antioxydants). L'impact de différentes variables, dont l'âge, l'indice de masse corporelle (IMC), le statut en vitamine D, la consommation d'alcool, l'intensité d'exposition au soleil et le phototype sur la DMO a été testé à l'aide de modèles de régression.

Résultats. – Aucun lien significatif n'a été retrouvé entre la DMO et les variables documentant le statut en vitamine D et le taux de parathormone, ni le phototype. Cependant, les phototypes les plus clairs ont tendance à être associés avec les valeurs les plus basses de DMO. En revanche, la valeur moyenne de la DMO diminue significativement avec l'âge et augmente avec l'indice de masse corporelle et le niveau d'activité physique.

Conclusion. – Quel que soit leur phototype, les femmes à statut précaire en vitamine D devraient adopter une supplémentation en vitamine D associée à un apport adapté en calcium tout au long de l'année, sans négliger l'adoption de mesures de protection solaire et le maintien d'un niveau satisfaisant d'activité physique, pour améliorer leur densité osseuse et prévenir le risque fracturaire.

Abstract

Purpose. – The aim of this study was to test the influence of phototype and vitamin D status feature on the bone mineral density (BMD) of the femoral neck in a group of middle-aged women considered at risk of osteoporosis (low levels of vitamin D [25(OH)D3 < 78 nmol/L] and hyperparathyroidism [parathormone level > 36 pg/mL]).

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Methods. – This two-step study was conducted on 122 French women enrolled in the SUVIMAX (*supplémentation en vitamines et minéraux antioxydants*: antioxidant vitamin and mineral supplementation) cohort. The impact of various variables on BMD, including age, body mass index (BMI), vitamin D status, alcohol intake, sun exposure intensity and phototype was investigated using regression models.

Results. – No statistical link was found between BMD and the variables documenting vitamin D status and parathormone levels, nor phototype. Nevertheless, fair phototypes tended to be associated with lower BMD values. However, BMD decreased with age and increased with BMI and physical activity level.

Conclusions. – Whatever their phototype, adult women concerned about precarious vitamin D status should undergo a vitamin D supplementation in combination with an adequate calcium intake all year long and a proper sun protection. Moreover, a physical activity maintenance should provide an additional benefit for prevention of osteoporosis.

Mots clés : Densité minérale osseuse ; Phototype ; Vitamine D

Keywords: Bone mineral density; Phototype; Vitamin D

La densité minérale osseuse (DMO) diminue avec l'âge à partir de la périménopause chez la femme. Au delà d'un certain niveau, la diminution de la densité osseuse (ostéoporose) est associée à une augmentation progressive du risque et du nombre de fractures [1]. Par ailleurs, un lien entre la densité minérale osseuse et la pigmentation constitutive cutanée a été rapporté, la concentration cutanée en phéomélanine et eumélanine comptant parmi les déterminants de la production de vitamine D par la peau [2]. Une étude française conduite chez des volontaires adultes de la cohorte SUVIMAX (supplémentation en vitamines et minéraux antioxydants) n'a pas mis en évidence d'effet indépendant du phototype sur le niveau du taux sérique de vitamine D [3]. Dans cette étude, les déterminants du taux de vitamine D étaient l'intensité d'exposition au soleil et la latitude de la région d'habitation, ces deux facteurs étant connus pour être liés avec le phototype.

Une étude complémentaire à ces travaux a été conduite afin de rechercher les déterminants de la valeur de la DMO mesurée au niveau du col du fémur chez des femmes considérées à risque d'ostéoporose (statut précaire en vitamine D [25(OH)D3 < 78 nmol/L] et parathormone sérique (PTH) > 36 pg/mL). Ces seuils ont été fixés en se fondant sur les résultats d'une étude conduite au sein de la cohorte SUVIMAX explorant le statut en vitamine D [4]. Dans le cadre de cette étude complémentaire, l'influence éventuelle du phototype et des variations des taux de vitamine D et de PTH sur les valeurs de DMO a été évaluée.

1. Patients et méthodes

Entre novembre 1994 et avril 1995, 804 femmes âgées de 35 à 62 ans ont été sélectionnées de façon aléatoire parmi les 7713 femmes volontaires participant à la cohorte française SUVIMAX [5–7]. Ces femmes ont été recrutées parmi 20 villes françaises de neuf régions (latitude variant entre 43 et 51° nord). Leur statut en vitamine D et leur taux circulant de parathormone ont été mesurés [4]. Les méthodes de mesure biologique de la vitamine D et de parathormone ont été développées ailleurs [3,4]. Parmi ces 804 femmes, 221 présentaient un statut précaire en vitamine D [25(OH)D3 < 78 nmol/L] et un taux de parathormone dans des limites supérieures à la normale ou élevé (PTH > 36 pg/mL) [4]. Pour des contraintes budgétaires, la DMO a été mesurée et une seconde évaluation des taux sériques de vitamine D et de PTH réalisée sur un sous-échantillon, deux ans plus tard et à la même saison (période hivernale). Pour ce faire, 122 femmes ont été sélectionnées parmi les 221, de façon aléatoire, en fonction de la région de résidence. La DMO a été mesurée par absorptiométrie biphotonique à rayons X DXA (Dual X-ray Absorptiometry, Hologic QDR 2000, Hologic Inc, Waltham, MA, USA), au niveau du col du fémur.

L'effet sur les valeurs de la DMO des variables générales suivantes a été évalué : l'âge à l'inclusion dans l'étude, l'indice de masse corporelle (IMC), le statut ménopausique, la durée écoulée depuis la ménopause, la prise d'un traitement hormonal de substitution ou de périménopause, le tabagisme (fumeur et ancien fumeur vs non-fumeur), le phototype selon la classification de Césarini (phototype clair : I, II, IIIa, IIIb, vs foncé : IV, V, VI) [8], l'autoappréciation de l'intensité d'exposition au soleil au cours de la vie (aucune ou faible vs modérée ou intense), la région de résidence (nord vs sud de la France), le niveau d'activité physique (> 3 METs vs ≤ 3 METs) et la consommation journalière d'alcool (évaluée en g/j). La définition d'une activité physique modérée ou intense correspond à une activité de loisir de plus de 3 METs pendant au moins plus d'une heure par semaine (un MET, ou équivalent métabolique, est définit par le rapport du coût énergétique d'une activité physique donnée à la dépense énergétique au repos). La consommation journalière d'alcool a été transformée en variable ordinale à trois niveaux en fonction de la valeur médiane des consommatrices d'alcool : consommation nulle, consommation d'alcool faible [0–12,87 g/j], modérée ou élevée [12,87 g/j et plus].

Par ailleurs, l'effet sur la DMO des variables spécifiques suivantes a été évalué : le statut en vitamine D (nmol/L) et en parathormone (pg/mL) en 1995 et 1997, le rapport du taux sérique en vitamine D de 1997 sur celui de 1995 et l'écart entre les taux sériques de PTH en 1997 et en 1995. Les distributions de vitamine D et de parathormone présentant une forte dissymétrie, une transformation logarithmique des données a été réalisée afin d'en normaliser les distributions. Par ailleurs, le niveau des apports nutritionnels ayant été évalué sur un sous-échantillon de la cohorte SUVIMAX, ces informations étaient disponibles pour 72 femmes de notre étude. De ce fait, la den-

sité nutritionnelle en calcium (mg/1000 kcal) et la densité nutritionnelle en vitamine D (µg/1000 kcal) ont été utilisées uniquement dans un but descriptif.

L'effet de chaque variable sur la valeur de la DMO a d'abord été testé grâce à un modèle de régression linéaire simple. Ensuite, un modèle de régression linéaire multiple a été construit pour évaluer simultanément l'effet des variables ayant montré un effet significatif à l'étape précédente, et celui des facteurs conventionnels ayant un risque individuel connu.

2. Résultats

La description de l'échantillon est rapportée dans le Tableau 1. Les modèles de régression univariés sont présentés dans les Tableaux 2 et 3. L'analyse univariée a montré un effet significatif de l'IMC ($p < 0,04$), de l'âge à l'inclusion ($p < 0,02$), de la ménopause ($p < 0,02$) — les femmes ménopausées présentant en moyenne des valeurs plus basses de DMO (valeur moyenne ± écart-type, 0,86 ± 0,11 vs 0,91 ± 0,10), du niveau d'activité physique ($p < 0,05$) — les sujets ayant une activité physique supérieure à 3 METs présentant des valeurs plus élevées (0,91 ± 0,10 vs 0,87 ± 0,11). Des tendances non statistiquement significatives ont été trouvées pour le tabagisme ($p = 0,10$) — les fumeuses et anciennes fumeuses présentant des valeurs plus élevées (0,91 ± 0,09 vs 0,87 ± 0,12), la consommation journalière d'alcool ($p = 0,13$) — les sujets ne consommant pas d'alcool présentant des valeurs plus basses (0,87 ± 0,11) par rapport à ceux ayant une consommation d'alcool faible (0,90 ± 0,10) et ceux ayant une consommation d'alcool modérée ou élevée (0,91 ± 0,10), et le phototype ($p = 0,10$) — les phototypes clairs présentant des valeurs plus basses de DMO (0,88 ± 0,10 vs 0,91 ± 0,11).

Dans le modèle de régression multiple (Tableau 4), aucun lien significatif n'a été trouvé avec le statut en vitamine D en 1997 ($p = 0,58$), le rapport du statut en vitamine D de 1997 sur celui de 1995 ($p = 0,99$) et l'écart entre les taux de PTH de 1997 et de 1995 ($p = 0,71$). En revanche, un effet défavorable de l'âge ($p < 0,002$) et favorable de l'IMC ($p < 0,001$) a été retrouvé ainsi qu'un effet protecteur lié à une activité physique supérieure à 3 METs ($p < 0,02$). Par ailleurs, le lien avec le phototype n'est pas statistiquement significatif lorsque l'on ajuste sur les autres variables ($p = 0,12$). Ni la ménopause ni les différents termes d'interaction entre les facteurs retenus n'ont montré d'effet significatif sur les valeurs de DMO. De ce fait, la ménopause et les interactions n'apparaissent pas dans le modèle de régression multiple.

Tableau 1
Description de la population, moyenne ± écart-type, ou pourcentage (%)

Variables	Effectif	Moyenne ± écart-type ou pourcentage
Âge (années)	122	47,8 ± 6,4
Indice de masse corporelle	122	23,4 ± 4,1
Région de résidence	122	
Nord de la France	77	63,1 %
Sud de la France	45	36,9 %
Phototype selon la classification de Césarini	122	
Clair (I, II, IIIa, IIIb)	84	68,9 %
Foncé (IV, V, VI)	38	31,2 %
Statut ménopausique	122	
Ménopause	55	45,1 %
Non-ménopause	67	54,9 %
Durée écoulée depuis la ménopause (mois)	121	32,5 ± 60,5
Traitement hormonal (substitution et périménopause)	121	
Oui	36	29,8 %
Non	85	70,2 %
Activité physique	122	
Inférieure ou égale à 3 METs	61	50,0 %
Supérieure à 3 METs	61	50,0 %
Tabagisme	122	
Fumeur ou ancien fumeur	55	45,1 %
Non-fumeur	67	54,9 %
Autoappréciation de l'intensité d'exposition solaire au cours de la vie	113	
Aucune ou faible	40	35,4 %
Modérée ou intense	73	64,6 %
Consommation journalière d'alcool	115	
Nulle	49	42,6 %
Faible	33	28,7 %
Modérée ou élevée	33	28,7 %
Taux sérique de vitamine D en 1995 (nmol/L)	122	42,5 ± 20,5
Taux sérique de vitamine D en 1997 (nmol/L)	121	54,9 ± 28,6
Taux sérique de parathormone en 1995 (pg/mL)	122	44,3 ± 15,6
Taux sérique de parathormone en 1997 (pg/mL)	121	39,0 ± 15,3
Densité nutritionnelle en vitamine D (µg/1000 kcal)	72	1,3 ± 0,8
Densité nutritionnelle en calcium (mg/1000 kcal)	72	512,4 ± 168,4
Densité minérale osseuse	122	0,89 ± 0,11

Tableau 2
Modèles de régression univariés – variables générales

		Variables	Effectif	Valeur de *p*
Constante	1,0722			
Coefficient	–0,0039	× *Âge à l'inclusion*	122	0,0113
Constante	0,7707			
Coefficient	0,0050	× *Indice de masse corporelle*	122	0,0326
		Région de résidence	122	
Constante	0,8774			
Coefficient	0,0165	Si nord de la France	77	0,4123
	0	Si sud de la France	45	
		Phototype selon la classification de Césarini	122	
Constante	0,9113			
Coefficient	–0,0340	Si clair (I, II, IIIa, IIIb)	84	0,1044
	0	Si foncé (IV, V, VI)	38	
		Statut ménopausique	122	
Constante	0,9093			
Coefficient	–0,0476	Si ménopause	55	0,0139
	0	Si non-ménopause	67	
Constante	0,8961			
Coefficient	–0,0002	× *Durée écoulée depuis la ménopause (en mois)*	121	0,1710
		Traitement hormonal	121	
Constante	0,8608			
Coefficient	0,0400	Si pas de traitement hormonal		0,0593
	0	Si traitement hormonal (substitution et périménopause)	121	
		Activité physique	122	
Constante	0,8681			
Coefficient	0	Si inférieure ou égale à 3 METs	61	0,0412
	0,0395	Si supérieure à 3 METs	61	
		Tabagisme	122	
Constante	0,9053			
Coefficient	0	Si fumeur ou ancien fumeur	55	0,1032
	–0,0317	Si non-fumeur	67	
		Autoappréciation de l'intensité d'exposition solaire au cours de la vie	113	
Constante	0,8904			
Coefficient	0	Si aucune ou faible	40	0,8805
	–0,0032	Si modérée ou intense	73	
		Consommation journalière d'alcool	115	0,1294
Constante	0,8663			
Coefficient	0	Si nulle	49	
	0,0294	Si faible	33	0,2198
	0,0473	Si modérée ou élevée	33	0,0496

3. Discussion

Les résultats de notre étude n'ont pas permis d'établir l'existence d'un lien significatif entre la DMO et la variation sur deux ans du taux de PTH et du statut en vitamine D chez des femmes à risque d'ostéoporose défini selon nos critères. De même, aucun lien significatif n'a pu être mis en évidence entre le phototype et la valeur de DMO. Dans cet échantillon et après ajustement multiple, les facteurs déterminants de la valeur de DMO étaient l'âge (impact défavorable de l'âge), l'IMC (impact favorable des valeurs plus élevées) et le niveau d'activité physique (impact favorable d'une activité physique déclarée modérée ou intense).

Le statut en vitamine D est un facteur limitant de la densité minérale osseuse mesurée au niveau du col fémoral chez l'adulte présumé en bonne santé, en particulier chez la femme d'âge moyen [9]. Cette observation est cohérente avec la mise en évidence de l'effet bénéfique de l'augmentation modérée des ingesta en vitamine D sur la correction de la perte osseuse objectivée en fin de période hivernale chez l'adulte [10].

Un déficit en vitamine D a été retrouvé non seulement chez la personne âgée vivant à domicile ou en institution [11], mais

Tableau 3
Modèles de régression univariés–variables spécifiques

		Variables	Effectif	Valeur de p
Constante	0,8834			
Coefficient	0,0186	× Rapport taux sérique de vitamine D en 1997 sur taux sérique de Vitamine D en 1995	121	0,4658
Constante	0,8866			
Coefficient	–0,0001	× Écart entre les taux sériques de parathormone en 1997 et ceux de 1995	121	0,9344
Constante	0,8252			
Coefficient	0,0374	× Taux sérique de vitamine D en 1997 (en nmol/L)	121	0,4021
Constante	0,9200			
Coefficient	–0,0250	× Densité nutritionnelle en vitamine D (en µg/1000 kcal)	72	0,1053
Constante	0,9469			
Coefficient	–0,0001	× Densité nutritionnelle en calcium (en mg/1000 kcal)	72	0,0988

Tableau 4
Modèle de régression multiple ($n = 120$[a])

		Variables	Valeur de p
Constante	0,9194		
Coefficients de l'équation de régression	+0,0002	× Rapport taux sérique de vitamine D en 1997 sur taux sérique de Vitamine D en 1995	0,9937
	+0,0003	× Écart entre les taux sériques de parathormone en 1997 et ceux de 1995	0,7095
	+0,0255	× Taux sérique de vitamine D en 1997 (en nmol/L)	0,5778
	+0,0084	× Indice de masse corporelle (en kg/cm^2)	0,0007
	–0,0056	× Âge à l'inclusion (en années)	0,0005
	+0	Si phototype foncé (IV, V, VI)	0,1172
	–0,0315	Si phototype clair (I, II, IIIa, IIIb)	
	+0	Si activité physique inférieure ou égale à 3 METs	0,0188
	+0,0437	Si activité physique supérieure à 3 METs	

[a] Deux données manquantes (une pour le taux sérique de vitamine D en 1997 et une pour le taux sérique de parathormone en 1997).

également chez l'adulte et en population générale française [4]. Parmi 1569 sujets adultes français présumés en bonne santé de la cohorte SUVIMAX, près de 15 % avaient des valeurs de vitamine D sérique inférieures au seuil de 30 nmol/L [4]. Un des facteurs déterminant cette présentation était la latitude de la région d'habitation — les valeurs les plus basses étant retrouvées dans le nord et les plus élevées dans le sud, illustrant la différence géographique de gradient d'intensité d'ensoleillement. Comme chez la personne âgée présentant un déficit vitaminique avéré, le statut précaire en vitamine D était associé à une augmentation de la production de parathormone. Mais chez ces adultes, le taux sérique de parathormone restait à un niveau relativement proche d'un plateau de 36 pg/mL tant que la valeur de vitamine D sérique restait supérieure au seuil de 78 nmol/L [4,12]. Ces résultats, compatibles avec l'installation d'un hyperparathyroïdisme secondaire modéré, ont défini les seuils utilisés dans notre étude. Par ailleurs, ces résultats ont été repris et commentés comme prédicteurs du remodelage tissulaire osseux associé à une diminution de la densité minérale osseuse et au risque fracturaire [12].

Le seuil définissant une valeur individuelle basse de vitamine D sérique ne fait pas l'objet de consensus. Un taux de 100 à 150 nmol/L pourrait être nécessaire au maintien de l'intégrité du tissu osseux [4]. Le choix de cette valeur seuil est cohérent avec les propositions issues d'autres travaux en faveur d'un taux de 75 nmol/L [13], ce qui est quasiment la valeur retenue dans notre enquête, ou d'un taux situé entre 50 et 100 nmol/L en vue de la prévention de l'ostéoporose et de l'hyperparathyroïdisme secondaire associé [14,15]. En France, les produits laitiers n'ont fait l'objet de mesures d'enrichissement en vitamine D que depuis 2001. L'augmentation des apports alimentaires en vitamine D reste un moyen nécessaire mais non suffisant pour atteindre un seuil de 100 à 150 nmol/L [16] sans le recours à une supplémentation médicamenteuse.

Le rôle du statut pondéral devrait faire l'objet d'investigations spécifiques, en particulier en étudiant le lien entre maigreur et risque fracturaire. Par ailleurs, une augmentation acceptable et adaptée du niveau d'activité physique devrait compter parmi les mesures visant à améliorer la densité osseuse et la prévention du risque fracturaire chez la femme adulte. Cette recommandation vaut particulièrement pour les pays du nord compte tenu du lien existant entre les phototypes clairs et l'exposition solaire [3]. L'absence de significativité statistique du lien retrouvé entre le phototype et la densité minérale osseuse vont dans le sens des résultats précédemment rapportés [3]. En effet, les relations entre phototype, statut en vitamine D et photoexposition sont complexes : les phototypes

clairs synthétisant plus volontiers la vitamine D cutanée. Cependant, cette synthèse est réalisée à des doses très faibles d'UV toujours sous-érythémateuses au-delà desquelles la vitamine D est transformée en composés biologiquement inactifs [17]. De plus, compte tenu du risque de photovieillissement et de carcinogenèse cutanée associé à l'exposition solaire, quel que soit leur phototype, les individus à statut précaire en vitamine D devraient adopter une supplémentation en vitamine D associée à un apport adapté en calcium tout au long de l'année, sans négliger l'adoption de mesures de protection solaire [17].

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Annexe 7. Article sur la supplémentation en antioxydants et le risque de cancers cutanés

Antioxidant supplementation increases the risk of skin cancer in women but not in men

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Antioxidant Supplementation Increases the Risk of Skin Cancers in Women but Not in Men[1]

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Abstract

This research aimed to test whether supplementation with a combination of antioxidant vitamins and minerals could reduce the risk of skin cancers (SC). It was performed within the framework of the Supplementation in Vitamins and Mineral Antioxidants study, a randomized, double-blinded, placebo-controlled, primary prevention trial testing the efficacy of nutritional doses of antioxidants in reducing incidence of cancer and ischemic heart disease in the general population. French adults (7876 women and 5141 men) were randomized to take an oral daily capsule of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 μg selenium, and 20 mg zinc) or a matching placebo. The median time of follow-up was 7.5 y. A total of 157 cases of all types of SC were reported, from which 25 were melanomas. Because the effect of antioxidants on SC incidence varied according to gender, men and women were analyzed separately. In women, the incidence of SC was higher in the antioxidant group [adjusted hazard ratio (adjusted HR) = 1.68; $P = 0.03$]. Conversely, in men, incidence did not differ between the 2 treatment groups (adjusted HR = 0.69; $P = 0.11$). Despite the small number of events, the incidence of melanoma was also higher in the antioxidant group for women (adjusted HR = 4.31; $P = 0.02$). The incidence of nonmelanoma SC did not differ between the antioxidant and placebo groups (adjusted HR = 1.37; $P = 0.22$ for women and adjusted HR = 0.72; $P = 0.19$ for men). Our findings suggest that antioxidant supplementation affects the incidence of SC differentially in men and women. J. Nutr. 137: 2098–2105, 2007.

Introduction

Melanoma and nonmelanoma skin cancers (SC),[10] namely squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), are the most common forms of malignancy in the Caucasian population (1) and sun exposure is thought to be the main established risk factor for all 3 types of tumor (2). An aging population, more intense exposure to UV rays due to depletion of the ozone layer, and sun exposure habits would appear to favor a higher incidence of skin malignancy (3).

Numerous studies have demonstrated the role of reactive oxygen species, also called free radicals, in skin carcinogenesis and the potential protective effect of antioxidants (4). Formation of free radicals in the skin can be enhanced by UV radiation. The cutaneous system has a very efficient interlinked antioxidant defense system for counteracting UV-induced oxidative stress. However, excessive exposure to sunlight or other sources of UV light can overwhelm the skin's antioxidant capacity. A potentially interesting strategy for preventing UV exposure damage could be to boost the endogenous antioxidant system by oral intake of antioxidant vitamins and minerals. Although clinical trials have showed contradictory findings (5–7), oral antioxidant pills have been recommended for the prevention of sunburns and for their supposed photoprotective properties.

In particular, it has been suggested that nutrients such as β-carotene, ascorbic acid, vitamin E, selenium, and zinc may prevent such harmful effects of UV exposure because of their antioxidant ability (8). Clinical trials testing the impact of supplementation with high doses of antioxidants over long periods have, however, failed to reveal beneficial effects on the incidence of SC (9,10). For example, the Nutritional Prevention of Cancer trial, a double-blind, randomized clinical trial, was designed to test whether selenium (200 μg/d) could prevent nonmelanoma SC (NMSC) in 1312 individuals with an individual

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[10] Abbreviations used: BCC, basal cell carcinoma; HR, hazard ratio; MSC, melanoma skin cancer; NMSC, nonmelanoma skin cancer; SC, skin cancer; SCC, squamous cell carcinoma; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants study.

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history of SC recruited in the eastern United States. The initial analysis, which was conducted after 6.4 y of intervention, showed a significant inverse association between supplementation and all types of cancer incidence (10). However, a subsequent analysis found the protective effect of selenium supplementation on all types of cancer incidence to be attenuated (11). Surprisingly, the last analysis conducted after 13 y of supplementation suggested a link between selenium supplementation and an increased risk of NMSC (12).

There is some evidence that a combination of antioxidants may have a more powerful free radical scavenging effect than individual molecules, due to complementarity and synergy in their mechanism of action (13). In addition, metabolic interrelationships also exist between antioxidant nutrients with beneficial mutual protection and regeneration.

The Supplementation in Vitamins and Mineral Antioxidants (SU.VI.MAX) study was a primary-prevention trial designed to assess whether a daily supplementation with antioxidant vitamins and minerals, at nutritional doses, could reduce the incidence of the most prevalent chronic diseases in industrialized countries causing premature death, cancer, and ischemic heart disease in middle-aged men and women. A double-blind, randomized, placebo-controlled design was used to compare medium-term outcome (5–10 y) in subjects receiving antioxidant supplementation or not. The framework of the SU.VI.MAX study allowed data to be collected relating to markers of oxidative stress and thus provided an opportunity to study the relationship between antioxidant effects relating to the intervention and cancer incidence.

Our aim was to test, in the context of the SU.VI.MAX study, the impact of a combination of antioxidant nutrients on the incidence of melanoma and NMSC in a large sample of middle-aged individuals from the French general population.

Subjects and Methods

Setting and study design. Details concerning the study rationale, design, methods, and study sample of the SU.VI.MAX trial have been reported previously (14,15). Briefly, the target population was a sample of adults aged 35–60 y for women and 45–60 y for men recruited from the French general population in France. The sample was thus not restricted to high-risk subjects. Enrolled subjects had to "declare themselves free of any severe pathology that might limit participation for 8 y including cancers and cardiovascular disease." The SU.VI.MAX study used a randomized, double-blinded, placebo-controlled design. The participants were randomized to take a capsule containing a combination of antioxidants [120 mg vitamin C (sodium ascorbate), 30 mg vitamin E (dl-α-tocopherol), 6 mg β-carotene, 100 μg selenium (selenium-enriched yeast), and 20 mg zinc (zinc gluconate)] or a matching placebo in a single oral daily capsule. The study cohort was stratified by gender, age group, smoking habits, and residence area. Random treatment allocation was performed by block-sequence generation stratified by gender and age group. Subjects were treated throughout the follow-up period.

The SU.VI.MAX study was approved by a medical ethics committee (Comité Consultatif pour la Protectiondes Personnes participant à la Recherche Biomédicale n°706) of Paris-Cochin, and the National Committee for the Protection of Privacy and Civil Liberties Comité National Informatique et Liberté (n°334641), which advocates that all medical information be confidential and anonymous. The participants signed an informed consent form allowing the investigators to perform screening tests to identify cancer or cardiovascular disease and to communicate the results to their physician.

Follow-up of the participants. The participants underwent a yearly medical visit, which consisted in alternate years either of taking a blood sample or of a clinical examination (physical examination, electrocardiogram, blood pressure measurement, visual acuity examination, anthropometrical measurements, fecal occult blood testing for subjects over 45 y, smear test for all women, and a screening mammogram for women over 50 y). Moreover, the participants were also expected to provide monthly information on treatment compliance, dietary intake, and any health event by completing computerized questionnaires. In the absence of monthly contact for >6 mo, or if the participant missed the yearly visit, an investigation was launched to determine the reasons and to resume contact. If necessary, an inquiry was conducted among neighbors and the participant's physician. Causes of death were provided by families, physicians, or hospitals. At the end of the interventional trial (September 1, 2002), vital status and possible causes of death were confirmed through the National Death Registry.

Antioxidant status. At baseline and every 2 y, 35 mL of venous blood samples after 12-h fasting were taken from each participant. Serum antioxidant concentrations were measured on a randomized sub-sample of 1134 subjects stratified by sex, age, treatment group, and geographic location. All biochemical analyses were performed in the same reference laboratory. Vitamin C status was evaluated by serum ascorbic acid (ascorbate) determination using an automated method based on the principle of continuous flow segmented by air bubbles (16). Serum levels of retinol, β-carotene, and α-tocopherol were measured by HPLC using the Biotek-Kontron HPLC system (17). Serum levels of zinc and selenium were determined using flame atomic absorption spectrometry (Perkin Elmer 3110 for zinc and selenium and Perkin Elmer 4100 ZL for selenium) (18,19).

Endpoints of the SU.VI.MAX trial. Whatever the source of information (see paragraph above on follow-up), once an event was suspected, all relevant records, including results of diagnostic tests and procedures, were collected from physicians, hospitals, or directly from the participants. The primary outcomes of the SU.VI.MAX trial were first fatal and nonfatal major ischemic cardiovascular events and first cancer events during the follow-up. However, BCC and SCC of the skin were not considered in the cancer outcomes as defined in the study protocol. Each event was reviewed by expert committees who were unaware of the treatment assignment. In the case of cancer events, these were ascertained by pathologist reports and reviewed by a committee of oncologists [details in (14)].

Endpoints of the analysis of SC. The outcomes of the present analysis were first event of melanoma at any stage, SCC and BCC of the skin, and other types of SC (International Classification of Diseases, 10th revision, Clinical Modification, codes C43, C44, D03, D04). Moreover, all SC, which were ascertained by pathologist reports, were also reviewed by an expert committee of dermatologists, who were unaware of the treatment assignment.

Assessment of sun exposure. Two questionnaires on sun exposure were administered to participants in 1997 and 2001. The content of the 2 questionnaires was similar, with one part that investigated sun exposure and protection over the past year and another part that assessed lifetime sun exposure and protection (20). Over 64% of the questionnaires of the first survey were returned (4824 women and 3260 men). Following the next survey, we collected 1332 additional questionnaires (800 women and 532 men). The final analysis was conducted on a sub-sample of 9293 volunteers (3751 men and 5542 women) who answered the following question (global self-assessment) "How would you describe the intensity of your skin's exposure to the sun during your lifetime?" with the responses none, mild, moderate, or severe. For the purpose of this analysis, responses were dichotomized as severe vs. moderate, mild, or none. Moreover, the latitude of the place of residence was also taken into account in the analysis.

Statistical methods. All subjects who participated in the SU.VI.MAX study were evaluated in the present analysis. Statistical analyses were performed using SAS software version 8.2 (SAS Institute). Descriptive analysis was performed by gender and treatment group (UNIVARIATE and FREQ procedures). Quantitative variables were expressed as mean ± SD.

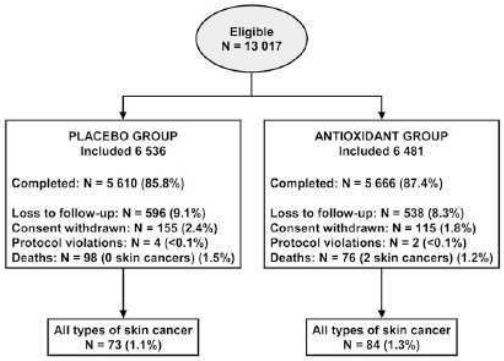

FIGURE 1 Flow of participants by treatment group. Percentages are relative to the number of subjects included in each group.

Baseline characteristic means were compared using ANOVA (GLM procedure). Relationships between antioxidant levels at baseline were assessed using Pearson correlation coefficients (CORR procedure). Percentages of individuals lost to follow-up in each treatment group were compared using the Pearson test (FREQ procedure, option CHISQ) (21).

The duration of follow-up for each participant was defined as the time from randomization until the occurrence of the first event (diagnosis of SC, death, or date of last contact). The analysis was conducted under the intention-to-treat principle.

The proportion of volunteers remaining free of event since randomization was described using Kaplan-Meier survival curves (22). Outcome analysis consisted of frequency comparisons, logrank tests (23), and Cox proportional hazard regression models (24). Cox models allowed a comparison of the effect of the different study variables, namely treatment group, age, smoking status, dwelling latitude, self-assessed lifetime sun exposure, and antioxidant status at baseline. For each outcome, we estimated hazard ratios (HR) with their 95% CI. In the next step, we tested the treatment effect controlling for variables that influenced outcome using a multivariate model to generate adjusted HR. However, as lifetime sun exposure was documented only for a sub-sample of the cohort, this item could not be included in the multivariate analysis. In addition, interaction terms between levels of each antioxidant at baseline and treatment group were also tested. Furthermore, to estimate the effect of antioxidant levels at baseline on each outcome in the antioxidant and the placebo groups, each multivariate Cox model obtained at the last step was rebuilt within each treatment group.

The number of participants was expected to allow detection of a 25% difference in incidence of the outcome (1-tailed $\alpha = 5\%$; 1-tailed $\beta = 90\%$).

Results

Study sample characteristics. A total of 13,017 volunteers, 7876 women and 5141 men, were randomized to take the capsule containing a combination of antioxidants or a matching placebo. The participants entered the trial between October 12, 1994 and April 30, 1995. Subsequently, 270 subjects (2%; 115 in the antioxidant group and 155 in the placebo group) withdrew their written consent on the very day of the enrollment visit or within the next 3 d, because they could not meet the constraints of the protocol. In addition, 6 subjects were excluded from the study because they did not fall within the specified age range. The flow of participants by treatment group is shown in Figure 1. The median follow-up time was 7.5 y for a total of 89,441 person-years (44,866 in the antioxidant group and 44,574 in the placebo group).

The 2 treatment groups did not differ with respect to capsule intake, which was 79% in each treatment group. Compliance was confirmed on a random sub-sample of ~1000 participants. In these subjects, plasma levels of the biochemical markers β-carotene, vitamin C, and selenium significantly increased in the antioxidant group 2 and 7 y after randomization. Furthermore, the 2 treatment groups differed significantly 2 and 7 y after randomization with respect to all markers except serum zinc [details in (14,15)].

The antioxidant and placebo groups were balanced for most baseline variables, notably smoking habits, alcohol consumption,

TABLE 1 Baseline antioxidant status by gender and treatment group[1]

	Women		Men		Group	Gender
Variable	Placebo	Antioxidants	Placebo	Antioxidants	*P*-value[3]	
n[2]	3964	3912	2572	2569		
Age, *y*	47.1 ± 6.6	47.1 ± 6.6	51.8 ± 4.7	51.8 ± 4.6	0.99	<0.0001
n	3869	3844	2508	2520		
Serum β-carotene, μmol/L	0.67 ± 0.47	0.68 ± 0.52	0.46 ± 0.36	0.46 ± 0.31	0.55	<0.0001
n	3643	3639	2395	2421		
Serum vitamin E, μmol/L	30.93 ± 7.53	31.07 ± 7.83	32.23 ± 8.50	32.28 ± 8.34	0.49	<0.0001
n	3665	3644	2404	2422		
Serum vitamin C, μmol/L	59.33 ± 28.56	59.16 ± 25.72	49.73 ± 23.79	49.68 ± 29.63	0.84	<0.0001
n	2847	2857	1836	1854		
Serum selenium, μmol/L	1.08 ± 0.19	1.09 ± 0.19	1.13 ± 0.20	1.14 ± 0.20	0.05	<0.0001
n	3287	3267	2128	2191		
Serum zinc, μmol/L	12.88 ± 1.86	12.79 ± 1.84	13.51 ± 1.85	13.50 ± 1.83	0.09	<0.0001
n	3288	3267	2129	2193		
BMI	22.9 ± 3.0	22.8 ± 0.5	25.2 ± 3.0	25.2 ± 3.0	0.99	0.302
n	3674	3672	2402	2417		

[1] Values are means ± SD.
[2] Data were missing to a variable extent for each variable (up to 28% for serum vitamin C levels) and the actual sample size is therefore indicated for each variable.
[3] No significant interactions between group and gender were detected.

occupational status, education level, marital status, and BMI (14). The treatment groups were also balanced for self-assessed lifetime sun exposure. In women, the frequency of severe exposure was 11.2% in the placebo group and 11.1% in the antioxidant group, whereas in men, it was 11.8% in the placebo group and 12.2% in the antioxidant group.

Within each gender, the ages of the treatment groups were comparable, with women being 47.1 ± 6.6 y old and men being 51.8 ± 4.7 y old. Serum levels of antioxidants did not differ between the treatment groups at baseline, except for selenium (Table 1). However, serum levels of antioxidants at baseline differed significantly between genders. Serum β-carotene concentrations were correlated with serum levels of vitamin C ($r = 0.26$; $P < 0.0001$) and vitamin E ($r = 0.24$; $P < 0.0001$).

Incidence of SC. Overall, 157 validated cases of SC occurred in 81 women and 76 men. These corresponded to 25 melanoma SC (MSC) in 16 women and 9 men. The remaining 132 NMSC were represented by 115 BCC in 57 women and in 58 men, 13 SCC in 4 women and in 9 men, and 4 cases of other types of SC, namely Bowen disease in 4 women (Table 2).

The overall incidence rate of SC did not differ between the treatment groups ($P = 0.35$). However, when segregated by gender, the frequency of SC in women was higher in the antioxidant group ($P = 0.02$). Fifty-one women developed SC in the antioxidant group compared with 30 in the placebo group. There was no such difference in the frequency of SC between treatment groups in men (43 cases in the placebo group and 33 in the antioxidant group; $P = 0.25$).

The incidence of melanoma did not differ between the treatment groups in men (6 cases in the placebo group and 3 cases in the antioxidant group; $P = 0.51$) but was higher in the antioxidant group in women (3 cases in the placebo group and 13 cases in the antioxidant group; $P = 0.01$).

The incidence of NMSC did not differ between treatment groups in either men (37 cases in the placebo group and 30 cases in the antioxidant group; $P = 0.39$) or women (27 cases in the placebo group and 38 cases in the antioxidant group; $P = 0.15$).

Due to the differential effects of antioxidant supplementation according to gender, all subsequent analyses were performed by gender.

Variables associated with the incidence of all SC. According to actuarial survival analysis of the cumulative incidence of SC, the difference between the treatment groups increased regularly over time in women, with a higher incidence in the antioxidant group (logrank test, $P = 0.02$) (Fig. 2*A*). Among men, the incidence of SC did not differ between the treatment groups (logrank test, $P = 0.22$). This differential effect according to gender was confirmed by a Cox proportional hazard regression model, in which there was a significant interaction between gender and treatment group ($P = 0.01$) in addition to

TABLE 2 Distribution of SC types in women and men in the placebo and antioxidant treatment groups[1]

	Women			Men		
Cancer type	Placebo	Antioxidant	P^2	Placebo	Antioxidant	P^2
n	3964	3912		2572	2569	
	n (%)			*n* (%)		
Melanoma	3 (0.08)	13 (0.3)	0.01	6 (0.2)	3 (0.1)	0.51
NMSC	27 (0.68)	38 (0.9)	0.15	37 (1.4)	30 (0.1)	0.39
BCC	23 (0.5)	34 (0.8)	NA	32 (0.012)	26 (0.1)	NA
SCC	3 (<0.1)	1 (<0.1)	NA	5 (0.1)	4 (0.1)	NA
Other SC	1 (<0.1)	3 (<0.1)	NA	None	None	—
Total SC	30 (0.7)	51 (1.3)	0.02	43 (1.6)	33 (0.1)	0.25

[1] Data are number of cases (percent of study group).
[2] Significant differences between treatment groups (χ^2 test) are indicated as probability values.
NA, Nonapplicable.

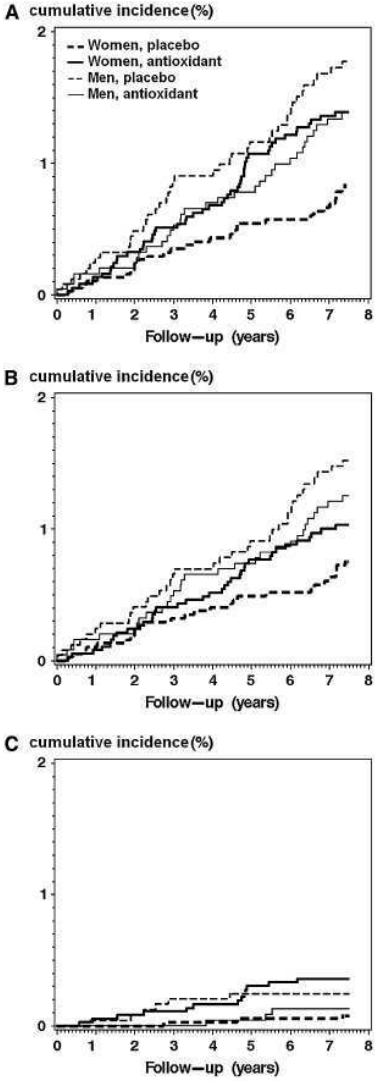

FIGURE 2 Kaplan-Meier curves for cumulative incidence of skin cancers: all types of skin cancer (*A*); nonmelanoma skin cancer (*B*); and melanoma skin cancers (*C*). Placebo/women: $n = 3964$; placebo/men: $n = 2572$; antioxidant/women: $n = 3912$; antioxidant/men: $n = 2569$. The 2 groups differed in the incidence of all SC ($P = 0.02$; logrank test) and the incidence of MSC ($P = 0.01$; logrank test).

TABLE 3 Cox proportional hazard regression models for SC occurrence by gender

	Women, $n = 7876$			Men, $n = 5141$		
	n[1]	HR (95% CI)	P[3]	n[1]	HR (95% CI)	P[3]
Univariate analysis						
Treatment group	7876	1.70 (1.08; 2.67)	0.021	5141	0.75 (0.48; 1.19)	0.22
Age, *y*	7874	1.06 (1.03; 1.10)	<0.001	5141	1.08 (1.03; 1.13)	0.0015
Current smoking	7361	1.05 (0.56; 1.91)	0.87	4815	0.90 (0.46; 1.76)	0.76
Dwelling latitude, *degree*	7711	0.99 (0.88; 1.10)	0.80	5025	1.00 (0.89; 1.12)	0.99
Lifetime sun exposure	5542	2.23 (1.26; 3.95)	0.006	3751	1.36 (0.69; 2.67)	0.37
Multivariate analysis[2]						
Treatment group		1.68 (1.06; 2.65)	0.027		0.69 (0.43; 1.10)	0.11
Age, *y*		1.06 (1.02; 1.10)	0.001		1.08 (1.03; 1.13)	0.0017
Current smoking		1.21 (0.68; 2.21)	0.53		0.96 (0.49; 1.87)	0.89
Dwelling latitude, *degree*		0.97 (0.87; 1.08)	0.57		0.98 (0.88; 1.10)	0.79

[1] Data were missing to a variable extent for each variable (up to 29% for lifetime sun exposure) and the actual sample size is therefore indicated for each variable.
[2] The multivariate analysis was only conducted on the 12,004 subjects for whom data on all relevant variables were available.
[3] Probability values were calculated with the Wald test.

gender and treatment group effects (data not shown). Therefore, subsequent analyses were conducted for each gender separately. Univariate and multivariate Cox proportional hazard regression models were generated for the number of cases of all SC (Table 3). In the univariate model, antioxidant treatment group (HR = 1.70; $P = 0.02$), older age (HR = 1.06; $P = 0.0005$), and greater lifetime sun exposure (HR = 2.23; $P = 0.006$) were associated with a higher probability of developing SC in women. Treatment group (adjusted HR = 1.68; $P = 0.02$) and age (adjusted HR = 1.06; $P = 0.001$) were retained as independent variables associated with cancer risk in the multivariate model. In men, age was the only variable associated with increased cancer risk identified in either the univariate or the multivariate analysis (HR = 1.08; $P = 0.001$ in both cases).

Variables associated with the incidence of melanoma. Actuarial survival analysis of the cumulative incidence of melanoma was also performed (Fig. 2*C*). In women, the difference between the treatment groups increased regularly over time, with a higher incidence in the antioxidant group (logrank test, $P = 0.01$), whereas the incidence of SC in men did not differ between groups (logrank test, $P = 0.31$). In women, antioxidant treatment group was the only variable associated with a higher probability of developing melanoma in both the univariate (HR = 4.32; $P = 0.02$) and multivariate (adjusted HR = 4.31; $P = 0.02$) Cox proportional hazard regression models (Table 4). No such association was identified in men.

Variables associated with the incidence of NMSC. According to the actuarial survival analysis, the 2 treatment groups did not differ in terms of the cumulative incidence of NMSC in either women (logrank test, $P = 0.17$) or men (logrank test, $P = 0.36$) (Fig. 2*B*). In the univariate Cox proportional hazard regression model, older age (HR = 1.09; $P = 0.0001$) and greater lifetime sun exposure (HR = 2.19; $P = 0.01$) were significantly associated with a higher probability of developing SC in women, as was older age in men (HR = 1.08; $P = 0.003$) (Table 5). In the multivariate model, only age was retained as an associate variable (adjusted HR = 1.09; $P = 0.0001$ in women and HR = 1.08; $P = 0.004$ in men).

TABLE 4 Cox proportional hazard regression models for melanoma occurrence by gender

	Women, $n = 7876$			Men, $n = 5141$		
	n[1]	HR (95% CI)	P[3]	n[1]	HR (95% CI)	P[3]
Univariate analysis						
Treatment group	7876	4.32 (1.23; 15.16)	0.02	5141	0.49 (0.12; 1.97)	0.31
Age, *y*	7874	0.95 (0.87; 1.02)	0.15	5141	1.09 (0.95; 1.25)	0.22
Current smoking	7361	1.75 (0.56; 5.43)	0.33	4815	2.83 (0.71; 11.32)	0.14
Dwelling latitude, *degree*	7711	1.12 (0.85; 1.48)	0.42	5025	0.93 (0.68; 1.27)	0.65
Lifetime sun exposure	5542	2.36 (0.65; 8.56)	0.19	3751	1.42 (0.17; 12.20)	0.74
Multivariate analysis[2]						
Treatment group		4.31 (1.23; 15.13)	0.02		0.49 (0.12; 1.97)	0.32
Age, *y*		0.95 (0.88; 1.03)	0.19		1.09 (0.95; 1.13)	0.19
Current smoking		1.53 (0.49; 4.80)	0.46		3.05 (0.76; 12.23)	0.11
Dwelling latitude, *degree*		1.12 (0.85; 1.49)	0.42		0.98 (0.68; 1.27)	0.64

[1] Data were missing to a variable extent for each variable (up to 29% for lifetime sun exposure) and the actual sample size is therefore indicated for each variable.
[2] The multivariate analysis was only conducted on the 12,004 subjects for whom data on all relevant variables were available.
[3] Probability values were calculated with the Wald test.

TABLE 5 Cox proportional hazard regression models for NMSC occurrence by gender

	Women, $n = 7876$			Men, $n = 5141$		
	n[1]	HR (95% CI)	P[3]	n[1]	HR (95% CI)	P[3]
Univariate analysis						
Treatment group	7876	1.41 (0.86; 2.30)	0.17	5141	0.80 (0.49; 1.29)	0.35
Age, *y*	7874	1.09 (1.05; 1.13)	0.0001	5141	1.08 (1.03; 1.13)	0.003
Current smoking	7361	0.89 (0.44; 1.81)	0.75	4815	0.70 (0.32; 1.53)	0.37
Dwelling latitude, *degree*	7711	0.96 (0.85; 1.08)	0.50	5025	1.01 (0.90; 1.14)	0.85
Lifetime sun exposure	5542	2.19 (1.16; 4.16)	0.01	3751	1.35 (0.66; 2.75)	0.40
Multivariate analysis[2]						
Treatment group		1.37 (0.83; 2.28)	0.220		0.72 (0.44; 1.18)	0.19
Age, *y*		1.09 (1.05; 1.13)	0.0001		1.08 (1.02; 1.13)	0.004
Current smoking		1.11 (0.54; 2.27)	0.77		0.74 (0.34; 1.62)	0.44
Dwelling latitude, *degree*		0.94 (0.83; 1.06)	0.29		0.99 (0.88; 1.12)	0.91

[1] Data were missing to a variable extent for each variable (up to 29% for lifetime sun exposure) and the actual sample size is therefore indicated for each variable.
[2] The multivariate analysis was only conducted on the 12,004 subjects for whom data on all relevant variables were available.
[3] Probability values were calculated with the Wald test.

Discussion

This study describes the effect of treatment with a combination of antioxidant nutrients on the incidence of SC over a median follow-up time of 7.5 y. In contrast to most of the interventional primary prevention trials performed to date, which have used pharmacological doses of individual antioxidants (or pairs of antioxidants), the current study used a combination of mineral and vitamin antioxidants administered at doses commonly used as nutritional supplements in European countries.

Our results show that the effect of antioxidant supplementation on the incidence of SC varies according to gender. The incidence of all types of SC and melanomas was higher in the group of women receiving antioxidant supplementation compared with the placebo group. Such an effect was not observed in men. These results can be compared with the main results of the principal analysis of the SU.VI.MAX trial (14), which showed that antioxidant supplementation was associated with a significant reduction in the overall incidence of all-site cancers in men with no effect in women. It should, however, be noted that, in the present analysis, the incidence of SC was lower in the men receiving antioxidants, although this difference did not reach statistical significance. This may reflect underpowering of the study for the detection of differences in the incidence of relatively rare events such as SC.

Our findings may be attributed in part to gender differences in nutrient metabolism. Although the molecular basis for this metabolic difference is poorly characterized, it may result from hormone-sensitive expression of genes coding for cellular transporters of vitamins and antioxidants involved in the metabolism of carcinogens or in regulation of the cell cycle. For example, it has been hypothesized that the relative importance of low selenium status as a risk factor for cancer might differ between men and women due to sex- or gender-related factors that influence tumor biology (25).

Gender-dependent differences in the handling of antioxidants in the skin may also contribute to the effect of antioxidant supplementation on SC incidence in women. For example, women tend to have higher concentrations of antioxidants in the skin due to a higher intake of dietary vitamin C and β-carotene and the presence of a larger reservoir of subcutaneous adipose tissue in which to store lipophilic antioxidants such as β-carotene (26). In our study, men had significantly lower average serum levels of several antioxidants, particularly β-carotene, than women. This may be related to the capacity of circulating β-carotene to reflect recent or current carotenoid intake from dietary fruits and vegetables (14,27), which may be lower in men.

The discrepancy between our results and experimental data in animals that has suggested an anticarcinogenetic effect of antioxidants with respect to SC may be explained by differences in the timing of the intervention. In animal models of cutaneous carcinogenesis, the diet is enriched in antioxidants prior to irradiation with UV-A or UV-B, leading to a predominantly beneficial effect of treatment on DNA protection. In contrast, in the SU.VI.MAX trial and related primary prevention studies, antioxidants are given only after many years of exposure to sunlight or other risk factors. At this stage, it may be too late for antioxidants to prevent DNA damage, whereas increased antioxidant exposure might exert a negative influence on antitumoral immunity, angiogenesis, or apoptosis. For example, antioxidants have been reported to interfere with the ability of natural killer lymphocytes to destroy tumor cells in animal models (28). This detrimental effect late in the disease process might explain why we observed a more pronounced effect upon melanoma, which requires a longer time frame over which to develop compared with other SC types and is presumed to be mainly induced by exposure to solar radiation during infancy. For other SC types, a protective effect of antioxidants with respect to sun exposure during the time of the study could counterbalance a detrimental effect on the development of preexisting precancerous lesions induced before the trial began, which could explain the absence of effect of antioxidant supplementation observed in our study.

Previous observational surveys, which have evaluated the relationship between antioxidant status, estimated by serum biomarkers, and the risk of SC have yielded contradictory results. Some studies reported a negative association between serum levels of α-tocopherol, carotenoids, or selenium and the risk of melanoma or NMSC (29–31), whereas others did not find any such protective effect (32–35). In contrast, a recent Italian study of a community-based cohort reported that the incidence rate of melanoma was nearly 4 times higher in individuals exposed to a high selenium intake (provided by tap water) than in unexposed individuals (36). Several large randomized trials evaluating the impact of β-carotene supplementation did not

reveal any beneficial effect on the development of SC in community-based populations (9,32–34,37,38) or in patients with antecedents of NMSC (39).

In addition, some trials have evaluated gender effects with respect to the potential benefits of dietary supplementation with individual antioxidants. For example, in the Nutritional Prevention of Cancer study, the initial data analysis showed that the beneficial effect of supplementation with selenium on the incidence of all types of cancer was restricted to men, specifically to those with the lowest baseline serum selenium concentrations (12). This differential effect of selenium supplementation according to gender is consistent with the results of previous case-control studies (40) and prospective studies conducted on all types of cancers (41).

There are several methodological limitations to the present study. For example, BCC occurrence was not considered as a primary endpoint within the framework of the SU.VI.MAX core trial and our analysis thus corresponds to a post hoc analysis. In addition, the pertinence of the analysis of the incidence of melanoma is limited by the small number of cases.

In conclusion, our findings suggest that dietary supplementation with vitamins and trace element antioxidants may not always provide beneficial effects. This issue is important, given the vast quantities of antioxidant pills that are sold in certain countries. This is particularly true among sunseekers and women in northern countries, where the use of such pills is reputed to prevent solar damage to the skin. In this respect, our study indicates that regular intake of such nutrients, especially at doses taken by consumers of supplements in northern countries, may be associated with harmful effects. Clearly, high doses of specific nutrients given to middle-aged adults over a period of several years do not have an equivalent biological impact to the lifelong intake of a broad palette of nutrients from a well-balanced diet. It therefore appears crucial to better define the safety profile of such nutrients to be able to assess accurately the risk-benefit relationship associated with their use as dietary supplements.

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Annexe 8. Article sur l'incidence des cancers cutanés 5 années après l'arrêt de la supplémentation.

Incidence of skin cancers during 5-year follow-up after stopping antioxidant vitamins and mineral supplementation
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Incidence of skin cancers during 5-year follow-up after stopping antioxidant vitamins and mineral supplementation ☆

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ABSTRACT

Context: In the SU.VI.MAX study, antioxidant supplementation for 7.5 years was found to increase skin cancer risk in women but not in men.
Objective: To investigate the potential residual or delayed effect of antioxidant supplementation on skin cancer incidence after a 5-year post-intervention follow-up.
Design, setting and participants: Assessment of skin cancer including melanoma and non-melanoma during the post-intervention follow-up (September 2002–August 2007). The SU.VI.MAX study was a double-blind, placebo-controlled, randomised trial, in which 12,741 French adults (7713 women aged 35–60 years and 5028 men aged 45–60 years) received daily a placebo or a combination of ascorbic acid (120 mg), vitamin E (30 mg), β-carotene (6 mg), selenium (100 μg) and zinc (20 mg), from inclusion in 1994 to September 2002.
Main outcome measures: Total skin cancer incidence, including melanoma, squamous cell carcinoma and basal cell carcinoma.
Results: During the post-intervention period, 10 melanomas appeared in women and 9 in men (26 and 18, respectively, for the total period of supplementation + post-supplementation). Six squamous cell carcinomas were found in women and 15 in men (10 and 25, respectively, for the total period). Finally, 40 basal cell carcinomas appeared in women and 36 in men (98 and 94, respectively, for the total period). Regarding potential residual or delayed effects of supplementation in women, no increased risk of melanoma was observed during the post-intervention follow-up period. No delayed effects, either on melanoma or non-melanoma skin cancers, were observed for either gender.

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Conclusions: The risk of skin cancers associated with antioxidant intake declines following interruption of supplementation. This supports a causative role for antioxidants in the evolution of skin cancers.

1. Introduction

Skin cancers (SC) are the most common malignancies occurring in Caucasian populations.[1–3] During the last decades, the steadily increasing incidence of skin cancers has brought much attention to the process by which these tumours develop and how they can be prevented. Skin is constantly exposed to ultraviolet (UV) radiation as well as to an array of environmental pollutants, such as components of cigarette smoke, which lead to the production of reactive oxygen species (ROS).[4] This phenomenon is responsible for accelerated sun-induced cutaneous changes (photoaging) in the areas exposed to UV radiation. Moreover, ROS are considered as potent inducers of structural alterations in DNA leading to mutations, the first step in tumour development.[5] Consequently, agents that inhibit ROS formation or activity may potentially enhance endogenous defence systems and thus prevent or reverse photodamage. Although clinical trials have provided contradictory findings concerning their efficacy, oral antioxidant pills have been proposed for the prevention of sunburn and for their supposed photoprotective properties.[6]

However, in contrast with this hypothesis, the results of the SU.VI.MAX study indicated a significantly higher incidence of total skin cancers and melanomas in the group of women receiving a combination of antioxidants compared to placebo after a median follow-up time of 7.5 years. Conversely, in men, no significant differences were found between the treatment groups.[7] In order to investigate possible residual or delayed effects of antioxidant supplementation on skin cancer incidence, the SU.VI.MAX cohort was followed-up 5 years after the end of the double-blind supplementation period.

2. Patients and methods

2.1. Study design and population

The study rationale, design, methods, study sample, endpoint ascertainment and initial findings of the SU.VI.MAX trial have been reported elsewhere.[8,9] Briefly, the target population was a sample of adults aged at inclusion between 35 and 60 years for women and between 45 and 60 years for men recruited from the French general population in France. The SU.VI.MAX study used a randomised, double-blind, placebo-controlled design. The participants were randomised to take a capsule containing a combination of antioxidants (120 mg vitamin C (sodium ascorbate), 30 mg vitamin E (DL-α-tocopherol), 6 mg β-carotene, 100 μg selenium (selenium-enriched yeast) and 20 mg zinc (zinc gluconate) or a matching placebo, in a single daily oral capsule. A total of 13,017 volunteers, 7876 women and 5141 men, were included and randomly allocated. The participants entered the trial between 12th October 1994 and 30th April 1995. Of these, 270 subjects (2%: 115 in the antioxidant group and 155 in the placebo group) withdrew their written consent within 3 d of enrolment because they could not meet the constraints of the protocol. In addition, six subjects were excluded from the study as they did not fall within the specified age range. Thus, 12,741 subjects (7713 women aged 46.6 ± 6.6 years and 5028 men aged 51.3 ± 4.7 years) contributed to the analyses. The primary analyses were performed on outcome events validated on 1st September 2002 (median follow-up time of 7.5 years). At the end of the intervention phase, the participants were informed of the results of the trial. No recommendations were given concerning the use of supplements, but about having a balanced diet with at least five fruits or vegetables daily. Participants who were still alive at the end of the supplementation period (1st September 2002) and who had not dropped out of the study or been lost to follow-up (n = 11 054) were asked to participate in the post-supplementation follow-up. Subjects have now been followed up for an additional 5 years (until the 1st September 2007), up to a total period of 13 years since beginning enrolment.

The protocol was approved by a medical ethics committee and the National Committee for the Protection of Privacy and Civil Liberties.

2.2. Follow-up during the post-intervention period

During the post-intervention follow-up, all major health events were identified from the 6-monthly questionnaires returned by the participants and from all additional information provided spontaneously by participants or their kin. Once a possible skin cancer was suspected, all relevant records, including results of diagnostic tests, procedures and pathology reports, were collected from the physicians and hospitals involved, or directly from the participants. End-points were validated only after review of all relevant information by a committee of specialised physicians who were blinded for supplementation assignment.

2.3. Study outcomes

The outcomes considered in the present analysis were all-stage melanoma, squamous cell carcinoma (SCC) of the skin, basal cell carcinoma (BCC) and other types of SC (International Classification of Diseases, 10th revision, Clinical Modification [ICD-10-CM], codes C43, C44, D03, D04). Moreover, all possible SC were removed surgically and evaluated by histopathology to ascertain the diagnosis. The pathologist's reports were subsequently reviewed by an expert committee of dermatologists, who were unaware of the treatment assignment. Whatever the original source of information, once an event was suspected, all relevant records, including

results of diagnostic tests and procedures, were collected from physicians, hospitals or directly from the participants.

2.4. Statistical methods

Statistical analyses were performed using SAS® software version 9.1.3 (SAS Institute Inc., Cary, NC).

Separate analyses were performed for each type of SC (BCC, SCC and melanoma). A subject with more than one cancer of a specific type was taken into account only once (only the first cancer occurrence), but if two different types of SC were identified, the subject was included for each type of cancer in the analysis. The duration of follow-up for each participant was defined as the time from randomisation until the occurrence of the first event (diagnosis of skin cancer, death or date of last contact). The analysis was conducted under the intention to treat principle. Due to the differential effects of antioxidant supplementation according to gender found during the intervention period,[7,9] all subsequent analyses were performed by gender.

The frequency of SC was described by treatment group (antioxidant group and placebo group) using the FREQ procedure, option CHISQ or EXACT.[10] The proportion of volunteers remaining event-free since randomisation was described using Kaplan–Meier survival analysis and compared between groups using the log-rank test.[11] These descriptive analyses were performed for both the intervention period and total period.

To test the effect of possible confounding factors (smoking status, dwelling latitude, sunburn during childhood,

Table 1 – Characteristics of the study population.

n	Women			Men		
	Placebo	Antioxidant	*p*	Placebo	Antioxidant	*p*
	3964	3912		2572	2569	
Age, mean (SD), years	47.1 (6.6)	47.1 (6.6)	1.00	51.8 (4.7)	51.8 (4.6)	0.99
Dwelling latitude, mean (SD), °	47.1 (1.9)	47.1 (1.9)	0.77	47.0 (2.0)	47.0 (2.0)	0.73
Body mass index, mean (SD), kg/m^2	22.9 (3.6)	22.8 (3.5)	0.44	25.2 (3.0)	25.2 (3.0)	0.84
Smoking status, %			0.89			0.83
Never	54.7	54.5		34.5	33.6	
Former	29.1	28.9		50.2	50.9	
Current	16.1	16.5		15.3	15.4	
Phototype[a] (I and II *versus* III and IV), %	34.3	33.9	0.77	17.1	19.1	0.13
Severe lifetime sun exposure[b] (yes *versus* no), %	11.1	11.6	0.54	12.1	12.5	0.74
Severe sunburn during childhood[c] (yes *versus* no), %	29.1	28.2	0.46	29.9	29.5	0.79
Severe sunburn during adulthood[c] (yes *versus* no), %	28.9	29.6	0.57	24.7	24.4	0.85

[a] Fitzpatrick classification[13]; data were available for 5432 women and 3668 men.
[b] Data were available for 5428 women and 3693 men.
[c] Data were available for 5542 women and 3751 men for sunburn during childhood and for 5010 women and 3426 men for sunburn during adulthood.

Table 2 – Occurrence of skin cancers by gender and treatment group.

Cancer type	Women		*p*[c]	Men		*p*[c]
	Placebo N = 3964	Antioxidant N = 3912		Placebo N = 2572	Antioxidant N = 2569	
Basal cell carcinoma						
Intervention period[a]	24 (0.6%)	34 (0.9%)	0.17	32 (1.2%)	26 (1.0%)	0.43
Post-intervention period[b]	21 (0.6%)	19 (0.6%)	0.75	15 (0.7%)	21 (0.9%)	0.40
Total period[b]	45 (1.1%)	53 (1.4%)	0.38	47 (1.8%)	47 (1.8%)	1.00
Squamous cell carcinoma						
Intervention period[a]	3 (0.1%)	1 (<0.1%)	0.62	6 (0.2%)	4 (0.2%)	0.75
Post-intervention period[b]	3 (0.1%)	3 (0.1%)	1.00	6 (0.3%)	9 (0.4%)	0.60
Total period[b]	6 (0.2%)	4 (0.1%)	0.75	12 (2.1%)	13 (2.3%)	0.84
Melanoma						
Intervention period[a]	3 (0.1%)	13 (0.3%)	0.0114	6 (0.2%)	3 (0.1%)	0.51
Post-intervention period[b]	6 (0.2%)	4 (0.1%)	0.54	4 (0.2%)	5 (0.2%)	1.00
Total period[b]	9 (0.2%)	17 (0.4%)	0.11	10 (0.4%)	8 (0.3%)	0.64

[a] Number of cases (% of study group participants).
[b] Number of cases (% of follow-up participants).
[c] Probability value (χ^2 test or Fisher's exact test, as appropriate).

phototype, self-assessed lifetime sun exposure on the outcomes), a series of Cox regression models adjusted for age was performed using the PHREG procedure. Age-adjusted hazard ratios (adjusted HR) with their 95% confidence intervals (95% CI) were estimated from these models.

A possible residual or delayed effect of antioxidant supplementation during the post-intervention period was tested using multivariate Cox regression models with treatment as a time-dependent variable adjusted for confounding variables according to the following equation[12]:

$$h(t) = h_0(t) * \exp(\beta_1^* \text{ treatment}(t) + \beta_2^* \text{ group} + \beta_i^* Z)$$

In the model, the 'Treatment(t)' variable was defined as antioxidant supplementation (yes or no) and the 'Group' variable as the original randomisation group (antioxidant or placebo group). The 'Treatment(t)' variable was always equal to 0 for the subjects in the placebo group whatever the period, whereas, for the subjects in the antioxidant group, it was equal to 1 during the intervention period and equal to 0 during the post-intervention period. The variable 'Group' was equal to 1 for the subjects in the antioxidant group and was equal to 0 for the placebo group. Thus, the relative risk of the variable 'Group' corresponds to the residual or late effect of the supplementation during the post-intervention period. The term 'Z' was the set of possible confounding factors. The results are expressed as relative risks (RR), adjusted for confounders, together with their 95% CI.

3. Results

3.1. Study sample characteristics

As described previously,[9] the antioxidant and placebo groups were balanced for most baseline variables, notably smoking habits, body mass index (BMI), Fitzpatrick phototype[13] and items related to sun exposure (Table 1).

During the post-intervention phase, 271 subjects withdrew consent or were lost to follow-up (follow-up time: 1.4 ± 1.6 years) and 158 subjects died. The median follow-up time was 12.5 years for a total of 138,616 person–years (68,576 in the antioxidant group and 70,040 in the placebo group).

3.2. Incidence of BCC

The frequency of BCC is presented in Table 2. At the end of the intervention period, 116 subjects, 58 women and 58 men, presented a validated case of BCC. In women, the frequency of BCC was 0.9% in the antioxidant group, compared to 0.6% in the placebo group. Five years later, the total number of subjects with a BCC had increased by 76–192 subjects (98 women and 94 men). The cumulative incidence of BCC according to treatment group and gender is presented in Fig. 1A and B. During the post-intervention period, the cumulative incidence curve for the antioxidant group remained above the placebo group in both genders, although the inter-group difference appeared to decrease after 5 years of follow-up. The between-group effect over the total period (1994–2007) was not significant for either gender (log-rank tests: $p = 0.47$ for women and $p = 0.87$ for men). Table 3 presents the results of the Cox regression analysis. For both genders, no significant residual or delayed effect of supplementation was found on the risk of BCC during the post-intervention period (RR = 0.70 [95% CI: 0.48–1.65] for women; 1.22 [0.64–2.33] for men).

3.3. Incidence of SCC

Fourteen subjects (4 women and 10 men) presented a SCC at the end of the intervention period, and 35 subjects (10 women and 25 men), at the end of the post-intervention period

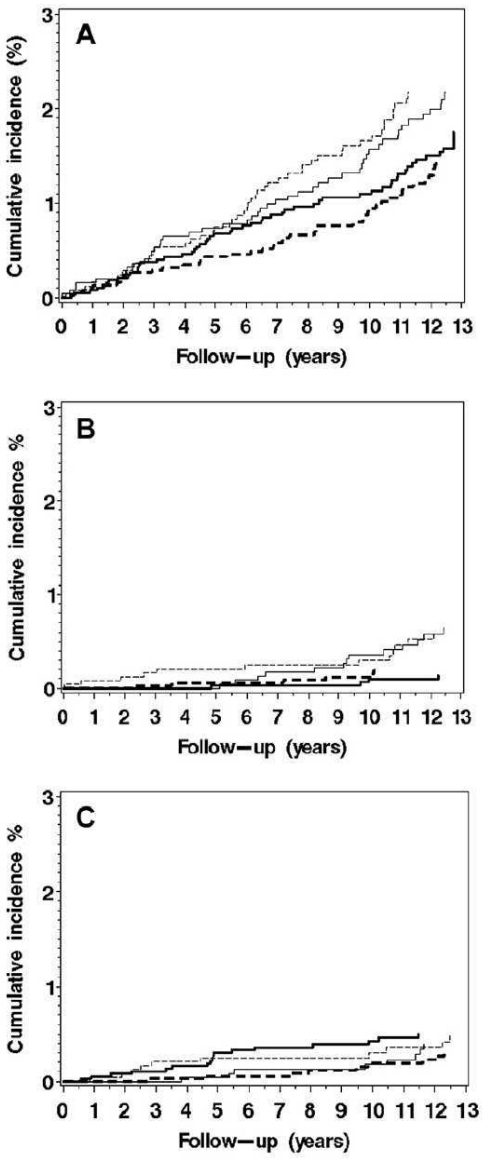

Fig. 1 – Cumulative incidence over the entire study period (intervention and post-intervention) of: (A) basal cell carcinoma; (B) squamous cell carcinoma of the skin, and (C) melanoma.

Table 3 – Residual or delayed effect of antioxidant supplementation during the post-intervention period for each skin cancer, by gender.

Cancer type	Placebo *n*	Antioxidant *n*	Relative risk (95% CI)	*p*
Basal cell carcinoma				
Men	47	47	1.22 (0.64–2.33)	0.54
Women	45	53	0.70 (0.48–1.65)	0.70
Squamous cell carcinoma				
Men	12	13	1.38 (0.49–3.84)	0.54
Women	6	4	0.95 (0.19–4.67)	0.95
Melanoma				
Men	10	8	1.15 (0.31–4.27)	0.83
Women	9	17	0.64 (0.18–2.27)	0.49

Relative risks refer to the residual treatment effect during the post-intervention period as described in Section 2.

(Table 2). The cumulative incidence curves of the antioxidant group and the placebo group are presented in Fig. 1C and D. These curves crossed over during the follow-up period for both genders. The between-group effect for the total period (1994–2007) was not significant for either gender (log-rank test: $p = 0.50$ for women, $p = 0.93$ for men). No significant effect of supplementation was found on the risk of SCC during the post-intervention period for either gender (RR = 0.95 [95% CI = 0.19–4.67] for women; 1.38 [0.49–3.84] for men) (Table 3).

3.4. *Incidence of melanoma*

At the end of the intervention period, the total number of subjects with a melanoma was 25 (16 women and 9 men). In women, the frequency of melanoma was significantly higher in the antioxidant group than in the placebo group (Table 2). At the end of the post-intervention study, the total number of subjects with a melanoma skin cancer was 44 (26 women and 18 men) and, for women, the frequency of melanoma was higher in the antioxidant group than in the placebo group, although this difference was not significant. Fig. 1E and F present the cumulative incidence of melanoma. In women, the difference between the treatment groups increased over time, with a numerically, but not significantly, higher incidence in the antioxidant group (log-rank test: $p = 0.13$), whereas the incidence was comparable in men (log-rank test: $p = 0.57$). A significant residual or delayed effect of supplementation on the risk of melanoma during the post-intervention period was not seen for either gender (RR = 0.64 [95% CI = 0.18–2.27] for women and 1.15 [0.31–4.27] for men) (Table 3).

4. Discussion

In the original analysis of skin cancer risk in the SU.VI.MAX trial, an excess risk of skin cancers was found in the antioxidant treatment group in women.[7] The present analysis shows that this elevated risk recedes when antioxidant supplementation is stopped. This observation argues in favour of a causative role of supplementation in the appearance of skin cancers reported in the original study. In addition, the post-intervention follow-up period did not reveal any delayed protective effects of supplementation on skin cancer.

Our results are at least partly consistent with previous lung cancer prevention trials performed in a community setting, which have assessed the impact of supplementation with antioxidants and conducted a follow-up post-intervention extension.[14,15] Both for these lung cancer studies, and for skin cancer in the SU.VI.MAX study, the rapidity with which tumour incidence rises following initiation of vitamin or antioxidant supplementation, suggests an effect on the growth of pre-clinical tumours, rather than induction of *de novo* tumours.[16] Indeed, it is unlikely that tumours of the skin may develop over a time period as short as the supplementation periods of these trials.[4] This is particularly the case for melanoma, whose earliest pathogenetic events are believed to occur early in life with a very long subclinical silent period. Moreover, in all these studies, the elevated risk of cancer declined after stopping the supplementation, over a 4-year period in the lung cancer trials and over 5 years in the SU.VI.MAX trial.

Our findings of risk differences between men and women should be interpreted with caution because of the relatively small number of events within subgroups of skin cancer. However, in a recent review it has been proposed that gender differences in oxidative stress caused by radical oxygen species (ROS) may underlie survival differences between male and female for melanoma as it is firmly recognised that males express lower amounts of anti-oxidant enzymes, resulting in more oxidative stress than females.[17] Other hypotheses to explain this difference are that women may have more skin fat tissue in which antioxidants and vitamins are stored[4,18] as well as hormonal factors may also influence susceptibility to skin cancers.[19,20]

Despite a large body of knowledge on cell-culture systems and in animal models demonstrating substantial efficacy,[21] previous studies of prevention strategies based on the administration of antioxidants to restore free-radical homeostasis, have so far failed to provide unequivocal evidence that any antioxidant agent or strategy is effective in preventing oxidative injury-mediated carcinogenesis in human populations.[9,15,21–23]

A recent meta-analysis of randomised clinical trials concluded that there was no evidence to support a preventive effect of antioxidant supplementation on cancer and cautioned that this may in certain cases be harmful.[24] One possible explanation for the contradictory results in such trials is that the genetic background may be a confounding factor for cancer development.[19,25] In light of these disappointing findings, it was subsequently shown in studies assessing the growth of various tumour cells *in vitro* that the efficacy of certain antitumoural drugs was actually diminished in the presence of antioxidants.[26] Hence, most drugs used for the treatment of cancers proceed through the production of ROS.[27] It is possible that for cancers with a long preclinical development period, such as melanoma, antioxidants interfere with scavenging of pre-clinical tumour cells by macrophages releasing ROS. Further research on ROS containing pathways, genetic susceptibility, pharmacokinetics, skin bioavailability and concentrations are issues still to be addressed in order to elucidate the overall impact of ROS pathways on carcinogenesis.

The principal limitation of this study is the relatively low number of events, due to the low overall incidence of melanoma,[28] which compromises the precision with which the risk can be determined. A further limitation that should be noted is that the analysis of the incidence rates of confirmed BCC was performed *post hoc*. Finally, we have no explicit data on any possible voluntary antioxidant intake during the post-intervention follow-up period, even though this was not encouraged by the study investigators at the end of the SU.VI.MAX trial.

In conclusion, the current study demonstrated that the risk of skin cancers associated with antioxidant intake declined following interruption of supplementation. This supports a causative role for antioxidants in the evolution of skin cancers. Systematic antioxidant supplementation should be avoided in at-risk individuals for skin cancers, such as those with a lifetime history of excessive sun exposure, since it may be harmful to them.

Author contributions

Dr.(s)/Pr.(s) Ezzedine, Hercberg, Galan, Kesse-Guyot, Malvy had full access to all of the data in the study and take(s) full responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Dr.(s)/Pr.(s) Ezzedine, Hercberg, Galan, Guinot, Malvy

Acquisition of the data: Dr.(s)/Pr.(s) Ezzedine, Hercberg, Galan.

Analysis and interpretation of the data: Dr.(s)/Pr.(s) Ezzedine, Latreille, Kesse-Guyot, Galan, Hercberg, Guinot, Malvy.

Drafting of the manuscript: Dr.(s)/Pr.(s) Ezzedine, Malvy, Guinot.

Critical revision of the manuscript for important intellectual content: Dr.(s)/Pr.(s) Hercberg, Galan, Kesse-Guyot.

Statistical analysis: Dr.(s)/Pr.(s) Guinot, Latreille, Ezzedine, Malvy.

Obtained funding: Dr.(s)/Pr.(s) Hercberg, Galan.

Administrative, technical, or material support: Pr. Hercberg.

Study supervision: Pr. Hercberg.

Conflict of interest statement

None declared.

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Annexe 9. Article sur variants du gène MC1R et couleur de la peau

MC1R gene polymorphism affects skin color measured by reflectance in a population of French adult women

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MC1R Gene Polymorphism Affects Skin Color and Phenotypic Features Related to Sun Sensitivity in a Population of French Adult Women

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ABSTRACT

The melanocortin-1 receptor (*MC1R*) gene is known to play a major role in skin and hair pigmentation and to be highly polymorphic in Caucasians. This study was performed to investigate the relationships between *MC1R* gene polymorphisms and skin color in a large sample of French middle-aged Caucasian women. The codons 60 to 265 and the codon 294 of the *MC1R* gene were sequenced in 488 women. The skin color was measured on the inner side of the forearm using a spectrophotometric instrument. Fifteen variants were identified: Arg151Cys, Arg160Trp, Arg142His, Asp294His, Ile155Thr, Asp84Glu, Val60Leu, Val92Met, Arg163Gln, Ser83Pro, Thr95Met, Pro256Ser, Val265Ile, Ala166Ala and Gln233Gln. Women carrying Arg151Cys, Asp294His, Arg160Trp and Asp84Glu variants had a significantly higher reflectance in the red region, which indicates a lower level of functional melanin. This association was the most pronounced for women carrying Asp84Glu. In contrast, no significant difference was observed for other variants. Moreover, associations between *MC1R* polymorphisms and the risks of experiencing sunburn and of having freckles were found independently of skin color. Our findings support the hypothesis that *MC1R* polymorphisms do not necessarily alter the skin color but should sensitize the skin to UV-induced DNA damage.

INTRODUCTION

Hemoglobin and melanin are among several molecules that influence human skin color. Variation in skin pigmentation can be explained by the amount and type of melanin, eumelanin and phaemelanin, as well as the number, size and distribution of melanosomes (1,2). The melanocortin-1 receptor (*MC1R*) is a 317 amino acid G-protein-coupled receptor, whose activation by α-melanocyte stimulating hormone (α-MSH) leads to transcription of microphthalmia transcription factor (3). This in turn activates the transcription of a number of enzymes involved in melanin synthesis, leading eventually to upregulation of the synthesis of eumelanin (4,5).

The *MC1R* gene (MIM# 155555), which is localized on chromosome 16q24.3, is the first gene that has been identified to play a major role in determining skin pigmentation in normal Caucasian populations (6). This gene is highly polymorphic and diminished function alleles of the *MC1R* gene are associated with a switch from synthesis of eumelanin to synthesis of phaeomelanin in follicular melanocytes (7,8).

Previous authors have reported a link between fair skin and various alleles, *i.e.* Arg142His, Arg151Cys, Arg160Trp, Asp294His, Asp84Glu and Ile155Thr (3,9–16). In these studies, the skin color was generally assessed using clinical scales (such as dark, medium, light) or through sun sensitivity using Fitzpatrick's skin type. These scales are often used because they are well-known and easy to fill out, but they provide only indirect assessment of the skin pigmentation. Instrumental methods, such as diffuse reflectance spectroscopy, are recognized to provide a more objective assessment of skin pigmentation based on the spectral reflectance of the skin at each wavelength across the visible waveband (17–20). However, studies using these instrumental methods to investigate the association with *MC1R* gene variants are rare and based on restricted wavelengths (14–16).

Thus, the principal objective of our study was to investigate the relationships between the *MC1R* gene polymorphisms and the skin color assessed objectively by reflectance spectroscopy taking into account the information of the whole spectrum. The second objective was to study the links of these polymorphisms with phenotypic features related to sun sensitivity in a large sample of French middle-aged Caucasian women.

MATERIALS AND METHODS

Study population. This study was performed in the context of the SU.VI.MAX cohort (SUpplémentation en VItamines et Minéraux Anti-oXydants), a longitudinal national study conducted in France, which investigated the relationships between nutrition and health in the context of the most prevalent chronic illnesses in industrialized

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countries. The design, objectives and methodology of this study are described in detail elsewhere (21,22). The SU.VI.MAX study was approved by the ethical committee for studies in human subjects (CCPPRB no. 706) of Paris-Cochin, and the "Comité National Informatique et Liberté" (CNIL no. 334641) which is responsible for data confidentiality. The SU.VI.MAX cohort included 13 017 subjects representative of the French adult middle-aged population for most sociodemographic features. The electronic data capture and storage system provided an opportunity to conduct cross-sectional surveys.

This cross-sectional study was conducted from October 2002 to January 2003. All the women from Paris area were requested to participate in this research. Among them (n = 2257), 570 women, aged 44–70 years, agreed to take part in this study and provided informed consents. These women were asked to follow strict skin care instructions. In particular, they did not apply any cosmetic or makeup during the 12 h prior to reflectance measurements.

Eighty-two women were excluded from the analysis due to major protocol violations, which could have a possible impact on the skin color measurement, and some technical problems: recent UV exposure (within 1 month before the beginning of the study, 44 women), recent use of self-tanning products (within 3 weeks before the beginning of the study, two women), presence of skin diseases (seven women with vitiligo and two with psoriasis), non-Caucasian origin (10 women), technical problems encountered during reflectance measurement (20 women) and insufficient blood sample for DNA extraction (13 women).

MC1R gene polymorphisms. Blood samples were obtained from each women, lysed in NASBA lysis buffer (Organon Teknika BV, Boxtel, The Netherlands) and stored at −80°C until assay. Genomic DNA was isolated by silica-based extraction as previously described (23). The PCR analysis was performed using 0.3 μg of genomic DNA in 25 μL of final reaction buffer as previously described using a GeneAmp PCR System 2400 thermal cycler (Perkin-Elmer, Branchburg, NJ; [24]). We used primers MC1Ramp2f: 5′-CCTGGA GGTGTCCATCTCTG-3′ and MC1Ramp2r: 5′-TGTGGAAGGCG TAGATGAGG-3′, which yielded an amplicon that spans nucleotides 1546388 to 1547187 in the NCBI genomic reference sequence NT_010542.15 and corresponds to nucleotides 746 to 1545 of the mRNA reference sequence for MC1R (NM_002386.2). The resulting PCR products were sequenced by MWG-BIOTECH AG (Ebersberg, Germany) using the Comfort Read® procedure and the primers: MC1Ramp1f: 5′-ATCTCTGACGGGCTCTTCCT-3′ and MC1Ramp1r: 5′-CGTAGATGAGGGGGTCGAT-3′ which span the nucleotides 759 to 1536 of the mRNA reference sequence. Codons 60–265 were all covered by the PCR and sequencing with these primers. Codon 294 was genotyped using "Hybridization Probe" technology (25). Briefly, the genomic DNA was amplified using the primers MC1R_294His_F 5′-CTCACACTCATCGTCC-3′ and MC1R_ 294His_R 5′-GCACACTTAAAGCCGC-3′ in the presence of sensor probe "MC1R_294His_sens" 5′-TGCAATGCCATCATCCACCC-fluorescein and anchor probe "MC1R_294His_anch" LCRed640-TCATCTACGCCTTCCACAGCCAG-Phosphate. After amplification, the reaction mixture was gradually heated from 40 to 80°C and fluorescence in the LCRed640 Channel was measured continuously. A melting curve analysis was performed on the fluorescence signals resulting from FRET between anchor and sensor probe. The sensor probe was designed to be a match to the C variant (corresponding to His in the amino acid sequence). Melting temperatures of 67°C for the match (C, His allele) and 59°C for the mismatch (G, Asp allele) allowed discrimination of the alleles and were validated by sequencing. Analysis of the data obtained by sequencing was performed at the Departments of Dermatology of the University of Edinburgh and of the Medical University of Vienna.

Features related to sun sensitivity. During a medical examination, investigators trained beforehand by a senior dermatologist collected five skin features related to sun sensitivity that are commonly used for phototyping (26,27): natural hair color at the age of 20 (white, red, blond, chestnut, brown or black), skin color in winter (whitish, light, darkish), freckles (no, some, frequent), sunburn event frequency (always, frequent, rare/exceptional, no) and suntan intensity (no, slight, light/dark, dark, very dark, black).

Skin color measurements. Skin color was measured on the inner side of the forearm using a Konica Minolta CM2600d (Osaka, Japan) spectrophotometer under controlled environmental conditions: room temperature (mean ± SD) 21 ± 3°C, relative humidity 37 ± 5%. The inner side of the forearm was chosen because this area is relatively well hidden from sun exposure and has been widely used in other studies to assess constitutive pigmentation. The spectrophotometer recorded automatically the spectral reflectance of the skin at each wavelength across the visible waveband: 360–740 nm, with a 10 nm spectral interval. It is a compact portable integrating sphere spectrophotometer which uses the D/8 geometry conforming to CIE No.15, ISO 7724/1, ASTM E1164, DIN 5033 Tei17, and JIS Z8722–1982 (diffused illumination/8° viewing system) standards. Light from pulsed xenon lamps diffuses on the inner surface of the integrating sphere and illuminates the skin uniformly. A double-beam system enables to measure both incident and reflected light and to compensate the source intensity variations. An illuminated viewfinder enables the user to place the measuring area (8 mm diameter) on the skin and to reduce pressure on the skin. Daily, before the series of measurements, the instrument was calibrated according to the recommendation of the provider. The Specular Component Included mode was used and the data analyzed was the average of three repeated measurements.

Statistical analysis. All statistical analyses were performed using SAS® software release 9.1.3 (SAS Institute Inc., Cary, NC) and DTM Data and Text Mining software release 3.0 (available at: http://www.lebart.org).

The nine most common *MC1R* gene variants were analyzed. Among these variants, six have previously been reported in the literature (3,16) as major penetrant "R" (Arg151Cys, Arg160Trp, Arg142His, Asp294His, Ile155Thr and Asp84Glu), and three as minor penetrant "r" (Val60Leu, Val92Met and Arg163Gln). Therefore, the data were first analyzed by grouping the variants in three categories: "WT," wild-type homozygous; "Minor," that is to say only minor variants (r/r and r/WT); and "Major" with at least one major variant (R/r, R/WT and R/R). In a second step, the specific contribution of each variant was analyzed individually. The variants were classed as either homozygote (var_1/var_1), heterozygote (var_1/WT) or compound heterozygote (var_1/var_2). The relationships between the skin features related to sun sensitivity and the gene polymorphisms were tested using logistic regression analyses.

The mean spectral curves were drawn for homozygous WT and each *MC1R* polymorphism, as well as for pooled "Minor" and "Major" variants. The relationships between the *MC1R* gene polymorphisms and the percentages of reflected light were then studied using principal component analysis method (PCA). This technique belongs to factor analysis methods, which allow a set of quantitative variables (in our case, the percentages of reflected light) to be reduced into a small number of derived variables that are uncorrelated, which account for decreasing proportions of the total variance in the data (28). These new variables, called principal components, are defined as linear functions of the original variables which are weighted according to their relative importance. Each principal component is interpreted by examining the partial contribution of each original variable (the percentages of reflected light) to the loading of variance onto the component. Furthermore, these principal components can be used as axes systems to generate graphical displays on which the individuals can be mapped. These maps allow the relationships between different categories of interest to be evaluated (in our case, individuals grouped according to *MC1R* gene polymorphisms). The bootstrap resampling method enables to estimate the variability of the position of these categories on the map: replicates of the population are first generated by randomly drawing with replacement, and the replicated elements are mapped on the axes system to describe these variabilities (29). Thus, the location of a particular subgroup on the map is described as an ellipse representing the area of the map where the center of gravity of the subgroup of interest can be located with 95% confidence. When the ellipses of two subgroups are well separated on the map, the locations of the groups of individuals are significantly different, which indicate that the skin color of individuals carrying different *MC1R* gene polymorphisms are also significantly different.

RESULTS

MC1R gene variants

Fifteen different *MC1R* gene variants were identified in our population (Table 1), including two synonymous changes, four

Table 1. Allelic frequencies of the *MC1R* polymorphisms.*

		Major diminished function						Minor diminished function			Rare variants	Variants frequency (n = 976)	Genotype frequency (n = 488)
	WT	Arg151Cys	Arg160Trp	Asp294His	Arg142His	Asp84Glu	Ile155Thr	Val60Leu	Val92Met	Arg163Gln			
WT†	171	38	21	8	11	4	3	78	51	23	4	583 (59.7)	412 (84.4)
Major diminished function													
Arg151Cys	38	3	0	0	3	1	1	8	3	1	0	61 (6.2)	58 (11.9)
Arg160Trp	21	0	2	1	0	0	1	4	2	1	0	34 (3.5)	32 (6.6)
Asp294His	8	0	1	0	0	1	0	2	2	0	0	14 (1.4)	14 (2.9)
Arg142His	11	3	0	0	0	0	0	2	0	0	0	16 (1.6)	16 (3.3)
Asp84Glu	4	1	0	1	0	0	0	0	0	0	0	6 (0.6)	6 (1.2)
Ile155Thr	3	1	1	0	0	0	0	3	1	0	0	9 (0.9)	9 (1.8)
Minor diminished function													
Val60Leu	78	8	4	2	2	0	3	11	7	7	1	134 (13.7)	123 (25.2)
Val92Met	51	3	2	2	0	0	1	7	2	3	0	73 (7.5)	71 (14.5)
Arg163Gln	23	1	1	0	0	0	0	7	3	2	1	40 (4.1)	38 (7.8)
Rare variants‡	4	0	0	0	0	0	0	1	0	1	0	6 (0.6)	6 (1.2)

*The rows and columns are the *MC1R* genotype on each allele. †In the WT category, true genomic WT are pooled with synonymous variants (Ala166Ala and Gln233Gln), which are mutations in the DNA sequence that do not lead to a change in the amino acid sequence of the protein. ‡Rare variants were Ser83Pro, Thr95Met, Pro256Ser and Val265Ile.

rare variants, *i.e.* with a frequency less than 0.5% of all alleles, and nine more common variants (Val60Leu, Val92Met, Arg151Cys, Arg163Gln, Arg160Trp, Arg142His, Asp294His, Ile155Thr and Asp84Glu). Moreover, 171 (35%) women were wild-type homozygous (consensus sequence), 184 (38%) women were carriers of only minor variants (Minor) and 127 (26%) women of at least one major variant (Major).

Features related to sun sensitivity

The percentage of women presenting each phenotypic feature related to sun sensitivity and their corresponding risk are presented in Table 2 for the various *MC1R* gene variants. As expected, some statistically significant associations were found. Women carrying at least one major variant (Major) had higher risks of having fair hair (OR [95% CI], 5.83 [2.74–12.38]), light skin color (4.94 [2.31–10.55]), freckles (4.07 [2.48–6.67]), light suntan intensity (3.64 [2.15–6.18]) and frequent sunburn (3.12 [1.83–5.32]) than WT homozygous women. In addition, women carrying only minor variants (Minor) had a significantly higher risk of freckles (1.94 [1.23–3.04]), and showed a trend to a higher risk of sunburn events (1.60 [0.96–2.68]). Among the major diminished function variants (R), Arg151Cys, Arg160Trp and Asp294His were significantly associated or tended to be associated with all the sun-sensitive skin features. Moreover, the Arg142His, Ile155Thr and Asp84Glu polymorphisms were found to be significantly associated with freckles and the frequency of sunburn. The Arg142His polymorphism tended to be associated with the hair color and the suntan intensity. For the minor diminished function variants (r), the carriers of Val60Leu and Val92Met polymorphisms had an increased risk of having freckles and experiencing sunburn events. Furthermore, the Val92Met and, to a lesser extent, the Val60Leu polymorphisms were linked to suntan intensity. No relationship was found between the sun-sensitive skin features and the Arg163Gln variant.

Skin color

The mean spectral curves for the *MC1R* gene polymorphisms grouped as "WT," "Minor" and "Major" are shown in Fig. 1. The "Minor" and "WT" curves are contingent, whereas the "Major" curve is displaced upward from the two other curves, which means a lighter skin color for the carriers of the major variants. The mean spectral curves for the nine most common *MC1R* gene polymorphisms are shown in Fig. 2. The curve of each R variant lies above that of the WT population (Fig. 2a–f). However, the Arg151Cys, Asp84Glu and Asp294His curves are well separated and the farthest from the WT curves (Fig. 2a–c), whereas the Arg160Trp, Ile155Thr and Arg142His curves are less well separated and closer to the WT curve (Fig. 2d–f). On the contrary, the curve of each minor diminished function variant and the WT curve are almost overlaid (Fig. 2g–i).

The PCA allowed the reflectance at 10 nm intervals to be reduced into two principal components accounting for 96% of the total variance observed in the individuals' spectrum. Figure 3 presents the contribution of each wavelength to the two first principal components. The first principal component (PC1) has similar positive weightings for all wavelengths. It describes the variation of reflectance in average, which

Table 2. Percentage of women presenting each phenotypic feature related to sun sensitivity and their corresponding risk for the various *MC1R* gene variants.

MC1R gene variants	Hair color Red/Blond/Chestnut		Freckles Frequent/Some		Skin color Whitish/Light		Suntan intensity No/Slight/Light		Sunburn event frequency Frequent/Always	
	%§	OR (95% CI)	%	OR (95% CI)	%	OR (95% CI)	%	OR (95% CI)	%	OR (95% CI)
WT*	69	1	27	1	72	1	51	1	18	1
Minor†	68	0.97 (0.62–1.51)	42	1.94 (1.23–3.04) **	72	0.99 (0.62–1.57)	57	1.30 (0.85–1.99)	26	1.60 (0.96–2.68) *t*
Major‡	93	5.83 (2.74–12.38)***	60	4.07 (2.48–6.67)***	93	4.94 (2.31–10.55)***	79	3.64 (2.15–6.18)***	41	3.12 (1.83–5.32)***
r variants										
Val60Leu	73	1.21 (0.72–2.02)	50	2.67 (1.62–4.38)***	74	1.07 (0.63–1.81)	60	1.49 (0.92–2.39)	32	2.12 (1.23–3.68)**
Val92Met	68	0.94 (0.52–1.71)	48	2.49 (1.40–4.44)**	80	1.56 (0.79–3.07)	66	1.91 (1.07–3.41)*	31	2.01 (1.06–3.80)*
Arg163Gln	76	1.46 (0.64–3.30)	32	1.25 (0.58–2.69)	71	0.94 (0.43–2.05)	50	0.98 (0.48–1.98)	24	1.39 (0.60–3.23)
R variants										
Arg151Cys	93	5.99 (2.06–17.43)**	60	4.00 (2.14–7.53)***	93	5.08 (1.74–14.83)**	80	3.91 (1.89–8.08)***	43	3.35 (1.73–6.49)***
Arg160Trp	94	6.57 (1.51–28.51)*	58	3.75 (1.70–8.28)***	97	11.50 (1.52–86.79)*	77	3.35 (1.37–8.19)**	32	2.13 (0.91–4.98) *t*
Arg142His	87	3.17 (0.69–14.40) *t*	69	5.96 (1.96–18.12) **	81	1.66 (0.45–6.10)	69	2.15 (0.72–6.45) *t*	44	3.47 (1.20–10.07)*
Asp294His	93	5.88 (0.75–46.13) *t*	64	4.88 (1.55–15.34)**	93	4.98 (0.63–39.18) *t*	93	12.69 (1.62–99.20)*	50	4.47 (1.46–13.69)**
Ile155Thr	100	NA	67	5.42 (1.30–22.60)*	100	NA	100	NA	67	8.93 (2.11–37.76)**
Asp84Glu	100	NA	83	13.56 (1.54–119.21)*	100	NA	67	1.95 (0.35–10.95)	67	8.93 (1.56–51.05)*

OR = odds ratio; CI = 95% confidence interval. *WT homozygous. †r/r and r/WT. ‡R/R, R/r and R/WT. §Percentage of women presenting the grouped category among the women carrying the variants. Probability of Wald test: *t* = trend $P > 0.05$ and < 0.20; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. NA = test not applicable due to the low frequency of this polymorphism.

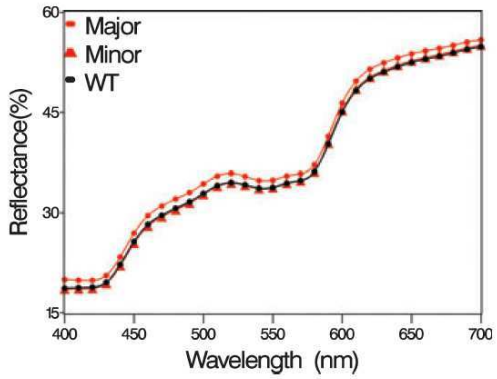

Figure 1. Mean spectral curves for *MC1R* gene variants described as WT (WT homozygous), Minor (r/r and r/WT) and Major (R/R, R/r and R/WT).

measures essentially the skin lightness. Thus, higher PC1-positive values denote a higher reflectance in average, and vice versa. Most of the variation between the individuals' spectrum is explained by PC1 (88%). On the contrary, the PC2 coefficients vary according to wavelengths, with low and negative values until 590 nm and high and positive values at longer wavelengths, which correspond to the red region of the spectrum. Therefore, PC2 represents the variation in the spectral shape (8% of the total information). Positive values of PC2 denote a tendency toward red skin color and vice versa. These components were used as an axis system to visualize the difference in skin reflectance between carriers of *MC1R* gene variants (Figs. 4 and 5a–i). The confidence ellipse of the "Major" variants is well separated on the first component from the confidence ellipses of the "Minor" and "WT" (Fig. 4). Hence, the "Major" variants have higher positive value of PC1 than "WT." Thus, women carrying at least one major variant have a significantly lighter skin than women carrying only minor variants or carrying WT homozygous. In addition, the "Minor" variants are above the "WT" on the map, but as their confidence ellipses overlap, there is no significant difference between them. In Fig. 5, the nine most common variants are highlighted with their 95% confidence ellipse. Women carrying the Arg151Cys, Asp84Glu and Asp294His variants have significantly higher positive value on PC1 than WT homozygous women (Fig. 5a–c). Therefore, these women have a significantly lighter skin color than the WT women. In addition, the Asp84Glu variant is the farthest from the WT, which indicates that the skin color of the women carrying this variant is the lightest among all the variants. The confidence ellipse of the Arg160Trp variant is above the confidence ellipse of the WT homozygous (Fig. 5d), suggesting that women carrying this variant have a significantly higher reflectance in the red region than WT homozygous women. Concerning the remaining variants (Ile155Thr, Arg142His, Val60Leu, Arg163Gln and Val92Met), no significant difference was found with the WT (Fig. 5e, f, g, h and i, respectively).

DISCUSSION

In this study, the distribution of the most frequent *MC1R* gene polymorphisms was investigated in a large sample of middle-aged, community-dwelling Caucasian women living in Paris. Little information is available in the literature on the distribution of these *MC1R* variants in French populations. However, it has been investigated in two skin cancer case–control studies (30,31). In these studies, the control group was composed of 172 unaffected members of melanoma-prone families (30), and of 151 individuals without any personal or familial history of skin cancer recruited in Parisian hospitals (31). The distribution of *MC1R* polymorphisms observed in our sample was consistent with that reported in these two groups. In particular, the Val60Leu and Val92Met polymorphisms were found to be the most frequent in the three studies.

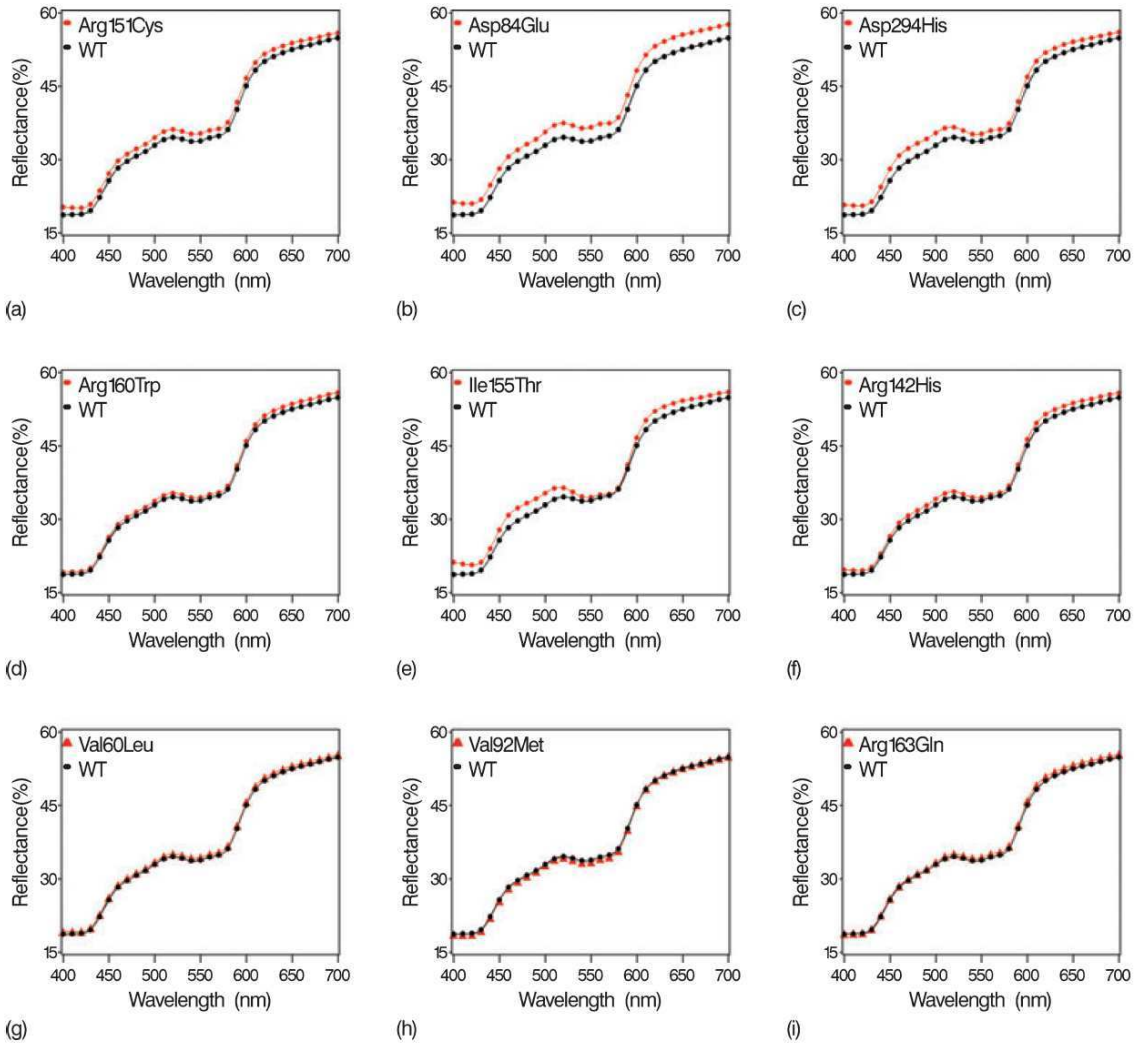

Figure 2. Mean spectral curves for the most common *MC1R* gene variants. (a) Arg151Cys *versus* WT. (b) Asp84Glu *versus* WT. (c) Asp294His *versus* WT. (d) Arg160Trp *versus* WT. (e) Ile155Thr *versus* WT. (f) Arg142His *versus* WT. (g) Val60Leu *versus* WT. (h) Val92Met *versus* WT. (i) Arg163Gln *versus* WT.

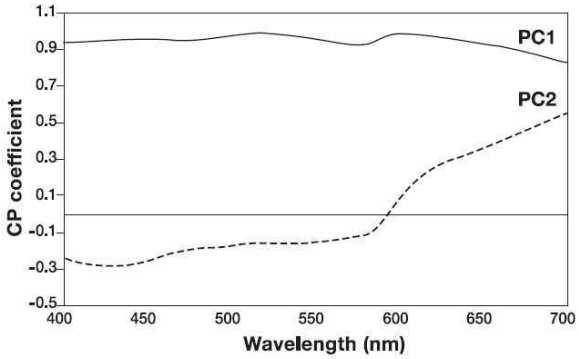

Figure 3. Contribution of each wavelength to the two first principal components PC1 and PC2. PC1 indicates the average reflectivity all along the spectrum and PC2 the reflectance in the red region of the spectrum.

In our population, eight of the nine most frequent variants were found to be associated with freckles. The strongest risk was observed for Asp84Glu, followed by Arg142His, Ile155Thr, Asp294His, Arg151Cys, Arg160Trp, Val60Leu and Val92Met variants. These associations, which have been also reported in a study conducted on a Dutch population (32), support a significant influence of the *MC1R* gene polymorphism on the development of freckles. In addition, our results suggest an association between the Ile155Thr variant and freckles, which has not been reported before.

The impact of *MC1R* gene polymorphism on the ability to tan and the risk of sunburn has been studied previously using Fitzpatrick's skin type in a study conducted in the Netherlands on a large population of 123 patients with melanoma, 453 patients with nonmelanoma skin cancer and 385 control subjects (12). The authors reported that the risk for carriers of

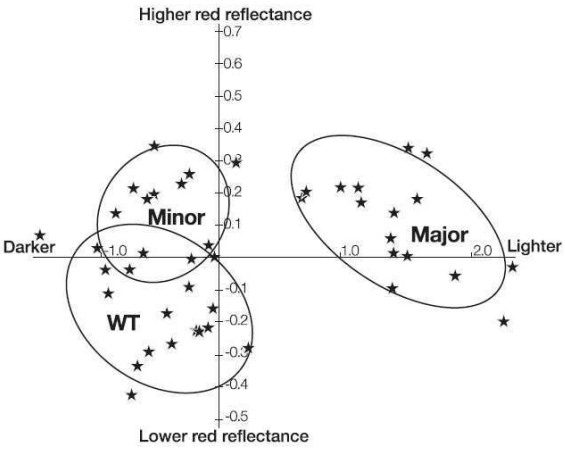

Figure 4. First principal plan of PCA: Difference in skin color between the *MC1R* gene variants described as WT (WT homozygous), Minor (r/r and r/WT) and Major (R/R, R/r and R/WT). The 95% confidence ellipses describe the uncertainty of the location of each category on the map. The points within the ellipses correspond to the centers of gravity of the replicates generated by the bootstrap method.

the Asp84Glu, Arg142His, Arg151Cys, Arg160Trp and Asp294His variants to have fair skin type (I–II, according to Fitzpatrick's skin type) was greater than 2.5 with respect to the WT genotype, whereas the relative risk for carriers of the Val60Leu, Val92Met and Arg163Gln variants was smaller than 2. In our study, all the variants except Arg163Gln were associated with sunburn events. The highest risks were found for Asp84Glu and Ile155Thr, followed by Asp294His, Arg142His, Arg151Cys, and after that Arg160Trp, Val60Leu and Val92Met polymorphisms. Moreover, women carrying the Asp294His, Arg151Cys, Arg160Trp and Val92Met polymorphisms had a significantly higher risk of being able to develop only a light tan. This risk is the strongest for Asp294His. Roughly, the same polymorphisms seem to be associated with the sunburn event frequency and with the suntan intensity. However, some differences were observed; for example, the Asp84Glu polymorphism was strongly associated with sunburn event frequency, but not associated with the ability to tan. A similar discrepancy, although less marked, was observed for carriers of the Arg142His and Val60Leu variants. These results suggest that the polymorphisms do not have an identical impact on the skin sensitivity to sunburn and the ability to tan.

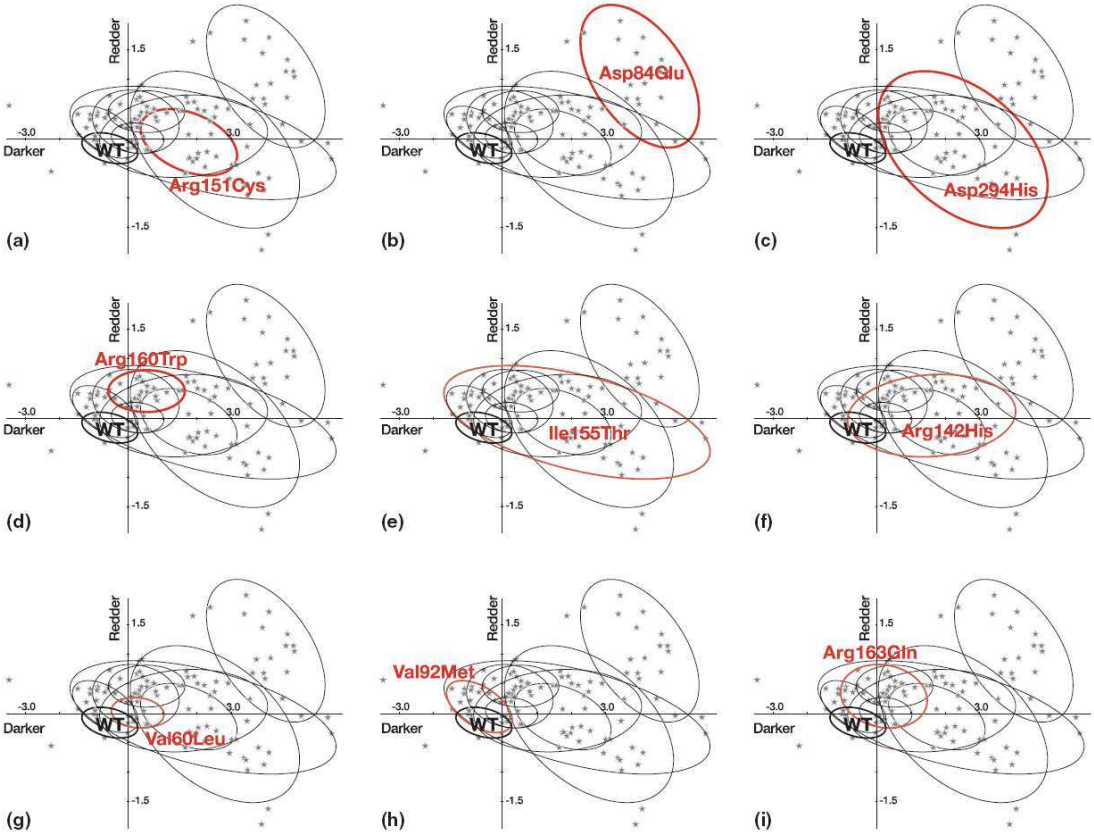

Figure 5. First principal plan of PCA: Difference in skin color between the most common *MC1R* gene variants. The 95% confidence ellipses describe the uncertainty of the location of each variant on the map. The points within the ellipses correspond to the centers of gravity of the replicates generated by the bootstrap method. (a) Arg151Cys *versus* WT. (b) Asp84Glu *versus* WT. (c) Asp294His *versus* WT. (d) Arg160Trp *versus* WT. (e) Ile155Thr *versus* WT. (f) Arg142His *versus* WT. (g) Val60Leu *versus* WT. (h) Val92Met *versus* WT. (i) Arg163Gln *versus* WT.

The effect of *MC1R* polymorphism on skin color has been investigated less extensively than its effect on hair color, in particularly using objective measures such as spectroscopy. In our study, skin color was assessed both with a categorical clinical rating scale (skin color in winter: whitish, light or darkish) and with reflectance measurements. Skin color in winter was found to be associated with the Arg160Trp and Arg151Cys polymorphisms, and a nonsignificant trend was found for the Asp294His variant. In an Australian study conducted in the general population of southeast Queensland (14), the effect of these polymorphisms on skin color (assessed as fair/pale, medium or olive/dark on the inner upper left arm) was studied. The authors found that all polymorphisms, except the Arg142His variant, increased the risk of having fair/pale skin, with the highest risk identified for the carriers of the Asp84Glu polymorphism, followed by Asp294His, Arg151Cys, Arg160Trp, Val92Met, Ile155Thr, Arg163Gln and Val60Leu. Both in this Australian study and in our own, the Arg160Trp, Arg151Cys and Asp294His polymorphisms were associated with a "fair/pale" or "whitish" skin, although this was not the case for the Arg142His polymorphism, which is known to have a marked influence on red hair color (12).

With respect to the skin color measured by reflectance spectroscopy, women carrying the Arg151Cys, Asp294His and Asp84Glu polymorphisms presented a lighter skin than WT homozygous women. Moreover, women carrying the Arg160Trp polymorphism had a significantly higher reflectance in the red region of the visible spectrum (600–700 nm). This region is known to be the most suitable for quantifying melanin levels. Indeed, based on the specific absorption spectra of the chromophores, absorption due to melanin outweighs absorption due to hemoglobin in blood in the red region (19). Therefore, women carrying one of these four polymorphisms have probably significantly lower amounts of functional melanin in the skin than WT homozygous women. This decrement could be explained by a lower concentration of melanin, impaired melanin quality or a suboptimal spatial distribution of melanin in the skin. This effect is most prominent for women carrying the Asp84Glu polymorphism. In contrast, no significant difference in skin reflectance was observed for the two other major variants (Arg142His and Ile155Thr) and for the minor variants (Val92Met, Val60Leu and Arg163Gln). In a recent study (16), the functional impact of these nine *MC1R* polymorphisms was evaluated *in vitro*, and these results compared to the skin color measured by reflectance on the inner arm (reflectance at 650 nm). The authors reported a dominant negative effect of all major diminished function variants (R variants), except for the Arg142His polymorphism, on the activity of the wild-type receptor. Two minor diminished function variants (Val60Leu and Arg163Gln) were also found to have a negative effect on the activity of the wild-type receptor, even if this effect was smaller than those observed with the R variants. In addition, the authors found that skin color was significantly lighter in individuals heterozygous for the Asp84Glu, Arg151Cys, Arg160Trp, Asp294His and Val92Met variants. Aside from the Val92Met polymorphism, our results are consistent with those reported by Beaumont *et al*. (16). In both studies, no significant association with fair skin color was found for the Arg142His variant. This finding is consistent with the absence of a dominant negative effect of this polymorphism on the activity of the wild-type receptor. In addition, the absence of a significant effect for the Val60Leu and Arg163Gln polymorphisms could be explained by a limited impact on the activity of the wild-type receptor, and supports the classification of these polymorphisms as minor diminished function variants.

As expected, in our study the results obtained using reflectance measurements of the skin are consistent with those based on the clinical assessment. The reflectance measurements also provide an objective estimation of the impact of each polymorphism on the skin color and highlight the major influence of the Asp84Glu polymorphism. Moreover, our findings indicate associations between *MC1R* polymorphisms and the risk of experiencing sunburn and of having freckles independently of skin color. These results suggest that the skin color measured with spectroscopy and the *MC1R* genotype are probably independent risk factors for the skin sensitivity to UV-induced DNA damage, as previously reported for melanoma risk factor studies (11,12,15,33). Therefore, a major application of our findings is the possible identification of individuals with a higher risk of sunburn, of photoaging and even skin cancer occurrence. However, further studies are needed to confirm our findings, especially for the polymorphisms with a low allelic frequency, and also to investigate the individual dose effect of each *MC1R* variant.

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Annexe 10. Article sur variants du gène MC1R et photo-vieillissement de la peau.

Functional MC1R gene variants are associated with an increased risk for severe photoaging of facial skin

Elfakir A, Ezzedine K, Latreille J, Ambroisine L, Jdid R, Galan P, Hercberg S, Gruber F, Malvy D, Tschachler E, Guinot C

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Functional MC1R-Gene Variants Are Associated with Increased Risk for Severe Photoaging of Facial Skin

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The objective of this study was to assess the association between melanocortin-1 receptor (MC1R) variants and the severity of facial skin photoaging. The study population comprised 530 middle-aged French women. A trained dermatologist graded the severity of facial skin photoaging from photographs using a global scale. Logistic regressions were performed to assess the influence of MC1R polymorphisms on severe photoaging with adjustment for possible confounders (demographic and phenotypic data and sun exposure intensity). Among the fifteen MC1R variants identified, the nine most common were V60L, V92M, R151C, R160W, R163Q, R142H, D294H, D84E, and I155T. One hundred and eighty-five individuals (35%) were WT homozygotes, 261 (49%) had one common variant, 78 (15%) had two common variants, and six (1%) had at least one rare variant. After adjustment for possible confounders, the presence of two common variants was already a risk factor for severe photoaging (AOR (95% confidence interval): 2.33 (1.17–4.63)). This risk reached 5.61 (1.43–21.96) when two major diminished-function variants were present. Surprisingly, the minor variant, V92M, was associated with increased risk of photoaging (2.57 (1.23–5.35)). Our results suggest that genetic variations of MC1R are important determinants for severe photoaging.

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INTRODUCTION

Aging of the skin is influenced both by intrinsic factors, such as chronological age, and by extrinsic or environmental factors, such as chronic UV exposure and smoking (Malvy *et al.*, 2000; Yaar and Gilchrest, 2007). Skin photoaging is defined as premature aging of the skin due to chronic sun exposure and presents characteristic morphological changes to both the epidermal and the dermal compartments (Rabe *et al.*, 2006; Yaar and Gilchrest, 2007). A number of phenotypic features influence the degree of photoaging, notably skin color (Kligman and Kligman, 1999; Malvy *et al.*, 2000) and skin phototype. Individuals with dark phototypes (III–IV) commonly exhibit more ''hypertrophic responses'' such as deep wrinkling, coarseness, and lentigines, whereas fair phototype individuals (I–II) generally show fewer wrinkles with epidermal atrophy, focal depigmentation, as well as dysplastic changes, such as actinic keratosis, non-melanoma, and melanoma skin cancers (Fitzpatrick, 1988; Yaar and Gilchrest, 2007).

Variation in human skin and hair color is due to differences in the absolute amounts and the relative proportions of black photoprotective eumelanin and red pheomelanin (Rees, 2003). During the last decade, a number of genes regulating pigmentation have been identified. Polymorphisms in the melanocortin-1 receptor (MC1R) gene (OMIM #155555) have been demonstrated to be major determinants of hair and skin color (Valverde *et al.*, 1995). The MC1R gene maps to chromosome 16q24.3 and encodes a seven-pass, transmembrane, G-protein-coupled receptor consisting of 317 amino acids (Chhajlani, 1996). Signaling through the receptor by α-melanocyte-stimulating hormone (α-MSH) activates adenylate cyclase, leading to increase in intracellular cAMP and transcriptional activation of a number of genes regulating melanin biosynthesis, notably members of the tyrosinase family (García-Borron et al., 2005). MC1R is highly polymorphic in Caucasian populations, and to date over 75 MC1R alleles have been identified (Wong and Rees, 2005; Beaumont *et al.*, 2007).

Numerous studies have established the association between MC1R mutations and phenotypic features such as red hair, fair skin, and freckles (Valverde *et al.*, 1995; Box *et al.*, 1997; Smith *et al.*, 1998; Flanagan *et al.*, 2000; Bastiaens *et al.*, 2001a; Duffy *et al.*, 2004), as well as links between

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Abbreviations: α-MSH, α-melanocyte stimulating hormone; AOR, adjusted odds ratio; BMI, body mass index; MC1R, melanocortin-1 receptor; Var, any MC1R variant; var, minor diminished-function MC1R variants; VAR, major diminished-function MC1R variants; WT, wild type

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some MC1R variants and solar lentigines (Bastiaens *et al.*, 2001a; Motokawa *et al.*, 2007). Furthermore, MC1R polymorphisms have been found to be related to sun sensitivity and low tanning ability in response to UV radiation, independently of skin color (Healy *et al.*, 2000). In addition, several studies have highlighted the association of MC1R variants with melanoma risk (Valverde *et al.*, 1996; Palmer *et al.*, 2000; Kennedy *et al.*, 2001; Matichard *et al.*, 2004; Stratigos *et al.*, 2006) and non-melanoma skin cancers again even after adjustment for pigmentation types (Box *et al.*, 2001; Bastiaens *et al.*, 2001b; Liboutet *et al.*, 2006). Since no study of the associations of MC1R polymorphisms and global photoaging has been reported to date, we set out to investigate the influence of MC1R variants on the severity of overall facial photoaging combining pigmentation irregularities, sagging, and wrinkles.

RESULTS

Characteristics of the study population

The analysis involved 530 women. The clinical and demographic features of the study population, and the association of these variables with the extent of facial skin photoaging are presented in Table 1. As expected, the extent of skin photoaging was strongly linked to age. By contrast, after adjustment for age, no significant association was observed for other variables, although there were trends for a higher risk of photoaging associated with light hair color, presence of freckles and high intensity of lifetime sun exposure, and for a lower risk in women with obesity.

Frequency of MC1R variants

Overall, 15 MC1R-gene variants were detected, including two synonymous changes (Table 2). The 13 non-synonymous variants all represented amino-acid substitutions, with no deletions or insertions being detected. Among these 13 MC1R variants, four variants were rare (S83O, T95M, P256S, V265I), with a frequency $<0.5\%$ of total alleles. Women carrying these MC1R variants ($n=6$) were excluded from further analyses. Of the remaining 524 subjects, 185 women (35%) were wild type (WT/WT) with no variant or synonymous variant allele, and 261 (50%) were heterozygous (WT/Var), bearing one of the nine most common non-synonymous MC1R gene variants (Table 2). In addition, 60 women (11%) were compound heterozygous for either minor (var1/var2; $n=19$, 4%) major (VAR1/VAR2; $n=10$, 2%), or mixed (VAR/var; $n=31$, 6%) variants, and 20 (4%) were homozygous for either major (5) or minor (15) variants (Table 2). The allelic frequencies observed here are consistent with those of previous studies of French populations (Matichard *et al.*, 2004; Liboutet *et al.*, 2006).

MC1R variants and skin photoaging

The associations between MC1R-gene variants and photoaging are presented in Table 3. After adjustment for age, carriers of variant polymorphisms on both copies of the MC1R gene presented a significantly increased risk of severe photoaging (adjusted odds ratio (AOR) (95% confidence interval) for Var/Var, 2.45 (1.30–4.61), as compared with WT/WT). This risk was considerably higher in the carriers of two major variants (VAR/VAR) (6.43 (1.76–23.47)), whereas in carriers of two minor variants (var/var) only a non-significant trend toward increased risk of photoaging was observed (Table 3). The same was true for heterozygous carriers (var/WT, VAR/WT, or VAR/var). When the different common polymorphisms were analyzed individually, three of the six major variants, namely R151C, R142H, and D84E, demonstrated significant association with photoaging, with AORs of 2.00 (1.02–3.92), 4.27 (1.26–14.52), and 16.10 (2.34–110.55), respectively. The R160W variant showed a similar trend, without reaching significance. Among the minor variants, only V92M showed significant association with photoaging (2.41 (1.22–4.75)) (Table 3). The risk associated with V92M was independent of the presence of another major or minor variant (not shown).

Multivariate logistic regression analyses were performed to take into account potential confounding factors such as smoking habits, body mass index (BMI), skin phototype, and age. In these analyses, presence of MC1R variants was retained as an independent risk factor for photoaging (Supplementary Table S1). The AOR values obtained in the multivariate models after adjustment for all potential confounding factors (Supplementary Table S1) were comparable to those obtained in the bivariate models adjusted only for age (Table 3).

DISCUSSION

Activation of the G-protein-coupled receptor, MC1R, by α-MSH stimulates the cAMP-signaling pathway in melanocytes and upregulates the synthesis of melanin through induction of the microphthalmia transcription factor. This leads to a switch from pheomelanin synthesis to synthesis of eumelanin, which is darker and thus more photoprotective (García-Borron *et al.*, 2005). The MC1R gene is highly polymorphic and several loss-of-function variants have been identified, which are associated with a red hair or pale skin phenotype, as well as with increased sun sensitivity (Rees, 2003). In addition, for several of these MC1R variants, associations with occurrence of non-melanoma skin cancer and melanoma have been found (Bastiaens *et al.*, 2001b; Duffy *et al.*, 2004; Healy, 2004). Since eumelanin plays a major role in protecting the skin against UV-B irradiation, we have studied here whether MC1R polymorphisms have an impact on the severity of global photoaging of the face.

In our sample of 524 French women, carriers of two loss-of-function MC1R variants (R151C, R160W, R142H, D294H, I155T, and D84E) had a more than sixfold increased risk for severe photoaging as compared with carriers of two WT alleles. Of the major variants, R142H, R151C, and D84E contributed the most to the risk; however, carriers of one single loss-of-function variant showed only a trend for association with photoaging, which did not reach significance.

Reduced physical protection of the skin due to disturbed MC1R signaling and decreased eumelanin synthesis (García-Borron *et al.*, 2005) would be the most obvious explanation for the increased risk for severe photoaging observed in the

Table 1. Distribution in photoaging severity expressed in frequency (percentage) and risk of severe photoaging related to each characteristic of the studied population

	Photoaging				
	Grades I–III (*n*=322)	Grades IV–VI (*n*=208)	*P*[1]	AOR[2]	(95% CI)[3]
Age group					
44–49	60 (18.6)	4 (1.9)	<0.001	1	/
50–54	82 (25.5)	20 (9.6)	0.02	3.66	(1.19–11.25)
55–59	115 (35.7)	45 (21.6)	<0.002	5.87	(2.02–17.09)
60–64	45 (14.0)	68 (32.6)	<0.001	22.66	(7.70–66.72)
65–70	20 (6.2)	71 (34.1)	<0.001	53.24	(17.25–164.32)
BMI classification					
Normal	217 (67.4)	135 (64.9)	0.16	1	/
Overweight	72 (22.4)	60 (28.8)	0.60	1.13	(0.71–1.80)
Obese	33 (10.2)	13 (6.3)	0.09	0.50	(0.23–1.10)
Smoking habits					
Non-smoker	178 (55.3)	122 (58.7)	0.35	1	/
Former smoker	105 (32.6)	65 (31.2)	0.39	1.22	(0.78–1.91)
Current smoker	39 (12.1)	21 (10.1)	0.18	1.53	(0.79 –2.97)
Menopausal status and HRT intake					
Not menopausal	99 (30.7)	15 (7.2)	0.74	1	/
Menopausal with HRT	159 (49.4)	122 (58.7)	0.45	0.75	(0.35–1.59)
Menopausal without HRT	64 (19.9)	71 (34.1)	0.61	0.81	(0.35–1.86)
Eye color					
Dark	101 (31.8)	64 (30.8)	0.69	1	/
Light	217 (68.2)	144 (69.2)		1.09	(0.71–1.69)
Hair color[4]					
Black	11 (3.4)	8 (3.8)	0.13	1	/
Dark brown	78 (24.5)	38 (18.3)			
Light brown	164 (51.6)	116 (55.8)		1.43	(0.90–2.29)
Blond	59 (18.6)	36 (17.3)			
Red	6 (1.9)	10 (4.8)			
Skin color					
Dark	70 (22.0)	53 (25.5)	0.91	1	/
Fair	248 (78.0)	155 (74.5)		0.97	(0.61–1.55)
Freckles					
None	193 (60.7)	114 (54.8)	0.053	1	/
Present	125 (39.3)	94 (45.2)		1.49	(0.99–2.25)
Suntan intensity					
None/slight/light	183 (58.5)	131 (63.3)	0.38	1	/
Dark/very dark	130 (41.5)	76 (36.7)		1.20	(0.80–1.81)

Table 1 continued on the following page

Table 1. Continued

	Photoaging				
	Grades I–III (*n*=322)	Grades IV–VI (*n*=208)	*P*[1]	AOR[2]	(95% CI)[3]
Sunburn event frequency					
None/rare	219 (70.0)	154 (74.4)	0.70	1	/
Frequent/constant	94 (30.0)	53 (25.6)		0.91	(0.58–1.44)
Skin phototype[5]					
IV	46 (14.3)	34 (16.3)	0.22	1	/
III	123 (38.2)	72 (34.6)			
II	124 (38.5)	79 (38.0)		1.29	(0.86–1.93)
I	29 (9.0)	23 (11.1)			
Lifetime sun exposure intensity					
Low–moderate	212 (66.7)	132 (63.5)	0.13	1	/
High	106 (33.3)	76 (36.5)		1.38	(0.91–2.11)

Abbreviations: AOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; HRT, hormone replacement therapy.
[1]Probability value of Wald test.
[2]AORs are adjusted for age, except for age group.
[3]95% CI.
[4]Hair color was grouped into two categories (light vs. dark) for calculation of AORs.
[5]Skin phototype was grouped into two categories (I–II vs. III–IV) for calculation of AORs.

Table 2. Frequency (percentage) of MC1R-gene variants[1]

	MC1R-gene variants												
		Major diminished-function						Minor diminished-function			Rare	Variant	Genotype
	WT	R151C	R160W	D294H	R142H	I155T	D84E	V60L	V92M	R163Q	variants	frequency (*n*=1,060)	frequency (*n*=530)
WT[2]	185	38	21	11	13	3	4	94	53	24	4	635 (59.9)	450 (84.9)
Major diminished-function variants													
R151C	38	3	0	0	3	2	1	7	3	2	0	62 (5.8)	59 (11.1)
R160W	21	0	2	2	0	1	0	4	3	1	0	36 (3.4)	34 (6.4)
D294H	11	0	2	0	0	0	1	3	2	0	0	19 (1.8)	19 (3.6)
R142H	13	3	0	0	0	0	0	2	0	0	0	18 (1.7)	18 (3.4)
I155T	3	2	1	0	0	0	0	3	1	0	0	10 (0.9)	10 (1.9)
D84E	4	1	0	1	0	0	0	0	0	0	0	6 (0.6)	6 (1.1)
Minor diminished-function variants													
V60L	94	7	4	3	2	3	0	11	7	7	1	150 (14.2)	139 (26.2)
V92M	53	3	3	2	0	1	0	7	2	3	0	76 (7.2)	74 (14.0)
R163Q	24	2	1	0	0	0	0	7	3	2	1	42 (4.0)	40 (7.5)
Rare variants[3]	4	0	0	0	0	0	0	1	0	1	0	6 (0.6)	6 (1.1)

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; MC1R, melanocortin-1 receptor; WT, wild type.
[1]The rows and columns represent the MC1R genotype on each allele.
[2]The WT category includes both WT and synonymous variants (A166A and Q233Q), which are mutations in DNA sequence that do not modify the amino-acid sequence of the protein.
[3]Rare variants correspond to S83P, T95 M, P256S, and V265I.

Table 3. Distribution in photoaging severity expressed in frequency (percentage) and risk of severe photoaging according to MC1R polymorphisms

	Photoaging				
	Grades I–III (*n*=317)	Grades IV–VI (*n*=207)	*P*[1]	AOR[2]	(95% CI)[3]
WT/WT	119 (37.5)	66 (31.9)	—	1	—
MC1R genotype			0.02		
WT/Var	157 (49.5)	104 (50.2)	0.18	1.36	(0.87–2.13)
Var/Var	41 (12.9)	37 (17.9)	<0.01	2.45	(1.30–4.61)
Major/minor variant classification			0.03		
Only minor variant(s)[4]	122 (38.5)	81 (39.1)	0.17	1.40	(0.87–2.25)
One major variant[5]	71 (22.4)	50 (24.2)	0.11	1.56	(0.90–2.68)
Two major variants[6]	5 (1.6)	10 (4.8)	<0.01	6.43	(1.76–23.47)
Minor diminished-function variants[7]					
V60L	87 (27.4)	51 (24.6)	0.57	1.18	(0.67–2.07)
V92M	38 (12.0)	36 (17.4)	0.01	2.41	(1.22–4.75)
R163Q	25 (7.9)	14 (6.8)	0.33	1.54	(0.64–3.70)
Major diminished-function variants[8]					
R151C	32 (10.1)	27 (13.0)	0.04	2.00	(1.02–3.92)
R160W	17 (5.4)	17 (8.2)	0.11	2.02	(0.85–4.81)
D294H	13 (4.1)	6 (2.9)	0.61	1.35	(0.42–4.29)
R142H	7 (2.2)	11 (5.3)	0.02	4.27	(1.26–14.52)
I155T	6 (1.9)	4 (1.9)	0.96	1.04	(0.20–5.37)
D84E	3 (0.9)	3 (1.4)	<0.01	16.10	(2.34–110.55)

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; MC1R, melanocortin-1 receptor; var, minor diminished-function MC1R variant; Var, any MC1R variant; WT, wild type.
[1]Probability value of Wald test.
[2]AORs are adjusted for age.
[3]95% CI.
[4]var1/WT, var1/var2, and var1/var1 are pooled together.
[5]VAR/WT and VAR/var are pooled together.
[6]VAR1/VAR1 and VAR1/VAR2 are pooled together.
[7]var1/WT, var1/var1, and var1/var2 are pooled together.
[8]VAR1/WT, VAR1/VAR1, and VAR1/VAR2 are pooled together.

carriers of these loss-of-function MC1R variants. However, MC1R polymorphisms still remained a significant risk factor for severe skin photoaging even after adjustment for potential confounding from skin color and lifetime sun exposure. In addition, loss-of-function variants are associated with multiple features related to skin phototype (Healy *et al.*, 2000; Latreille *et al.*, 2009). Taken together, this suggests that mechanisms other than the diminished protective effect of natural pigmentation may contribute to heightened risk of photoaging. Similar observations have been made by Bastiaens *et al.* (2001b) when studying non-melanoma skin cancer. They reported that MC1R variants are determinants for increased risk independently of the fair skin and red hair phenotype. A possible mechanistic explanation for our finding could be that, in contrast to eumelanin, which serves as a scavenger for reactive oxygen species, pheomelanin is a potential source of reactive oxygen species (Bustamante *et al.*, 1993; Abdel-Malek *et al.*, 2008). A shift in the balance of melanin synthesis toward pheomelanin in the carriers of functional mutations of MC1R might thus increase the risk of cell and tissue damage through generation of reactive oxygen species. These mechanisms are very likely to affect both the function and survival of melanocytes, and could, thus, potentially contribute to the pigment changes associated with photoaging. It remains to be investigated whether changes in the pattern of melanin synthesis may contribute to other aspects of photoaging, particularly with respect to the dermal compartments (Rabe *et al.*, 2006; Yaar and Gilchrest, 2007). The finding that both eumelanin and pheomelanin can be released from melanocytes and can be detected in urine

and serum of healthy individuals, suggests that this might be plausible (Wakamatsu *et al.*, 2006). Apart from regulation of pigmentation in melanocytes, α-MSH, the principal endogenous activator of MC1R, exerts a wide variety of biological effects in other cell types, including keratinocytes and fibroblasts (Kiss *et al.*, 1995; Böhm *et al.*, 2006). Importantly, α-MSH is able to protect cells from UV-induced apoptosis, probably by enhancing DNA repair (Böhm *et al.*, 2005a). Indeed, it has been demonstrated for melanocytes that loss-of-function mutations of MC1R were associated with higher cyclobutane pyrimidine dimer levels, impaired nucleotide-excision repair, and increased susceptiblity to apoptosis after UV irradiation (Kadekaro *et al.*, 2005; Hauser *et al.*, 2006). It is, thus, tempting to speculate that loss-of-function MC1R variants may cause increased sun sensitivity and accumulation of sun damage not only by impaired repair capacity in melanocytes themselves, but also in other cell types within the skin. In this respect, both increased sun sensitivity (Healy *et al.*, 2000) and occurrence of skin neoplasms cannot be explained completely by the influence of MC1R functional variants on skin type (Kennedy *et al.*, 2001; Bastiaens *et al.*, 2001b). Finally, α-MSH has been reported to exert immunmodulatory activities (Böhm *et al.*, 2006). It is, therefore, conceivable that altered immunoregulatory activities of α-MSH in individuals with MC1R variants might contribute to the inflammation regularly observed in photoaged skin (Rabe *et al.*, 2006; Yaar and Gilchrest, 2007). Although an effect of MC1R genotype on the suppression of antigen-activated lymphocyte proliferation by α-MSH has been excluded (Cooper *et al.*, 2005), immunmodulatory effects on other cell types remain to be determined (Böhm *et al.*, 2005b, 2006), in particular in the context of UV exposure (April and Barsh, 2007). Nonetheless, it should be noted that, despite the presence of MC1R mRNA (Roberts *et al.*, 2007), MC1R protein has not been unequivocally demonstrated in cell types other than those of melanocytic lineage. For this reason, the potential contributions of these alternative functions of MC1R should be considered as hypothetical.

In addition to the effect observed with the major loss-of-function MC1R variants, an association was found between severe photoaging and one of the minor variants (V92M), this link being independent of the presence of another minor or major MC1R variant. This finding is surprising since presence of this variant does not reduce MC1R function *in vitro*, and this variant was considered by some as a ''pseudo-allele'' with no significant effect on eumelanin synthesis (Wakamatsu *et al.*, 2006). However, associations of this variant with development of solar lentigines in Japanese (Motokawa *et al.*, 2007), and development of basal cell carcinoma in French populations (Liboutet *et al.*, 2006) indicate that the reduced binding capacity of α-MSH to the V92M MC1R variant might have biological relevance *in vivo*. An alternative possibility would be that this variant is linked to a not yet identified mutation with functional consequences in a distant gene.

Our study has certain limitations. Although the sample size of our study was large enough to examine the association of each of the nine most common MC1R variants with photoaging, the small number of redheads did not provide adequate statistical power to perform a specific analysis for this subgroup. In addition, assessment of certain confounding factors, such as ''natural hair color at the age of 20'' and ''skin color without tanning'', relied on self-reporting and ''lifetime sun exposure intensity'' on self-evaluation, an approach that could introduce reporting bias. Finally, in our study, as in any other allelic association study, the associations between MCR1 variants and photoaging might also reflect associations with other markers due to linkage disequilibrium.

In conclusion, our study conducted with a large sample of French middle-aged women showed that MC1R-gene variants, particularly loss-of-function variants, are associated with strongly increased risk of severe photoaging independently of age, BMI, skin color, skin phototype, menopausal status, smoking habits, and lifetime sun exposure. These findings suggest that the MC1R gene plays a major role in skin photoaging.

MATERIALS AND METHODS

Study design and population

This cross-sectional study was performed in the context of the SU.VI.MAX cohort (SUpplémentation en VItamines et Minéraux AntioXydants—antioxidant vitamin and mineral supplementation), a longitudinal cohort study of French middle-aged adult volunteers, which focused on the relationships between nutrition and the incidence of a number of chronic diseases that are frequent in industrialized countries (Hercberg *et al.*, 1998, 2004). The SU.VI.MAX study protocol was approved by The Hospital Medicals Ethics Committee of Paris-Cochin (CCPPRB no. 706) and the ''Comité National Informatique et Liberté'' (CNIL no. 334641). The study was conducted according to Declaration of Helsinski Principles. All participants provided written, informed consent. The SU.VI.MAX cohort included 13,017 subjects who were representative of the French adult, middle-aged population for most sociodemographic features (Hercberg *et al.*, 1998). The electronic data capture and storage system provided an opportunity to conduct cross-sectional surveys.

This study was conducted in autumn/winter 2002–2003 with a sample of 570 middle-aged women (age range 44–70 years) who lived in Paris and had provided informed consent. Inclusion criteria were Caucasian origin, no known dermatological disorder, and no history of facial antiaging procedures such as injection of filling agents, laser intervention, or plastic surgery. Moreover, they were expected to follow specific skin care instructions, notably application of detergents or cosmetics to the face was not authorized for at least 12 hours before the study visit. On the day of the visit, they were first asked to complete a standardized questionnaire. Three standardized, high-resolution digital images (2008 × 3032 pixels) of the face were taken for each participant (one frontal view of the face and one of each profile) using a Kodak DCS 760 digital camera with a 105 mm camera lens (Kodak, Paris, France). The camera was mounted on a monopod and a specifically developed chair was used to allow standardized positions of the camera with respect to the face. Lighting conditions were standardized by means of two symmetrical lamps, which provided a continuous daylight spectrum, placed at 45 degrees to each side of the face.

Of the 570 volunteers who participated in the study, 28 were excluded from the analysis because they did not fulfill at least one inclusion criterion: 18 had a history of recent antiaging invasive procedures and 10 were non-Caucasian. Besides, MC1R genotyping was not successful for 12 individuals because either the DNA sample or PCR product could not be obtained. The final study population was, thus, composed of 530 individuals.

Assessment of general and phenotypic data

Data on age (in years), height (in meters), weight (in kg), smoking habits (never, former, current), and menopausal status (non-menopausal, menopausal with hormone replacement therapy, menopausal without hormone replacement therapy) were obtained on the day of the visit from a medical questionnaire during a standardized interview performed by trained interviewers. Natural hair color at the age of 20 was ascertained and classified as follows: red, blond, light brown, dark brown, or black. Eye color was recorded as brown/black, hazel, green, or blue/gray. Skin color in the winter was recorded as fair or dark; freckles as none or present; sunburn event frequency as none, rare, frequent, or constant; and suntan intensity as none, slight, light, dark, or very dark. Skin phototype was recorded according to Fitzpatrick's classification (Fitzpatrick, 1988) as follows: (''always burn, never tan'': skin type-I; ''always burn, then tan'': skin type-II; ''always tan, sometimes burn'': skin type-III; and ''always tan, never burn'': skin type-IV). BMI ($kg\,m^{-2}$) was calculated as weight divided by square of the height. Moreover, each participant was asked to complete a self-administered questionnaire relating to lifetime sun exposure behavior. From this questionnaire, a score quantifying lifetime sun exposure intensity was calculated. This score is a linear combination of five items (voluntary sun exposure, exposure of the body and face, exposure during the hottest hours of the day, intensity of self-reported lifetime sun exposure, and importance of sunbathing), which were weighted according to their contribution to the score. The design, validation, and description of this score have been described previously elsewhere (Guinot *et al.*, 2001).

Assessment of severity of photoaging

Each set of facial photographs was examined by a trained dermatologist to rate the severity of facial skin photoaging, using Larnier's six-grade ordinal scale of photodamage (Larnier *et al.*, 1994). Each grade is depicted by three reference photographs that illustrate the diversity and range of pigmentation disorders, wrinkling, and looseness.

Detection of MC1R-gene variants and sequence analysis

Blood samples were collected on the day of visit, lysed in NASBA lysis buffer (Organon Teknika BV, Boxtel, the Netherlands), and stored at −80 °C until assay. Genomic DNA was isolated by silica-based extraction as previously described (Boom *et al.*, 1990). PCR analysis was performed using 0.3 μg of genomic DNA in 25 μl of final reaction buffer, as previously described (Eckhart *et al.*, 2000), using a GeneAmp PCR System 2400 thermal cycler (Perkin-Elmer, Branchburg, NJ). The primers used were MC1Ramp2f: 5′-CCTGG AGGTGTCCATCTCTG-3′ and MC1Ramp2r: 5′-TGTGGAAGGCG TAGATGAGG-3′. They yielded an amplicon that spans nucleotides 1,546,388–1,547,187 in the NCBI genomic reference sequence NT_010542.15, and correspond to nucleotides 746–1,545 of the mRNA reference sequence for MC1R (NM_002386.2). The resulting PCR products were sequenced by MWG-BIOTECH AG (Ebersberg, Germany) by the Comfort Read procedure and the primers used were MC1Ramp1f: 5′-ATCTCTGACGGGCTCTTCCT-3′ and MC1Ramp1r: 5′-CGTAGATGAGGGGGTCGAT-3′, which span nucleotides 759–1,536 of the mRNA reference sequence. Codons 60–265 were all covered by PCR and sequencing with these primers. Codon 294 was genotyped by ''Hybridisation Probe'' technology (Bernard *et al.*, 1998). Briefly, genomic DNA was amplified using primers MC1R_294His_F 5′-CTCACACTCATCGTCC-3′ and MC1R_294His_R 5′-GCACACTTAAAGCCGC-3′ in the presence of sensor probe ''MC1R_294His_sens'' 5′-TGCAATGCCATCATCCA CCC–fluorescein and anchor probe ''MC1R_294His_anch'' LCRed640–TCATCTACGCCTTCCACAGCCAG–phosphate. After amplification, the reaction temperature was gradually increased from 40 to 80 °C and fluorescence in the LCRed640 Channel was continuously measured. A melting-curve analysis was performed on the fluorescence signals resulting from fluorescence resonance energy transfer between the anchor and sensor probe. The sensor probe was designed to be a match to the C-variant (corresponding to His in the amino-acid sequence). Melting temperatures of 67 °C for the match (C, His allele) and 59 °C for the mismatch (G, Asp allele) allowed discrimination of the alleles and were validated by sequencing. Data obtained by sequencing were analyzed at the Department of Dermatology, University of Edinburgh (UK) and at the Medical University of Vienna (Austria).

Statistical analyses

For the logistic analyses, skin photoaging severity was categorized as either ''mild to moderate'' (grades I–III on the Larnier scale, reference category) or ''severe'' (grades IV–VI). Similarly, eye color was categorized as light (blue/grey/green/hazel) or dark (brown/black), and the reported hair color at 20 was grouped as light (red/blond/light brown) or dark (dark brown/black). BMI was categorized as underweight–normal ($BMI < 25\,kg\,m^{-2}$), overweight ($25 \leqslant BMI < 30\,kg\,m^{-2}$), or obese ($BMI \geqslant 30\,kg\,m^{-2}$), according to WHO recommendations (WHO, 1995). The lifetime sun exposure intensity score was categorized into three equal-size groups (about 1/3 of the total sample in each group) using the quantile method (Milton, 1998). These corresponded to low (t1), moderate (t2), and high (t3) exposure. The lifetime sun exposure intensity score was categorized as either ''low-to-moderate exposure'' (score $<$ t2) or ''high exposure'' (score $\geqslant$ t2), to focus on the highest levels of lifetime sun exposure.

The presence of MC1R variants was defined by the presence of non-synonymous variants, that is to say nucleotide mutation in the DNA sequence of the gene, which led to a change in the amino-acid sequence of the protein. The MC1R polymorphisms investigated were the nine most common variants (V60L, V92M, R151C, R160W, R163Q, R142H, D294H, I155T, and D84E), which corresponded also to those previously described as diminished-function mutations (Aasland *et al.*, 2000; Wong and Rees, 2005; Beaumont *et al.*, 2007). The link between MC1R-gene variants and skin photoaging severity was analyzed by first pooling the nine most common non-synonymous MC1R-gene variants (Var), to study a possible gene dosage effect of MC1R (WT/WT, Var/WT, and Var/Var). Then, the major diminished-function variants (VAR) and minor diminished-function variants (var) were considered separately (WT/WT, only

minor variant(s), one major variant, and two major variants). The major diminished-function variants group included those variants that have been shown previously to cause significant impairment in receptor function and are strongly associated with red hair, fair skin, and skin cancer, namely, D84E, R142H, R151C, R160W, D294E, and I155T (Wong and Rees, 2005; Beaumont *et al.*, 2007). All remaining non-synonymous variants were considered minor diminished-function variants. Finally, the individual effect of each most common variant was evaluated (WT homozygotes, at least one specific variant).

Statistical analyses were performed using SAS software release 9.1.3 (SAS Institute, Cary, NC). The associations between severity of expression of skin photoaging and clinical and demographic variables were evaluated after adjustment for age using bivariate logistic regression analyses and AORs generated with their 95% confidence intervals. Next, bivariate logistic regression analyses with adjustment for age were performed to evaluate the association between severity of skin photoaging and the presence of MC1R-gene variants, and AORs were once again calculated. Finally, multiple logistic regression analyses were performed to take into account the possible confounding factors on the association between MC1R-gene variants and photoaging, and AORs were calculated with their 95% confidence intervals. All significance levels reported were two-sided with a threshold of <0.05 considered statistically significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Table S1. Risk of severe photoaging according to contributive MC1R polymorphisms, adjusted on all the variables

	Model 1 [4]			Model 2 [4]		
	P [1]	AOR [2]	[95% CI] [3]	P [1]	AOR [2]	[95% CI] [3]
MC1R genotype	0.05			-	-	-
WT/Var vs WT/WT	0.36	1.25	[0.78-2.00]	-	-	-
Var/Var vs WT/WT	0.02	2.33	[1.17-4.63]	-	-	-
Major/minor variant classification	-	-	-	0.09		
Only minor variant(s) vs. WT/WT	-	-	-	0.24	1.35	[0.82-2.20]
One major variant vs. WT/WT	-	-	-	0.30	1.37	[0.76-2.48]
Two major variants vs. WT/WT	-	-	-	0.01	5.61	[1.43-21.96]
Age (years)	<0.001	1.22	[1.16-1.28]	<0.001	1.22	[1.17-1.28]
BMI classification	0.12			0.14		
Overweight vs. normal	0.68	1.11	[0.68-1.79]	0.62	1.13	[0.70-1.84]
Obese vs. normal	0.06	0.45	[0.20-1.02]	0.07	0.48	[0.21-1.07]
Smoking habits	0.56			0.59		
Former smoker vs. no smoker	0.55	1.15	[0.72-1.84]	0.57	1.15	[0.72-1.83]
Current smoker vs. no smoker	0.31	1.44	[0.71-2.91]	0.33	1.42	[0.70-2.85]
Menopausal status and HRT	0.39			0.40		
Menopausal with HRT vs. no menopausal	0.89	0.94	[0.40-2.20]	0.65	0.82	[0.35-1.91]
Menopausal without HRT vs. no menopausal	0.56	1.30	[0.53-3.18]	0.79	1.13	[0.47-2.73]
Eye colour	0.79			0.80		
Light vs. Dark		1.07	[0.65-1.75]		1.07	[0.65-1.75]
Hair colour	0.33			0.36		
Red/blond/light vs. Dark brown/Black		1.33	[0.75-2.34]		1.31	[0.73-2.32]
Skin colour	0.18			0.19		
Fair vs. Dark		0.66	[0.36-1.22]		0.67	[0.37-1.22]
Freckles	0.14			0.17		
Present vs. None		1.41	[0.90-2.22]		1.38	[0.87-2.17]
Suntan intensity	0.63			0.64		
None/slight/light vs. Dark/very dark		1.15	[0.65-2.01]		1.14	[0.65-2.00]
Sunburn event frequency	0.54			0.59		
None/rare vs. Frequent/constant		1.18	[0.69-2.00]		1.16	[0.68-1.97]
Skin phototype	0.36			0.41		
I-II vs. III-IV		1.27	[0.76-2.13]		1.24	[0.74-2.08]
Lifetime sun exposure intensity	0.13			0.12		
High vs. low-moderate		1.43	[0.90-2.28]		1.45	[0.91-2.31]

[1] Probability value of Wald test
[2] Adjusted odds ratios (AOR) are adjusted for all the controlled variables
[3] 95% Confidence Interval
[4] The distribution in photoaging grades I-III/grades IV-VI account for 313/207 for both models

Table S1 (continued). Risk of severe photoaging according to contributive MC1R polymorphisms, adjusted on all the variables

	Model 3 [4]			Model 4 [4]			Model 5 [4]			Model 6 [4]		
	P [1]	AOR [2]	[95% CI] [3]	P [1]	AOR [2]	[95% CI] [3]	P [1]	AOR [2]	[95% CI] [3]	P [1]	AOR [2]	[95% CI] [3]
MC1R variants												
R151C [5] vs. WT/WT	-	-	-	-	-	-	-	-	-	-	-	-
R142H [5] vs. WT/WT	-	-	-	0.07	3.52	[0.89-13.89]	-	-	-	-	-	-
D84E [5] vs. WT/WT	-	-	-	-	-	-	0.008	19.81	[2.16-181.60]	-	-	-
V92M [6] vs. WT/WT	-	-	-	-	-	-	-	-	-	0.01	2.57	[1.23-5.35]
Age (years)	<0.001	1.26	[1.16-1.36]	<0.001	1.34	[1.21-1.47]	<0.001	1.31	[1.19-1.45]	<0.001	1.28	[1.19-1.39]
BMI classification	0.22			0.41			0.33			0.48		
Overweight vs. normal	0.68	0.85	[0.39-1.86]	0.72	0.84	[0.32-2.18]	0.72	0.84	[0.32-2.19]	0.89	0.95	[0.44-2.03]
Obese vs. normal	0.08	0.29	[0.07-1.18]	0.19	0.34	[0.07-1.71]	0.14	0.29	[0.06-1.51]	0.23	0.47	[0.14-1.59]
Smoking habits	0.02			0.07			0.06			0.08		
Former smoker vs. no smoker	0.01	2.51	[1.23-5.13]	0.03	2.56	[1.11-5.90]	0.02	2.87	[1.20-6.87]	0.02	2.29	[1.12-4.69]
Current smoker vs. no smoker	0.03	3.65	[1.11-12.05]	0.21	2.45	[0.60-10.01]	0.38	1.86	[0.46-7.46]	0.56	1.47	[0.41-5.26]
Menopausal status and HRT	0.65			0.74			0.50			0.96		
Menopausal with HRT vs. no menopausal	0.72	0.79	[0.21-3.00]	0.95	1.08	[0.11-10.53]	0.92	0.90	[0.13-6.27]	0.99	0.99	[0.25-3.94]
Menopausal without HRT vs. no menopausal	0.90	1.09	[0.27-4.33]	0.74	1.47	[0.14-15.06]	0.69	1.51	[0.20-11.26]	0.91	1.09	[0.25-4.80]
Eye colour	0.10			0.59			0.13			0.51		
Light vs. Dark		0.53	[0.25-1.13]		0.78	[0.31-1.94]		0.48	[0.19-1.23]		0.77	[0.36-1.67]
Hair colour	0.54			0.66			0.44			0.44		
Red/blond/light vs. dark brown/Black		1.31	[0.54-3.19]		1.24	[0.47-3.24]		1.49	[0.54-4.08]		1.37	[0.61-3.07]
Skin colour	0.91			0.62			0.48			0.32		
Fair vs. Dark		0.95	[0.39-2.35]		0.77	[0.27-2.16]		0.68	[0.24-1.96]		0.64	[0.27-1.54]
Freckles	-			0.63			0.40			0.40		
Present vs. None		-	-		1.24	[0.51-3.01]		1.47	[0.60-3.56]		1.37	[0.66-2.86]
Suntan intensity	0.54			0.45			0.18			0.17		
None/slight/light vs. Dark/very dark		1.30	[0.55-3.06]		1.47	[0.54-4.03]		1.97	[0.74-5.25]		1.80	[0.78-4.16]
Sunburn event frequency	0.43			0.90			0.69			0.55		
None/rare vs. Frequent/constant		1.41	[0.60-3.34]		1.07	[0.38-3.00]		1.24	[0.43-3.59]		1.30	[0.55-3.09]
Skin phototype	0.53			0.62			0.64			0.62		
I-II vs. III-IV		1.30	[0.57-2.98]		1.27	[0.50-3.24]		1.26	[0.48-3.27]		1.21	[0.56-2.64]
Lifetime sun exposure intensity	0.24			0.36			0.22			0.45		
High vs. low-moderate		1.53	[0.75-3.12]		1.48	[0.64-3.42]		1.70	[0.72-3.99]		1.32	[0.64-2.71]
Interaction terms R151C*Freckles	0.03											
R151C*freckles vs. no freckle	0.006	7.38	[1.80-30.26]									
WT*freckles vs. no freckle	0.64	1.22	[0.52-2.88]									

[1] Probability value of Wald test [2] Adjusted odds ratios (AOR) are adjusted for all the controlled variables [3] 95% Confidence Interval
[4] The distribution in photoaging grades I-III/grades IV-VI account for 148/93, 124/77, 120/69 and 155/102 for models 3, 4, 5, and 6, respectively
[5] VAR1/WT, VAR1/VAR1 and VAR1/VAR2 are pooled together [6] var1/WT, var1/var1 and var1/var2 are pooled together

Annexe 11. Article sur les facteurs de risques des lentigines et des éphélides

Freckles and solar lentigines have different risk factors in Caucasian women

Ezzedine K, Mauger E, Latreille J, Jdid R, Malvy D, Gruber F, Galan P, Hercberg S, Tschachler E, Guinot C

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ORIGINAL ARTICLE

Freckles and solar lentigines have different risk factors in Caucasian women

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Abstract

Background To date, few epidemiological data on the relationships between solar lentigines, freckles and behavioural and constitutional risk factors in Caucasian populations exist.

Objectives To investigate the potential impact of behavioural and phenotypic variables, as well as the MC1R genetic background, on the history of facial freckles and the severity of solar lentigines in Caucasian women.

Methods The severity of solar lentigines was graded from facial digital images of 523 French middle-aged women by a dermatologist and summarized by a score afterwards. The history of facial freckles was assessed and the sun-exposure behaviour was characterized using a six-category typology. Risk factors including MC1R polymorphism were evaluated using logistic regression models.

Results Two constitutive host factors were found to be independently associated with a history of facial freckles: frequent sunburns and the presence of diminished function variants of the MC1R gene. In addition to age, five factors were independently associated with solar lentigines: constitutive host factors (dark skin colour and tanning capacity), a history of freckles, sun-exposure behaviour and current intake of oral contraceptive or progestogen treatments.

Conclusion These results strengthen the hypothesis that solar lentigines are markers of photoaging, whereas freckles are mainly determined by genetic factors. The finding that hormonal treatment is associated with a higher risk for solar lentigines merits further investigations.

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Conflict of Interest

The authors declare no conflict of interest.

Funding Source

None.

Introduction

Solar lentigines are hyperpigmented macules with well-defined borders varying in size from a few millimetres to several centimetres in diameter, and in colour from light to dark brown.[1] They are mainly observed in Caucasians and in Asians of Mongolian extraction.[2,3] Solar lentigines usually appear after the age of 50 on chronic sun-exposed skin sites.[4] The different names used for solar lentigines, i.e. sun-induced freckles, sunburn freckles, freckles in adulthood, lentigo senilis, age spot and actinic lentigines reflect their well-established link with ageing and chronic sun exposure.[5]

Freckles are also pigmented spots with irregular but distinct borders, generally reddish to light brown in colour. In contrast to age spots, which tend to be distinctly pigmented all year round, pigmentation of freckles typically fades during the winter months.[6] In Caucasian populations, freckles commonly appear on the face, neck, chest and arms during childhood, partially disappear with age, and are more frequently observed in fair phototype individuals, in particular those with red hair.[6] Freckles and solar lentigines have different pathophysiological origins. Solar lentigines arise through proliferation of basal melanocytes and a subsequent

increase in melanization.[4] By contrast, freckles are the result of an increase in local melanin production.

Variations in normal skin pigmentation is due to several factors: (1) the ratios of eumelanin and phaeomelanin, (2) the number, size and distribution of melanosomes and (3) the activation state of melanocytes.[7,8] Melanin production and the ratio of eumelanin to phaeomelanin are regulated amongst other mechanisms by the action of melanocyte-stimulating hormone (MSH). MSH binds to the melanocortin-1 receptor (MC1R), a 317 amino acid G-protein coupled receptor encoded by the MC1R gene, localized on chromosome 16d24.3.[9,10] Some variations of this gene, leading to functional impairment of the receptor, have been identified as important determinants of hair and skin pigmentation in Caucasian populations.[11,12] To date, over 75 different MC1R alleles have been identified, making this gene one of the most variable human genes.[9,13.]

Up to now, several epidemiological studies have been published on the relationship between solar lentigines, freckles and behavioural and constitutional risk factors.[5,14,15] Here, we have investigated the impact of behavioural and phenotypic variables, as well as of the MC1R genetic background, on the history of facial freckles (self-reported) and the severity of solar lentigines (examination of photographs) in a large sample of French middle-aged Caucasian women.

Materials and methods

Study design and population

This cross-sectional study was performed to investigate skin ageing in the context of the SU.VI.MAX cohort (*SUpplémentation en VItamines et Minéraux Anti-oXydants* - antioxidant vitamin and mineral supplementation), a longitudinal cohort study, conducted in French middle-aged adults.[16] The SU.VI.MAX study protocol was approved by The Hospital Medicals Ethics Committee of Paris-Cochin (CCPPRB n°706) and the '*Commission Nationale de l'Informatique et des Libertés*' (CNIL n°334641). The study was conducted according to Declaration of Helsinki Principles. All participants gave their written, informed consent. The cohort included 13 017 subjects who were representative of the French adult middle-aged population for most sociodemographic features.[17] The electronic data capture and storage system provided an opportunity to conduct cross-sectional surveys.

This study was conducted in the autumn/winter 2002–2003. All the women from Paris area were requested to participate in this research. Among them (n = 2257), 570 women, aged 44–70 years, agreed to take part in this study and provided informed consents. These women were asked to follow specific skin-care instructions, notably application of detergents or cosmetics to the face was not authorized for at least 12 h before the study visit. Of the 570 women who entered the study, 47 were excluded from this analysis: history of recent antiageing invasive procedures (18 women) and non-Caucasian origin (28 women). In addition, the severity of solar lentigines could not be assessed for one woman who wore make-up that altered her natural facial skin colour. The final study population was thus composed of 523 individuals.

General and phenotypic data collected

Age (in years), height (in metres), weight (in kilograms), smoking habits (never, former, current), menopausal status and hormonal treatment (combined oral contraceptive and progestogen treatments) were obtained on the day of the visit from a medical questionnaire during a standardized interview. Body mass index (BMI in kg/m^2) was categorized as underweight-normal (BMI<25 kg/m^2), overweight (25 ≤ BMI <30 kg/m^2) or obese (BMI ≥30 kg/m^2), according to the WHO recommendations.[18] Skin phototype was recorded according to Fitzpatrick's classification, as follows: 'always burn, never tan' (skin type I), 'always burn, then tan' (skin type II), 'always tan, sometimes burn' (skin type III) and 'always tan, never burn' (skin type IV).[19] Natural hair colour at the age of 20, eye colour, skin colour in winter, sunburn event frequency, suntan intensity and history of facial freckles were also documented.[20.]

Skin colour was measured on the inner side of the forearm using a spectrophotometer (Konica Minolta CM2600d, Osaka, Japan) after 30 min of rest in controlled environmental conditions: room temperature (mean ± standard deviation, 21 ± 3 °C) and relative humidity (37 ± 5%). The results were expressed in the CIEL*a*b* colour system. The individual typological angle (ITA°) was then calculated for each woman and categorized into three equal size groups using the quantile method: dark (18.1°–35.8°), medium (35.9°–41.6°) and fair (41.7°–59.0°).[21,22.]

Sun behaviour typology

A self-administered sun behaviour questionnaire was specifically developed for the SU.VI.MAX study. This questionnaire was addressed to the cohort in 1997 and 2001.[23] Its reliability, which was tested by comparing the responses to the lifetime items of the 6027 volunteers who answered both surveys, was satisfactory showing a consistent stability of the responses. From the data collected in 1997, a series of scores – linear combinations of several correlated items weighted according to their contribution – were developed. The design, validation and formula for calculation of these scores have been described previously elsewhere.[24] Using the formula provided, a lifetime sun-exposure score was calculated for each woman, and the scores were categorized into three equal size groups using the quantile method.[22] In addition, a series of items related to sun-exposure patterns and sunburn events was used for this study.

Furthermore, the women who declared use of sun protection products were assigned to one of four sun-exposure behaviour groups: 'No voluntary sun exposure, and use of sun protection products whatever SPF' (T1), 'Voluntary sun exposure ≤2 h/day and use of sun protection products with SPF>20' (T2), 'Voluntary sun exposure ≤2 h/day and use of sun protection products with

SPF≤20' (T3) and 'Voluntary sun exposure >2 h/day and use of sun protection products whatever SPF' (T4).[25] Women who declared that they did not use sun-protection products were attributed to one of two additional groups: 'No use of sun-protection products and no voluntary sun exposure' (T0), or 'No use of sun-protection products and voluntary sun exposure' (T00). The decision tree for assignment of each individual in the resulting six-category sun behaviour typology is shown in Fig. 1.

MC1R gene polymorphisms

Blood samples were collected the day of the visit to study the polymorphism of the melanocortin 1 receptor (MC1R) gene. Analyses of the data obtained by sequencing were performed at the Departments of Dermatology at the University of Edinburgh (United Kingdom) and at the Medical University of Vienna (Austria). MC1R genotyping was not successful for 13 individuals because either the DNA sample or PCR product could not be obtained. Patterns of MC1R gene polymorphism in our population have been described elsewhere.[26] The nine most common variants were analysed (V60L, V92M, R151C, R160W, R163Q, R142H, D294H, I155T, D84E). Among these variants, six have previously been reported[9] as major penetrant 'R' and three as minor penetrant 'r'. Therefore, MC1R variants were pooled into three classes according to the presence of 'Major' or 'Minor' diminished receptor function polymorphisms: Wild-Type homozygote (WT/WT), presence of one or two 'Minor' variants (r/r and r/WT), presence of one 'Major' variant (R/r, R/WT) and presence of two 'Major variants' (R/R). Finally, the individual effect of each variant was also evaluated (WT/WT vs. at least one specific variant).

Outcome variables

High-resolution digital images of the face of each woman were taken under standardized conditions that have been described elsewhere.[26] Afterward, the current presence or absence of freckles was assessed, and the severity of solar lentigines was graded on the forehead and cheeks by a dermatologist using a specific six-grade scale with photographic illustrations.[27] Then, the global severity of SL was estimated by a score built using Principal Component Analysis and linear regression.[28] Each individual's score value was transformed to fit a range between 0 and 10. The formula for the SL score is as follows: 1.25 * grade on cheeks + 1.25 * grade on forehead (with grade0 = 0, grade1 = 1, grade2 = 2, grade3 = 3, grade>3 = 4, for each area). To be used as an outcome variable, the distribution of the score was then dichotomized according to its median value (low score vs. high score values). Besides, the history of facial freckles (HFF) was also used as a secondary outcome variable.

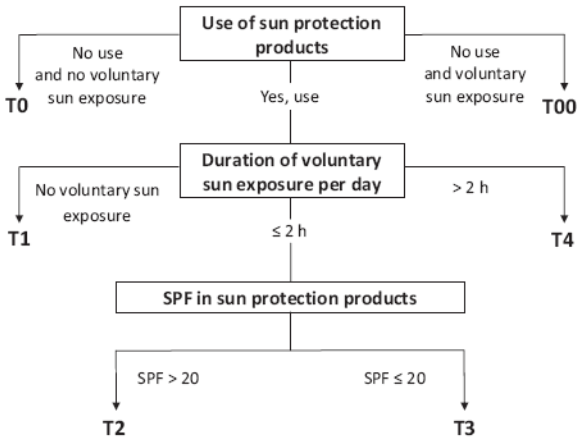

Figure 1 Decision tree for assignment in sun behaviour typology.

Statistical analyses

All the statistical analyses were performed using SAS® software release 9.1.3 (SAS Institute Inc., SAS Campus Drive, Cary NC 27513, USA). The relationships between sun behaviour typology and general and phenotypic data were explored using a chi-squared test or Fisher's exact test, and their relationships with SL severity scores and age using Pearson's correlation coefficients. Logistic regression analysis was performed to identify items potentially associated with each outcome variable. In the next step, the variables associated with each outcome variable were entered into a multivariate logistic regression model, and possible interaction terms were tested. Associations were expressed as adjusted odds ratios (AOR) together with their 95% confidence interval (95% CI) estimates.[28.]

Results

Women in our study were between 44- and 70-years old (mean ± standard deviation: 58 ± 6 years). The severity of solar lentigines was significantly associated with age ($r = 0.27$, $P < 0.0001$) and lifetime sun exposure ($r = 0.16$, $P < 0.0002$), but neither with sunburn during childhood nor sunburn during adulthood ($r = -0.06$, $P = 0.18$ and $r = -0.02$, $P = 0.59$, respectively). Besides, sunburn during childhood and sunburn during adulthood were positively associated ($r = 0.23$, $P < 0.0001$). Regarding the sun behaviour typology (Table 1), significant associations were found for hair and skin colours, suntan intensity, sunburn events frequency and skin phototype; as well as with items related to sun exposure.

Concerning history of facial freckles, HFF was significantly associated with fair skin colour and with fair phototypes (I, II, and III), and a trend was found for blonde and red hair colour (Table 2). As expected, HFF was also associated with the presence of freckles at the time of investigation: among the 213 women who reported HFF, 52 (24%) were found to present freckles at visual examination of the facial digital images. In addition, HFF was also significantly more frequent in women who declared to not tan at all or achieving only slight or light suntan intensity, than

Table 1 Sample description according to sun behaviour typology

		Sun behaviour typology*						*P* value†
		T1 (*n* = 113)	T2 (*n* = 56)	T3 (*n* = 99)	T4 (*n* = 57)	T0 (*n* = 49)	T00 (*n* = 53)	
Age (in years)		57.5 ± 0.6‡	56.7 ± 0.8	57.3 ± 0.6	56.6 ± 0.8	58.8 ± 0.9	59.1 ± 0.9	0.19
BMI classification	Underweight-normal	73 (65)§	37 (66)	68 (69)	44 (77)	32 (65)	34 (64)	0.64 F
	Overweight	31 (27)	15 (27)	24 (24)	11 (19)	11 (22)	11 (21)	
	Obese	9 (8)	4 (7)	7 (7)	2 (4)	6 (12)	8 (15)	
Smoking habits	Non-smoker	67 (59)	33 (59)	47 (47)	26 (46)	36 (73)	32 (60)	0.12
	Former smoker	35 (31)	16 (29)	36 (36)	21 (37)	12 (24)	14 (26)	
	Current smoker	11 (10)	7 (13)	16 (16)	10 (18)	1 (2)	7 (13)	
Menopausal status	Menopausal	84 (75)	43 (77)	77 (80)	38 (70)	42 (86)	44 (85)	0.33
	Non-menopausal	28 (25)	13 (23)	19 (20)	16 (30)	7 (14)	8 (15)	
Hormonal treatments	Never and past intake	103 (91)	51 (91)	96 (97)	51 (89)	47 (96)	48 (91)	0.31 F
	Current intake	10 (9)	5 (9)	3 (3)	6 (11)	2 (4)	5 (9)	
Eye colour	Blue/grey	32 (28)	16 (29)	28 (28)	14 (25)	12 (24)	9 (17)	0.68
	Green/hazel/brown/black	81 (72)	40 (71)	71 (72)	43 (75)	37 (76)	44 (83)	
Hair colour	Light & dark brown/black	89 (79)	42 (75)	70 (71)	52 (91)	42 (86)	44 (83)	0.04
	Blond/red	24 (21)	14 (25)	29 (29)	5 (9)	7 (14)	9 (17)	
Skin colour	Dark	17 (15)	11 (20)	28 (28)	15 (26)	12 (24)	20 (38)	0.03
	Fair	96 (85)	45 (80)	71 (72)	42 (74)	37 (76)	33 (62)	
Individual Typology Angle (tertiles)	Dark	25 (23)	20 (37)	41 (42)	24 (46)	7 (15)	20 (39)	0.01
	Intermediate	42 (38)	19 (35)	28 (29)	14 (27)	23 (48)	14 (27)	
	Fair	43 (39)	15 (28)	28 (29)	14 (27)	18 (38)	17 (33)	
Suntan intensity	Dark/very dark	25 (22)	22 (39)	52 (53)	29 (51)	10 (20)	33 (62)	<.001
	None/slight/light	88 (78)	34 (61)	47 (47)	28 (49)	39 (80)	20 (38)	
Sunburn event frequency	None/rare	80 (71)	36 (64)	85 (86)	37 (65)	40 (82)	37 (70)	0.01
	Frequent/constant	33 (29)	20 (36)	14 (14)	20 (35)	9 (18)	16 (30)	
Skin phototype	IV	10 (9)‡	6 (11)	20 (20)	9 (16)	12 (24)	7 (13)	<.001 F
	III	33 (29)	23 (41)	46 (46)	26 (46)	10 (20)	30 (57)	
	II	50 (44)	25 (45)	26 (26)	21 (37)	22 (45)	11 (21)	
	I	20 (18)	2 (4)	7 (7)	1 (2)	5 (10)	5 (9)	
Self-assessed lifetime sun-exposure intensity	None/low	49 (46)	7 (13)	13 (14)	4 (7)	29 (59)	9 (18)	<.001
	Moderate/high	57 (54)	48 (87)	83 (86)	52 (93)	20 (41)	41 (82)	
Consideration for lying in the sun	None/not important	108 (98)	34 (63)	57 (58)	24 (42)	48 (100)	40 (78)	<.001
	Very/extremely important	2 (2)	20 (37)	41 (42)	33 (58)	0 (0)	11 (22)	
Know the correct definition of sunburn		32 (28)	26 (46)	32 (32)	14 (25)	17 (35)	12 (23)	0.02 F

*Due to missing values and unforeseen inconsistencies, 96 women were not assigned in the typology.
†*P* value of χ^2; test or of Fisher's exact test (F). ‡Mean ± Standard Deviation. §Frequency and (%).

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Table 2 Risk factors for history of facial freckles

		History of freckles		P*	OR	[95% CI]
		No (n = 307)	Yes (n = 213)			
Age (in years)		57.9 ± 6.5†	57.5 ± 6.2	0.40	0.99	[0.96 – 1.02]
BMI classification	Underweight-normal	207 (60)‡	137 (40)	0.75	1	–
	Overweight	75 (57)	56 (43)	0.56	1.13	[0.75 – 1.70]
	Obese	25 (56)	20 (44)	0.55	1.21	[0.65 – 2.26]
Smoking habits	Non-smoker	174 (59)	121 (41)	0.26	1	–
	Former smoker	104 (62)	63 (38)	0.49	0.87	[0.59 – 1.29]
	Current smoker	29 (50)	29 (50)	0.21	1.44	[0.82 – 2.53]
Menopausal status	Menopausal	237 (60)	158 (40)	0.66	1	–
	Non-menopausal	64 (58)	47 (42)		1.10	[0.72 – 1.69]
Hormonal treatments	Never and past intake	287 (59)	196 (41)	0.52	1	–
	Current intake	20 (54)	17 (46)		1.24	[0.64 – 2.44]
Eye colour	Blue/grey	89 (64)	51 (36)	0.20	1	–
	Green/hazel/brown/black	218 (57)	162 (43)		1.30	[0.87 – 1.93]
Hair colour	Light & dark brown/black	249 (61)	159 (39)	0.08	1	–
	Blond/red	58 (52)	54 (48)		1.46	[0.96 – 2.22]
Skin colour	Dark	86 (75)	29 (25)	0.0001	1	–
	Fair	221 (55)	184 (45)		2.47	[1.55 – 3.93]
Individual Typology Angle (tertiles)	Dark	105 (63)	61 (37)	0.0288	1	–
	Intermediate	108 (64)	62 (37)	0.96	0.99	[0.63 – 1.54]
	Fair	84 (51)	81 (49)	0.0237	1.66	[1.07 – 2.57]
Current presence of freckles	None	282 (64)	161 (36)	<.0001	1	–
	Present	25 (33)	52 (68)		3.64	[2.18 – 6.10]
Suntan intensity	Dark/very dark	134 (65)	71 (35)	0.0182	1	–
	None/slight/light	173 (55)	142 (45)		1.55	[1.08 – 2.23]
Sunburn event frequency	None/rare	249 (67)	124 (33)	<.0001	1	–
	Frequent/constant	58 (40)	89 (61)		3.08	[2.08 – 4.57]
Sunburn during childhood	No	87 (70)	37 (30)	0.0032	1	–
	Yes	205 (55)	168 (45)		1.93	[1.25 – 2.98]
Sunburn during adulthood	No	22 (73)	8 (27)	0.11	1	–
	Yes	282 (58)	203 (42)		1.98	[0.86 – 4.53]
Skin phototype	IV	62 (82)	14 (18)	<.0001	1	–
	III	115 (60)	77 (40)	0.0010	2.97	[1.55 – 5.67]
	II	99 (50)	101 (51)	<.0001	4.52	[2.38 – 8.59]
	I	31 (60)	21 (40)	0.0073	3.00	[1.35 – 6.69]
Exposure during mountain sports§	No	191 (60)	126 (40)	0.46	1	–
	Yes	107 (57)	81 (43)		1.15	[0.80 – 1.65]
Exposure during nautical sports¶	No	232 (59)	164 (41)	0.80	1	–
	Yes	60 (60)	40 (40)		0.94	[0.60 – 1.48]
Exposure during hobbies**	No	168 (61)	109 (39)	0.19	1	–
	Yes	120 (55)	99 (45)		1.27	[0.89 – 1.82]
Nudism practice	No	282 (60)	186 (40)	0.23	1	–
	Yes	23 (51)	22 (49)		1.45	[0.79 – 2.68]
Artificial UV device Practice††	No	233 (59)	163 (41)	0.84	1	–
	Yes	63 (58)	46 (42)		1.04	[0.68 – 1.60]

Table 2 *Contiuned*

		History of freckles		P*	OR	[95% CI]
		No (*n* = 307)	Yes (*n* = 213)			
Exposure during professional activities‡‡	No	290 (59)	200 (41)	0.99	1	–
	Yes	13 (59)	9 (41)		1.00	[0.42 – 2.39]
Lifetime sun exposure (tertiles)	Low score values	100 (62)	61 (38)	0.43	1	–
	Medium score values	98 (55)	79 (45)	0.21	1.32	[0.86 – 2.04]
	High score values	109 (60)	73 (40)	0.67	1.10	[0.71 – 1.70]
Self-assessed lifetime sun-exposure intensity	None/low	84 (63)	50 (37)	0.32	1	–
	Moderate/high	223 (58)	163 (42)		1.23	[0.82 – 1.84]
Consideration for lying in the sun	None/not important	228 (59)	159 (41)	0.96	1	–
	Very/extremely important	71 (59)	49 (41)		0.99	[0.65 – 1.50]
Sun behaviour typology	T1	67 (59)	46 (41)	0.31	1	–
	T2	29 (52)	27 (48)	0.35	1.36	[0.71 – 2.58]
	T3	62 (63)	37 (37)	0.62	0.87	[0.50 – 1.51]
	T4	28 (49)	29 (51)	0.21	1.51	[0.79 – 2.86]
	T0	33 (67)	16 (33)	0.33	0.71	[0.35 – 1.43]
	T00	34 (64)	19 (36)	0.55	0.81	[0.41 – 1.60]
Major/minor variants classifications						
MC1R classification§§	WT/WT	134 (75)	44 (25)	–	–	–
	1 or 2 Minor variants	112 (58)	82 (42)	–	–	–
	Only 1 Major variant	52 (44)	65 (56)	–	–	–
	Two Major variants	0 (0)	15 (100)	–	–	–
MC1R classification	WT/WT	134 (75)	44 (25)	<.0001	1	–
	1 or 2 Minor variants	112 (58)	82 (42)	0.0004	2.23	[1.43 – 3.48]
	At least one Major variant	52 (39)	80 (61)	<.0001	4.69	[2.88 – 7.63]
Minor diminished function variants						
V60L	WT/WT	134 (75)	44 (25)	<.0001	1	–
	Var	68 (52)	63 (48)		2.82	[1.74 – 4.57]
V92M	WT/WT	134 (75)	44 (25)	0.0008	1	–
	Var	39 (53)	34 (47)		2.65	[1.50 – 4.71]
R163Q	WT/WT	134 (75)	44 (25)	0.19	1	–
	Var	24 (65)	13 (35)		1.65	[0.77 – 3.51]
Major diminished function variants						
R151C	WT/WT	134 (75)	44 (25)	<.0001	1	–
	Var	23 (41)	33 (59)		4.37	[2.32 – 8.22]
R160W	WT/WT	134 (75)	44 (25)	0.0003	1	–
	Var	14 (42)	19 (58)		4.13	[1.91 – 8.92]
D294H	WT/WT	134 (75)	44 (25)	0.0003	1	–
	Var	6 (32)	13 (68)		6.60	[2.37 – 18.40]
R142H	WT/WT	134 (75)	44 (25)	0.0002	1	–
	Var	5 (28)	13 (72)		7.92	[2.67 – 23.46]

Table 2 *Continued*

		History of freckles		P*	OR	[95% CI]
		No (n = 307)	Yes (n = 213)			
I155T	WT/WT	134 (75)	44 (25)	0.0059	1	–
	Var	3 (30)	7 (70)		7.10	[1.76 – 28.66]
D84E	WT/WT	134 (75)	44 (25)	0.0141	1	–
	Var	1 (17)	5 (83)		15.22	[1.73 – 133.82]

*P value of Wald test. †Mean ± standard deviation. ‡Frequency and (%), due to possible missing values the sum of the cell frequencies can be smaller than the total indicated in the top of the columns. §Hiking, climbing, mountaineering, snowshoeing, cross-country skiing, alpine skiing. ¶Rowing, kayaking, canoeing, windsurfing, water skiing, sailing, boat, sea fishing, angling. **Gardening, fishing, golf, tennis, cycling, jogging, hiking, horse riding, ††Ten volunteers who had artificial UV sessions for medical reasons were excluded. ‡‡Farmer, seasonal worker, postman, gym teacher, tennis instructor, coach travel, employee in camping, archaeologist. §§due to the distribution, no statistics can be computed, therefore the two last categories were pooled at least 1 Major variant.
CI, 95%, Confidence Interval; OR, Odd Ratio.

Table 3 Independent risk factors for history of facial freckles

		History of freckles*		P†	AOR‡	[95% CI]
		No (n = 298)	Yes (n = 206)			
MC1R classification	WT/WT	134 (75)§	44 (25)	<.0001	1	–
	1 or 2 Minor variants	112 (58)	82 (42)	0.0012	2.12	[1.35–3.33]
	At least one Major variant	52 (39)	80 (61)	<.0001	4.01	[2.43–6.60]
Sunburn event frequency	None/rare	240 (67)	119 (33)	<.0001	1	–
	Frequent/constant	58 (40)	87 (60)	<.0001	2.58	[1.71–3.90]

*Due to missing values this analyse was performed on 504 women.
†P values of Wald test.
‡Odds Ratios are adjusted on all items.
§Frequency and (%).
CI, 95% confidence interval.

in women declaring frequent sunburn and in those reporting sunburns in childhood. Concerning MC1R gene polymorphism, HFF was found to be significantly linked to the presence of diminished function variants, except for R163Q. After adjustment for multiple factors (Table 3), HFF remained significantly associated with self-reported frequency of sunburn and with the presence of diminished function variants, the 15 women with two major variants always reporting history of facial freckles.

As expected, solar lentigines severity was significantly associated with age (Table 4). They were also more severe in women with green, hazel, brown or black eyes, with light brown, dark brown and black hair colour, in women with darker skin colour (measured by ITA), in those with a history of freckles (HFF), and those with current presence of freckles. Solar lentigines were significantly more frequent in women who declared to achieve a dark or very dark suntan intensity, and in women with phototypes II and III. Significant associations were also found with current intake of combined oral contraceptive or progestogen treatments and the practice of nautical sports, and trends were observed for practice of other hobbies and professional activities involving with high sun exposure. In addition, significant positive associations were also found with lifetime sun exposure. The presence of SL was also significantly linked with sun behaviour typology with risk increasing from T2 to T4. About MC1R gene polymorphism, a significant association was only observed for the R160W variant. After adjustment for multiple factors (Table 5), SL remained significantly associated with age, and with dark and very dark suntan intensity, dark skin colour, a history of freckles, the two most risky behaviours regarding sun exposure (T3 and T4) and current intake of combined oral contraceptive or progestogen treatments.

Discussion

This study aimed to investigate the impact of behavioural and phenotypic variables, as well as the MC1R genetic background, on self-reported history of facial freckles and on directly observed severity of solar lentigines in a large sample of French middle-aged Caucasian women. To the best of our knowledge, this is the first study that combines such variables in the assessment of factors determining the severity of SL and the HFF.

Table 4 Age-adjusted risk factors for solar lentigines

		Solar lentigines		P*	AOR†	[95% CI]
		Low score (*n* = 260)	High score (*n* = 263)			
Age (in years)		56.4 ± 6.2‡	59.0 ± 6.3	<.0001	1.07	[1.04 – 1.10]
BMI classification	Underweight-normal	169 (49)§	176 (51)	0.43	1	–
	Overweight	65 (49)	68 (51)	0.64	0.91	[0.60 – 1.37]
	Obese	26 (58)	19 (42)	0.20	0.66	[0.35 – 1.25]
Smoking habits	Non-smoker	140 (47)	156 (53)	0.59	1	–
	Former smoker	91 (54)	78 (46)	0.43	0.85	[0.58 – 1.26]
	Current smoker	29 (50)	29 (50)	0.67	1.13	[0.63 – 2.03]
Menopausal status	Menopausal	187 (47)	210 (53)	0.16	1	–
	Non-menopausal	66 (59)	46 (41)		1.51	[0.85 – 2.69]
Hormonal treatments	Never and past intake	244 (50)	242 (50)	0.0056	1	–
	Current intake	16 (43)	21 (57)		2.83	[1.36 – 5.90]
Eye colour	Blue/grey	84 (60)	56 (40)	0.0027	1	–
	Green/hazel/brown/black	173 (46)	207 (55)		1.85	[1.24 – 2.78]
Hair colour	Blond/red	66 (59)	46 (41)	0.0241	1	–
	Light & dark brown/black	191 (47)	217 (53)		1.65	[1.07 – 2.54]
Skin colour	Fair	205 (51)	200 (49)	0.44	1	–
	Dark	52 (45)	63 (55)		1.18	[0.77 – 1.81]
Individual Typology Angle (tertiles)	Fair	94 (56)	73 (44)	0.0004	1	–
	Intermediate	91 (54)	79 (47)	0.33	1.25	[0.80 – 1.95]
	Dark	67 (40)	99 (60)	0.0001	2.45	[1.55 – 3.89]
Current presence of freckles	None	230 (52)	216 (48)	0.0355	1	–
	Present	30 (39)	47 (61)		1.72	[1.04 – 2.85]
History of freckles (self-declared)	None	183 (60)	124 (40)	<.0001	1	–
	Present	74 (35)	139 (65)		3.03	[2.08 – 4.41]
Suntan intensity	None/slight/light	170 (54)	145 (46)	0.0062	1	–
	Dark/very dark	87 (42)	118 (58)		1.66	[1.15 – 2.39]
Sunburn event frequency	None/rare	182 (49)	191 (51)	0.89	1	–
	Frequent/constant	75 (51)	72 (49)		1.03	[0.69 – 1.52]
Sunburn during childhood	No	58 (47)	66 (53)	0.85	1	–
	Yes	190 (51)	183 (49)		0.96	[0.63 – 1.46]
Sunburn during adulthood	No	13 (43)	17 (57)	0.68	1	–
	Yes	242 (50)	243 (50)		0.86	[0.40 – 1.82]
Skin phototype	I	36 (69)	16 (31)	<.0001	1	–
	II	105 (52)	97 (48)	0.0083	2.47	[1.26 – 4.85]
	III	76 (39)	117 (61)	<.0001	4.12	[2.09 – 8.12]
	IV	43 (57)	33 (43)	0.15	1.74	[0.81 – 3.74]
Exposure during mountain sports¶	No	164 (52)	153 (48)	0.15	1	–
	Yes	87 (46)	101 (54)		1.31	[0.90 – 1.89]
Exposure during nautical sports**	No	207 (52)	189 (48)	0.0221	1	–
	Yes	41 (41)	59 (59)		1.71	[1.08 – 2.70]
Exposure during hobbies††	No	150 (54)	127 (46)	0.07	1	–
	Yes	96 (44)	123 (56)		1.41	[0.98 – 2.02]
Nudism practice	No	234 (50)	234 (50)	0.44	1	–
	Yes	20 (44)	25 (56)		1.28	[0.68 – 2.38]
Artificial UV device practice‡‡	No	197 (50)	199 (50)	0.13	1	–
	Yes	49 (45)	60 (55)		1.40	[0.90 – 2.17]
Exposure during§§ professional activities	No	243 (50)	247 (50)	0.25	1	–
	Yes	9 (41)	13 (59)		1.69	[0.69 – 4.12]

Table 4 *Contiuned*

		Solar lentigines		P*	AOR†	[95% CI]
		Low score (*n* = 260)	High score (*n* = 263)			
Lifetime sun exposure (tertiles)	Low score values	97 (60)	64 (40)	0.0024	1	–
	Medium score values	81 (46)	96 (54)	0.0099	1.79	[1.15 – 2.79]
	High score values	79 (43)	103 (57)	0.0008	2.12	[1.36 – 3.31]
Self-assessed lifetime sun-exposure intensity	None/low	85 (63)	49 (37)	<.0001	1	–
	Moderate/high	172 (45)	214 (55)		2.32	[1.53 – 3.51]
Consideration for lying in the sun	None/not important	209 (54)	178 (46)	0.0003	1	–
	Very/extremely important	42 (35)	78 (65)		2.23	[1.45 – 3.44]
Sun behaviour typology	T1	69 (61)	44 (39)	0.0007	1	–
	T2	26 (46)	30 (54)	0.0454	1.98	[1.01 – 3.85]
	T3	43 (43)	56 (57)	0.0080	2.15	[1.22 – 3.79]
	T4	17 (30)	40 (70)	<.0001	4.25	[2.10 – 8.59]
	T0	28 (57)	21 (43)	0.83	1.08	[0.54 – 2.17]
	T00	29 (55)	24 (45)	0.66	1.16	[0.59 – 2.30]
Major/minor variants classifications						
MC1R classification	WT/WT	90 (50)	89 (50)	0.61	1	–
	1 or 2 Minor variants	102 (52)	93 (48)	0.82	0.95	[0.63 – 1.44]
	Only 1 Major variant	54 (46)	63 (54)	0.43	1.21	[0.75 – 1.96]
	Two Major variants	6 (40)	9 (60)	0.37	1.65	[0.55 – 4.95]
Minor diminished function variants						
V60L	WT/WT	90 (50)	89 (50)	0.30	1	–
	Var	75 (57)	57 (43)		0.78	[0.49 – 1.24]
V92M	WT/WT	90 (50)	89 (50)	0.38	1	–
	Var	33 (45)	40 (55)		1.29	[0.73 – 2.27]
R163Q	WT/WT	90 (50)	89 (50)	0.89	1	–
	Var	19 (51)	18 (49)		1.05	[0.51 – 2.18]
Major diminished function variants						
R151C	WT/WT	90 (50)	89 (50)	0.42	1	–
	Var	25 (45)	31 (55)		1.29	[0.70 – 2.37]
R160W	WT/WT	90 (50)	89 (50)	0.049	1	–
	Var	10 (30)	23 (70)		2.28	[1.00 – 5.16]
D294H	WT/WT	90 (50)	89 (50)	0.11	1	–
	Var	14 (74)	5 (26)		0.41	[0.14 – 1.22]
R142H	WT/WT	90 (50)	89 (50)	0.39	1	–
	Var	7 (39)	11 (61)		1.56	[0.57 – 4.31]
I155T	WT/WT	90 (50)	89 (50)	0.20	1	–
	Var	7 (70)	3 (30)		0.39	[0.09 – 1.66]
D84E	WT/WT	90 (50)	89 (50)	0.13	1	–
	Var	2 (33)	4 (67)		4.03	[0.67 – 24.19]

*P value of Wald test. †For each variable, Odds Ratio is age-adjusted, except for age. ‡95% confidence interval. §Mean ± Standard Deviation, ¶Frequency and (%), due to possible missing values the sum of the cell frequencies can be smaller than the total indicated in the top of the columns. **Hiking, climbing, mountaineering, snowshoeing, cross-country skiing, alpine skiing. ††Rowing, kayaking, canoeing, windsurfing, water skiing, sailing, boat, sea fishing, angling. ‡‡Gardening, fishing, golf, tennis, cycling, jogging, hiking, horse riding. §§Ten volunteers who had artificial UV sessions for medical reasons were excluded. ¶¶Farmer, seasonal worker, postman, gym teacher, tennis instructor, coach travel, employee in camping, archaeologist.
CI, Confidence Interval.

As reported previously, we found that development of freckles and solar lentigines was associated with distinct risk factors.[5,15,29] For example, age was found to be a significant risk factor for SL but not for HFF. In contrast, two independent constitutive host factors related to melanodeficiency were found to be associated with HFF: on one hand, these were frequent and constant sunburns; on the other, the presence of MC1R variants, with an increasing dosage effect according to the extent of reduction in

Table 5 Independent risk factors for solar lentigines

		Solar lentigines*		P†	AOR‡	[95% CI]
		Low score (*n* = 206)	High score (*n* = 206)			
Age		56.3 ± 6.3§	58.9 ± 6.2	<.0001	1.12	[1.08 – 1.17]
Suntan intensity	None/slight/light	141 (57)¶	107 (43)	0.0086	1	–
	Dark/very dark	65 (40)	99 (60)		1.96	[1.19 – 3.24]
History of freckles (self-declared)	None	149 (61)	97 (39)	<.0001	1	–
	Present	57 (34)	109 (66)		3.99	[2.48 – 6.41]
Individual Typology Angle (tertiles)	Fair	75 (56)	58 (44)	0.0210	1	–
	Intermediate	76 (55)	63 (45)	0.28	1.35	[0.78 – 2.34]
	Dark	55 (39)	85 (61)	0.0060	2.27	[1.26 – 4.06]
Sun behaviour typology	T1	68 (62)	42 (38)	0.0209	1	–
	T2	25 (46)	29 (54)	0.18	1.66	[0.79 – 3.49]
	T3	42 (43)	55 (57)	0.0211	2.12	[1.12 – 4.00]
	T4	16 (31)	36 (69)	0.0041	3.19	[1.44 – 7.03]
	T0	28 (58)	20 (42)	0.51	1.28	[0.61 – 2.72]
	T00	27 (53)	24 (47)	0.78	0.90	[0.42 – 1.93]
Hormonal treatments	Never and past intake	193 (51)	188 (49)	0.0044	1	–
	Current intake	13 (42)	18 (58)		3.73	[1.51 – 9.21]

*Due to missing values, this analysis was performed on 412 women.
†*P* values of Wald test.
‡Odds Ratios are adjusted on all items.
§Mean ± standard deviation.
¶Frequency and (%).
CI, 95% confidence interval.

function of the receptor, i.e. the effect was more pronounced in individuals carrying major variants than in those carrying minor variants. In addition to age, several other independent risk factors were identified for SL. These included constitutive host factors related to melanocompetency (dark skin colour and efficient tanning capacity), a history of facial freckles and behaviour related to sun exposure and sun protection (voluntary sun exposure of >2 h daily associated with sun-protection products and voluntary sun exposure of ≤2 h daily using sun protection-products with an SPF ≤20).

As previously reported, we found in age-adjusted analysis that darker Caucasian phototypes and their related phenotypic items, history of facial freckles and lifetime sun exposure, were positively associated with the severity of SL. Conversely, we did not find a significant link between a history of sunburn and the severity of SL. As expected, we found an association between a history of freckles and skin phototype. In addition, and as reported previously[30], a history of freckles was a more reliable variable than the current presence of freckles, as freckles tend to fade with age.

Concerning MC1R gene variants, only one variant, R160W, was found to be associated with a higher risk of SL. This major diminished function variant has been shown to be associated with a decreased ability to stimulate the production of cyclic AMP in response to melanocortin and with reduced cell-surface expression, suggesting impaired processing of the MC1R protein.[31,32] Moreover, Sanchez-Laorden *et al.* demonstrated that R151C and R160W variants show an aberrant anterograde trafficking with intracellular retention, which accounts for their functional impairment.[33] In addition, Cario-André *et al.* have demonstrated by electron microscopy that melanocytes in solar lentigines, but not in perilesional skin, showed an activated phenotype, with aberrant trafficking of melanosomes, leading to an accumulation of these organelles within the cell, which tended to aggregate into polymelanosomes.[4] Taken together, these findings may shed light on a possible role of R160W in melanosome transfer and in the activation of melanocytes. In this context, our results suggest a partly common pathway in the development of SL and facial freckles through the action of the R160W gene variant. However, we can presume that with greater numbers of carriers of other major diminished function variants than those we have in our study, we could have additional significant results.

Finally, we found a positive association between the severity of SL and current intake of hormonal treatments that persisted after adjustment for multiple factors. This association has not been reported previously and thus needs further investigation. However, hormonal replacement therapies have been implicated in the development of melasma, although the exact pathological mechanisms by which they act are not fully understood.[34–36] A recent

study has shown that melasma is caused by an increase in the number of melanosomes in the epidermis. These same authors have found that a subset of modulators of the Wnt pathway was upregulated in melasma.[35] In parallel, a microarray analysis has identified upregulation of the SFRP1 gene in SL. The protein encoded by this gene is part of the Wnt pathway, and may thus be a common pathophysiological intermediary for the development of both melasma and SL.[37] Moreover, like SL, melasma is more common in darker skin phototypes, suggesting that there may be a role for genetic variations associated with pigment production in melasma.[38,39] However, the possible impact of MC1R variants on melasma occurrence and severity remains to be investigated.

A notable finding of our study was that SL was found to be strongly associated with sun behaviour typology: the risk of SL increased with more risky sun-exposure behaviour. In contrast, such an association was not found for facial freckles. These observations reinforce the hypothesis that SL is a marker of skin photoaging, and thus of sun-exposure behaviour, whereas freckles are mainly determined by inherited factors.

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Annexe 12. Article sur l'étude GWAS et l'expression du vieillissement cutané

A genome-wide association study in Caucasian women points out for a role of the STXBP5L gene in facial photoageing

Le Clerc S, Taing L, Ezzedine K, Latreille J, Labib T, Coulonges C, Bernard A, Melak S, Carpentier W, Malvy D, Jdid R, Galan P, Hercberg S, Morizot F, Guinot C, Tschachler E, Zagury JF

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A Genome-Wide Association Study in Caucasian Women Points Out a Putative Role of the *STXBP5L* Gene in Facial Photoaging

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A genome-wide association study (GWAS) was conducted on 502 French middle-aged Caucasian women to identify genetic factors that may affect skin aging severity. A high-throughput Illumina Human Omni1-Quad beadchip was used. After single-nucleotide polymorphism (SNP) quality controls, 795,063 SNPs remained for analysis purposes. Possible stratification was first examined using the Eigenstrat method, and then the relationships between genotypes and four skin aging indicators (global photoaging, lentigines, wrinkles, and sagging) were investigated separately by linear regressions adjusted on age, smoking habits, lifetime sun exposure, hormonal status, and the two main Eigen vectors. One signal passed the Bonferroni threshold ($P=1.53\times10^{-8}$) and was significantly associated with global photoaging. It was also correlated with the wrinkling score and the sagging score. According to HapMap, this SNP, rs322458, was in linkage disequilibrium (LD) with intronic SNPs of the *STXBP5L* gene, which is expressed in the skin. In addition, it was also in LD with another SNP that increases the expression of the *FBXO40* gene in the skin. These two genes, which were not previously described in the context of aging, may constitute good candidates for the investigation of molecular mechanisms of skin photoaging.

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INTRODUCTION

Similar to other organs, skin ages owing to passage of time. Skin aging is influenced both by inherited intrinsic factors and by extrinsic or environmental factors, such as chronic UV exposure and smoking (Malvy *et al.*, 2000; Yaar and Gilchrest, 2007). Intrinsic aging is an ineluctable process and is due to the genetically determined natural degeneration of the cell functioning and loss of extracellular matrix with age (Yaar and Gilchrest, 1990). Its clinical phenotype on the skin is mainly characterized by fine wrinkles and dry, thin, and pale skin (Fisher *et al.*, 2002; Makrantonaki and Zouboulis, 2007).

The main factor responsible for extrinsic aging of the skin is UVR. UV-induced skin aging or photoaging is defined as the premature occurrence of signs of aging on the skin, and presents with characteristic morphological changes of both the epidermal and dermal compartments (Rabe *et al.*, 2006; Yaar and Gilchrest, 2007). A number of hereditary phenotypic features influence the severity of photoaging, most notably skin color (Kligman and Kligman, 1999; Malvy *et al.*, 2000), and skin phototype (Fitzpatrick, 1988). Individuals with dark phototypes (III–IV) commonly exhibit more "hypertrophic responses" such as deep wrinkling, coarseness, and lentigines, whereas fair phototype individuals (I–II) generally show fewer wrinkles with epidermal atrophy, focal depigmentation, as well as dysplastic changes, such as actinic keratosis, nonmelanoma, and melanoma skin cancers (Rabe *et al.*, 2006; Yaar and Gilchrest, 2007; Puizina-Ivić, 2008).

Up to now, the exploration of the genes affecting skin aging has remained limited to *MC1R* gene (Elfakir *et al.*, 2010; Suppa *et al.*, 2011), or to genes involved in genetic pathologies with accelerated skin aging (Rooryck *et al.*,

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Abbreviations: BMI, body mass index; GWAS, genome-wide association study; LD, linkage disequilibrium; SNP, single-nucleotide polymorphism; SU.VI.MAX, SUpplémentation en VItamines et Minéraux AntioXydants

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2008; Soufir *et al.*, 2010). A candidate gene approach has previously established associations between *MC1R* gene variants, particularly loss-of-function variants, with an increased risk of severe photoaging (Elfakir *et al.*, 2010). In addition, a few studies conducted in twin cohorts have explored the associations between environmental factors, skin aging, and gene expression (Plomin *et al.*, 1994; Shekar *et al.*, 2005, 2006; Christensen *et al.*, 2009).

To unravel new genetic associations with skin aging in a systematic way, we have undertaken a genome-wide study on a well-defined sample of Caucasian women from the SU.VI.MAX (*SUpplémentation en VItamines et Minéraux AntioXydants* (Antioxidant Vitamin and Mineral Supplementation)) cohort (Hercberg *et al.*, 2004). To the best of our knowledge, no genome-wide association study (GWAS) targeting skin aging in middle-aged women of European-derived ancestry has been previously reported.

RESULTS

Using the Illumina HumanOmni1-Quad BeadChips, we conducted a GWAS by testing associations between single-nucleotide polymorphisms (SNPs) and global skin photoaging on a large sample of French middle-aged women from the SU.VI.MAX cohort. After the various quality-control tests (see Materials and Methods), 795,063 genotyped SNPs were available for 502 women.

Table 1 describes the sample of women according to the severity of photoaging. We also computed the correlations between the age and the outcome variables (Table 2). We found that the correlations with age were all statistically significant ($P<0.0001$): 0.56 for the grade of photoaging, 0.61 for the score of wrinkling and the score of sagging, and 0.27 for the score of lentigines. Similarly, the correlations between the grade of photoaging and the other outcome variables were also statistically significant ($P<0.0001$): 0.78 for the score of wrinkling, 0.66 for the score of sagging, and 0.31 for the score of lentigines; the correlation between the score of wrinkling and the score of sagging reached 0.71 ($P<0.0001$; Table 2).

Our core association analysis focused on genotypic associations obtained using linear regressions, after correction for stratification and nongenetic skin aging factors. Figure 1 presents the distribution of the *P*-values obtained for each SNP along the chromosomes (Manhattan plot). One SNP located on the chromosome 3 (locus 3q13.33), rs322458, passed the Bonferroni threshold (6.28×10^{-8}) with $P=1.53\times10^{-8}$. According to HapMap, this SNP is in linkage disequilibrium (LD) with five SNPs positioned in intronic regions of the *STXBP5L* gene (rs470647, rs612545, rs617332, rs645045, and rs1795413), and with two intergenics SNPs (rs377374 and rs450614; Figure 2). A more refined analysis suggested that the effect was likely recessive. Indeed, when regrouping the individuals according to their grade of skin photoaging, the frequency of the homozygous rs3224587-AA genotype was clearly inversely proportional with photoaging severity (Figure 3): from 28% of homozygous subjects among grade 1 to 4% among grade 5. To further investigate the rs322458 SNP, we assessed its putative impact on each phenotype: lentigines, wrinkling, and sagging. No relationship was found with the lentigines score ($P=0.63$), whereas significant links were found with wrinkling and sagging scores (respectively, $P=5.6\times10^{-5}$ and $P=1.76\times10^{-4}$).

Moreover, bioinformatics databases were investigated for possible associations between SNPs and mRNA expression, regulation (splicing, polyadenylation, and miRNA), and also for putative transcription binding sites. According to Genevar (Nica *et al.*, 2011), the genotype rs470647-AA (rs470647 is in LD with the rs322458; see Figure 2) increases the expression in skin of a neighboring gene, *FBXO40* ($P=6\times10^{-4}$; Figure 4). The rs470647 SNP and *FBXO40* are at a distance of 683 kb.

To further investigate other possible associations, we also computed all the haplotypes based on two SNPs derived from both *STXBP5L* and *FBXO40* genes. Only three haplotypes were strongly associated with photoaging (Figure 4) and they implicated the rs322458 SNP. These haplotypes involved one exonic SNP and one 3′-untranslated region of the *STXBP5L* gene (respectively, rs17740066, $P=6.27\times10^{-9}$ and rs6782033, $P=3.96\times10^{-9}$), and one intronic SNP of the *FBXO40* gene (rs6775899, $P=9.52\times10^{-10}$). The rs17740066 and rs6782033 SNPs were in partial LD with rs322458 ($D'=1$); in other words, the G allele frequency of rs322458 SNP was identical with that of the haplotypes GG (rs322458–rs17740066) and GA (rs322458–rs6782033). Interestingly, the rs17740066 SNP corresponds to the Val855Ile protein variation and rs6782033 SNP corresponds to a putative binding site for a miRNA (hsa-mir-892b; Figure 4). There was no LD between the two SNPs, rs6775899 and rs322458 ($r^2=0.014$ and $D'=0.2$). However, the GA haplotype (rs322458–rs6775899) also exhibited a significant *P*-value ($P=9.52\times10^{-10}$), suggesting it might also be a haplotype of interest.

DISCUSSION

We have described here a GWAS investigating possible associations between SNPs and global skin photoaging. This research yielded an association for the rs322458 SNP connected to the *STXBP5L* gene with severity of skin photoaging, the rs322458-AA genotype being inversely linked with the severity of skin aging. This SNP was also associated with the wrinkle and sagging scores that are defined independently from the grade of photoaging, but it was not associated with the lentigines score, suggesting that: (1) its role in photoaging does not include pigmentary disorders; and (2) molecular mechanisms might be shared by sagging and wrinkling. According to the HapMap database, this SNP is also polymorphic in the Asian and African populations, and thus it would also be worth investigating these populations. As for any GWAS, additional genetic studies will be needed to affirm this association.

Another alias for *STXBP5L* is *LLGL4*, as it is homologous to the Lethal giant larvae (*Lgl*) drosophila gene (Katoh and Katoh, 2004). The protein coded by *STXBP5L* contains five WD40 repeats (or β-transducin repeats) and a C-terminal syntaxin-binding (STXB) domain. *Lgl* regulates epithelial polarity and, when mutated, may lead to tumor-like

Table 1. Description of the population according to photoaging severity

	Photoaging severity						
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5/6 [1]		
	N=43	*N*=86	*N*=174	*N*=150	*N*=49	Total, *N*=502	*P*-value of test
Age (years)	50.1±4.2[2]	54.1±5.0	56.8±5.5	60.9±5.6	62.6±5.2	57.6±6.4	<0.0001[3]
Lifetime sun exposure (score)	5.3±3.4	5.1±3.5	5.2±3.6	5.5±3.5	5.5±3.5	5.3±3.5	0.84[3]
BMI classification							0.49[4]
Normal	28 (8.4)[5]	57 (17.0)	121 (36.1)	94 (28.1)	35 (10.4)	335 (66.7)	
Overweight	9 (7.4)	19 (15.6)	37 (30.3)	45 (36.9)	12 (9.8)	122 (24.3)	
Obese	6 (13.3)	10 (22.2)	16 (35.6)	11 (24.5)	2 (4.4)	45 (9.0)	
Hormonal status							<0.0001[4]
Nonmenopausal	27 (28.7)	24 (25.5)	32 (34.0)	9 (9.6)	2 (2.2)	94 (18.7)	
Menopausal with HRT	9 (3.4)	40 (15.3)	98 (37.4)	92 (35.1)	23 (8.8)	262 (52.2)	
Menopausal without HRT	7 (4.8)	22 (15.1)	44 (30.1)	49 (33.6)	24 (16.4)	146 (29.1)	
Smoking habits							0.61[4]
Never	23 (8.0)	45 (15.7)	100 (35.0)	86 (30.1)	32 (11.2)	286 (57.0)	
Former smoker	15 (9.3)	34 (21.3)	50 (31.2)	47 (29.5)	14 (8.7)	160 (31.9)	
Current smoker	5 (8.9)	7 (12.5)	24 (42.8)	17 (30.4)	3 (5.4)	56 (11.1)	
Eye color							0.21[4]
Blue/gray	14 (10.3)	18 (13.2)	50 (36.8)	36 (26.5)	18 (13.2)	136 (27.2)	
Green/hazel/brown/black	28 (7.8)	68 (18.7)	122 (33.6)	114 (31.4)	31 (8.5)	363 (72.8)	
Hair color at 20 years							0.08[4]
Blond/red	4 (3.7)	20 (18.6)	40 (37.0)	28 (25.9)	16 (14.8)	108 (21.6)	
Light and dark brown/black	38 (9.7)	66 (16.9)	132 (33.8)	122 (31.2)	33 (8.4)	391 (78.4)	
Skin color without tanning							0.78[4]
Fair	35 (9.0)	65 (16.8)	136 (35.0)	113 (29.2)	39 (10.0)	388 (77.8)	
Dark	7 (6.3)	21 (18.9)	36 (32.4)	37 (33.3)	10 (9.0)	111 (22.2)	
History of facial freckles							0.40[4]
No	25 (8.5)	55 (18.7)	104 (35.4)	87 (29.6)	23 (7.8)	294 (58.9)	
Yes	17 (8.3)	31 (15.1)	68 (33.2)	63 (30.7)	26 (12.7)	205 (41.1)	
Suntan intensity							0.71[4]
None/slight/light	23 (7.7)	49 (16.3)	101 (33.7)	96 (32.0)	31 (10.3)	300 (60.1)	
Dark/very dark	19 (9.5)	37 (18.6)	71 (35.7)	54 (27.2)	18 (9.0)	199 (39.9)	
Sunburn event frequency							0.74[4]
None/rare	28 (7.8)	61 (17.0)	123 (34.4)	113 (31.6)	33 (9.2)	358 (71.7)	
Frequent/constant	14 (9.9)	25 (17.7)	49 (34.8)	37 (26.2)	16 (11.4)	141 (28.3)	

Abbreviations: BMI, body mass index; HRT, hormonal replacement therapy.
[1]As a single woman had grade 6, she had been grouped with grade 5 individuals.
[2]Mean ±SD.
[3]Analysis of variance (ANOVA) test.
[4]The χ^2 test.
[5]Frequency and (%): because of possible missing values, the sum of the cell frequencies can be smaller than the total indicated in the top of the columns.

phenotype development. According to bioinformatics analysis (UniProt, 2011), *STXBP5L* seems to be implicated in vesicle trafficking and could have a role in exocytosis ((Katoh and Katoh, 2004; UniProt, 2011). Interestingly, *STXBP5L* has previously been associated with liver fibrosis risk in Caucasians and with chronic hepatitis C infection (Li *et al.*, 2009). *STXBP5L* is expressed in several tissues, including the skin (Safran *et al.*, 2010), and is also expressed in lung carcinoid and germ cell tumors (Katoh and Katoh, 2004).

Bioinformatics database exploration pointed out the possible role of the SNP rs322458 in the skin expression of a neighboring gene, *FBXO40*. Haplotype analysis of the

Table 2. Correlation coefficients between age and outcome variables

	Age	Score of wrinkling	Score of sagging	Score of lentigines	Grade of photoaging
Age	1	0.61	0.61	0.27	0.56
Score of wrinkling		1	0.71	0.31	0.78
Score of sagging			1	0.26	0.66
Score of lentigines				1	0.31
Grade of photoaging					1

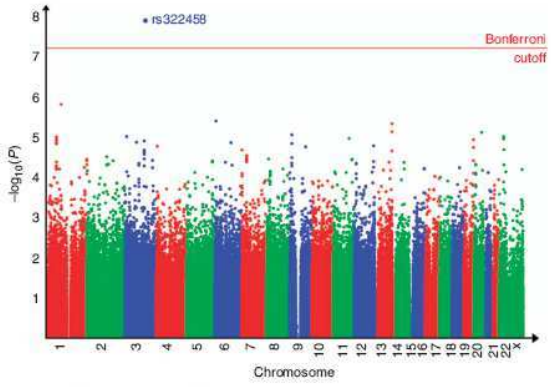

Figure 1. Manhattan plot of the association study with the photoaging score. Distribution of $-\log10(P)$ obtained for the associations tested between the genotypes and skin photoaging, according to Larnier's scale, along the human chromosomes (Manhattan plot).

STXBP5L and *FBXO40* gene region also revealed positive signals ($P \sim 10^{-9}$), bringing up a second hypothesis in which rs3322458 G allele in the dominant mode, possibly in combination with other alleles, might be implicated in the phenotype.

FBXO40 encodes a protein characterized by a 40 amino-acid F-box motif. This gene is expressed specifically in the muscle (Ye *et al.*, 2007), may function as a regulator involved in the postnatal myogenesis (Ye *et al.*, 2007), and has a role in muscle hypertrophy (Shi *et al.*, 2011). F-box proteins are involved in the SCF (Skp, Cullin, F-box containing) complex, known to act as protein–ubiquitin ligases (Skowyra *et al.*, 1997), and a recent study demonstrated that the SCF–F-box40 complex prevented skeletal muscle hypertrophy by limiting the IGF1 pathway in the muscle (Shi *et al.*, 2011).

Both *STXBP5L* and *FBXO40* were not known before for any skin function. How could they affect skin aging? *FBXO40* is linked with the IGF1 pathway known for its role in inflammation, and its direct link with myogenesis could also explain its impact on wrinkling and sagging severity. Knowing that

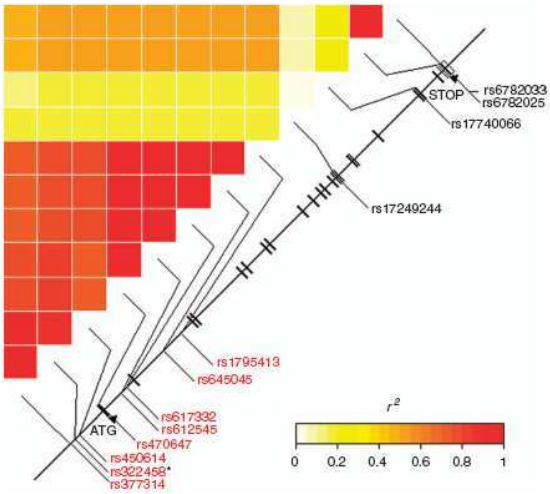

Figure 2. Genetic map of the *STXBP5L* gene. The single-nucleotide polymorphisms (SNPs) in high linkage disequilibrium (LD) with the SNP rs322458 are in red, and the exonic SNPs genotyped in the study are in black. The LD map (providing the r^2 coefficient between SNPs) is given below the genetic map. The SNP rs322458 is flagged with an asterisk (*).

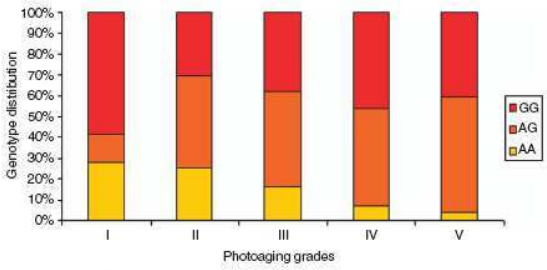

Figure 3. Distribution of the rs322458 genotypes in function of the photoaging severity.

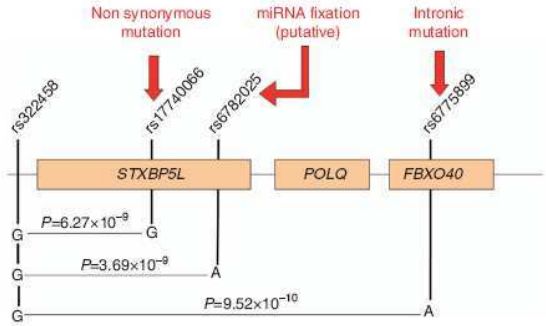

Figure 4. Haplotype analysis. All the two-single-nucleotide polymorphism (SNP) haplotypes of the region were computed using the Shape-IT software. Associations were computed, and the figure presents the three best signals obtained, which all involve the SNP rs322458.

photoaging is intimately associated with the occurrence of dysplastic skin changes, such as actinic keratosis as well as nonmelanoma and melanoma skin cancer, it is striking to see that *STXBP5L* has been linked to cancer (Katoh and Katoh, 2004; Li *et al.*, 2009). Therefore, the search for gene polymorphisms involved in photoaging may also help to identify risk factors for skin carcinogenesis.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was conducted to investigate skin aging in the context of the SU.VI.MAX cohort, a longitudinal cohort study, conducted in French middle-aged adults (Hercberg *et al.*, 1998). The protocol was approved by the Hospital Medicals Ethics Committee of Paris-Cochin (CCPPRB no. 706) and the "*Commission Nationale de l'Informatique et des Libertés*" (CNIL no. 334641). The study was conducted according to the Declaration of Helsinki Principles. All participants gave their written, informed consent. The SU.VI.MAX cohort included 13,017 volunteers who were representative of the French adult middle-aged population for most sociodemographic features (Hercberg *et al.*, 2004).

This study was conducted in the autumn/winter of 2002–2003. All women living in the Paris area were requested to participate in this research. Among them ($n=2,257$), 570 women, aged 44–70 years, agreed to take part in this study and provided informed consent. The participants were asked to follow specific skin care instructions; notably, application of detergents or cosmetics to the face was not authorized for at least 12 hours before the study visit. On the day of the visit, they were first asked to complete a self-administered questionnaire related to lifetime sun exposure behavior. Subsequently, three standardized, high-resolution digital images ($2,008 \times 3,032$ pixels) of the face were taken for each participant (one frontal view of the face and one of each profile), using a Kodak DCS 760 digital camera with a 105 mm camera lens (Kodak, Paris, France). The camera was mounted on a monopod and a specifically developed chair was used to allow standardized positions of the camera with respect to the face. Lighting conditions were standardized by means of two symmetrical lamps, which provided a continuous daylight spectrum, placed at 45° to each side of the face. Finally, a blood sample was collected for genetic analysis.

Assessment of skin aging features

The facial photographs was examined for each woman by a dermatologist, and the severity of global skin photoaging was rated using a six-grade ordinal scale (Larnier *et al.*, 1994), each grade being depicted by three reference photographs that illustrate the diversity and range of pigmentation disorders, wrinkling, and sagging. In addition, the severity of 12 age-related skin features was also assessed on forehead and on cheeks using specific ordinal photographic scales (Morizot *et al.*, 2002).

Outcome variables: phenotypes analyzed

The primary outcome variable is the global photoaging grade (1–6) and the secondary outcomes variables are the three independent scores: wrinkling, sagging, and lentigines scores. On the basis of the 12 age-related skin features, the global severity of wrinkling, sagging, and solar lentigines was estimated by three scores built using principal component analysis and linear regression methods (Jobson, 1992). Then, each individual's score values were transformed to fit a range between 0 and 10.

The solar lentigines score is computed as follows: $1.25\times$ severity on cheeks $+1.25\times$ severity on forehead (with grade $0=0$, grade $1=1$, grade $2=2$, grade $3=3$, and grade $>3=4$ for each skin area). The sagging score is based on four features: 0.87 when presence of bags under the eyes $+0.78\times$ severity of nasolabial fold (with grade $<3=1$, grade $3=2$, grade $4=3$, and grade $>4=4$) $+0.93\times$ severity of tissue slackening $+1.07\times$ severity of drooping eyelids (with grade $<3=1$, grade $3=2$, and grade $>3=3$ for the two preceding features). Finally, the wrinkling score is computed using the six remaining features: $-0.64+0.42\times$ severity of wrinkles above the upper lip (with grade $0=0$, grade $1=1$, grade $2=2$, grade $3=3$, and grade $4=4$) $+0.64\times$ severity of wrinkles under the eyes (with grade $<3=0$, grade $3=1$, grade $4=2$, and grade $5=3$) $+0.70\times$ severity of fine lines on cheek (with grade $0=0$, grade $1=1$, and grade $2=2$) $+0.44\times$ severity of furrows between eyebrows $+0.54\times$ severity of crow's feet $+1.06\times$ severity of coarse wrinkles on cheek (with grade $<2=0$, grade $2=1$, grade $3=2$, grade $4=3$, and grade $5=4$ for the three preceding features).

Covariables used for the statistical analysis: general and phenotypic data

To focus more specifically on the genetic factors affecting skin aging, several characteristics known to affect aging had to be taken into account: age (in years), body mass index (BMI; in $kg\,m^{-2}$), smoking habits (never, former, and current), and hormonal status (nonmenopausal, menopausal with hormone replacement therapy, and menopausal without hormone replacement therapy). BMI was categorized as underweight/normal (BMI $<25\,kg\,m^{-2}$), overweight ($25\leqslant BMI<30\,kg\,m^{-2}$), or obese (BMI $\geqslant 30\,kg\,m^{-2}$) according to the World Health Organization (WHO) recommendations (WHO, 1995). In addition, phenotypic data such as natural hair color at the age of 20 years, eye color, skin color in winter, sunburn event frequency, suntan intensity, and history of facial freckles were also collected. Moreover, lifetime sun exposure intensity was estimated by a score based on data collected by a self-reported questionnaire. This score is a linear combination of five items weighted according to their relative contribution to the score: voluntary sun exposure, exposure of the body and the facial skin, exposure during the hottest hours of the day, intensity of self-reported lifetime sun exposure, and consideration for sunbathing. The design, validation, and description of this score have been described previously (Guinot *et al.*, 2001).

Genotyping method

The 529 women were genotyped using Illumina Infinium HumanOmni1-Quad BeadChips (Illumina, San Diego, CA) that contain 1,140,419 markers. Genomic DNA (250 ng) was whole-genome amplified, fragmented, denatured, and hybridized on prepared HumanOmni1-Quad BeadChips for a minimum of 16 hours at 48 °C. Nonspecifically hybridized fragments were removed by washing, and the remaining specifically hybridized DNA was fluorescently labeled by a single base extension reaction and detected using a IScan scanner (Illumina). Normalized bead-intensity data obtained for each sample were loaded into GenomeStudio software (version 1.6.3; Illumina), which converted fluorescence intensities into SNP genotypes. For the analysis, we considered only SNPs, consequently

excluding the copy-number variations that represented 91,706 markers on the HumanOmni1-Quad BeadChips. Moreover, 2,182 SNPs were removed because they were located on the Y chromosome and they could not be analyzed as the population was composed of women.

Quality control

Using the GenomeStudio software (version 1.6.3; Illumina), we analyzed the crude genotyping data, and SNPs were filtered according to the following parameters. First, nine samples with a call rate (percentage of SNPs genotyped by sample) of <95% in the Illumina clusters were removed. Second, the SNPs with a call frequency (percentage of samples genotyped by SNP) of <99% were reclustered. Third, after reclustering, samples with a call rate <98% were deleted. This method has been already used in several studies (Le Clerc *et al.*, 2009; Limou *et al.*, 2009, 2010). The clustering step can create SNP genotyping errors, which can be prevented by following the Illumina procedure (http://www.illumina.com/Documents/products/technotes/technote_infinium_genotyping_data_analysis.pdf).

This method evaluates the quality of the newly created clusters according to several criteria, which can be manually checked and corrected as necessary. In total, after all the quality control steps were carried out, 56,479 SNPs with a call frequency of <98% (2% of missing data) were excluded. This procedure ensures reliable genotyping data with little missing data. Hardy–Weinberg equilibrium analysis was performed for each SNP in each group by using an exact statistical test implemented in PLINK software (Purcell *et al.*, 2007). Deviation from Hardy–Weinberg equilibrium in a group of patients suggests an error in genotyping. Thus, 3,866 SNPs, which were not in the Hardy–Weinberg equilibrium ($P<1.0\times10^{-3}$), were rejected in this way. We removed 191,123 SNPs with minor allele frequency <1% to avoid error of genotyping, leaving a total of 795,063 SNPs.

Identification of population stratification

To correct for possible population stratification, genotypes were analyzed using EIGENSTRAT utility of the EIGENSOFT package version 2.0 (Price *et al.*, 2006). The two first pass with the Eigenstrat software pointed out 18 outliers, which were removed from further analyses. Then, a third pass without outliers was performed to determine the Eigen vectors. In the statistical analysis, we used the top two Eigen vectors as covariables to correct for population substructure in the association analyses (Price *et al.*, 2006).

Statistical analysis

Of the 570 women who participated in the study, 68 were excluded from the analysis: 18 had a history of recent antiaging invasive procedures and 10 were observably non-Caucasian. In addition, one sample was removed because of insufficient DNA concentration, 12 samples were removed because the DNA was damaged, and nine samples were removed after quality control. Furthermore, 18 outliers appeared during the stratification analysis. Thus, the population investigated for our genome-wide association study was composed of 502 individuals.

The population was first described according to the severity of photoaging, using a series of analyses of variance for quantitative variables and using χ^2 tests for qualitative variables. In addition, Kendall rank correlation coefficients were calculated between age and each outcome variable, and between each pair of outcome variable (Armitage, 1971). Then, for the remaining 795,063 SNPs and 502 women, the associations between the genotypes and skin photoaging were tested. The statistical analysis was performed by a multivariate linear regression (PLINK software; Purcell *et al.*, 2007) in the genotypic mode, taking as covariables the two first Eigenstrat principal components and the potential confounding factors (smoking habits, BMI, hormonal status, lifetime sun exposure intensity, and age). The *P*-values were adjusted by the Bonferroni correction (statistical threshold $=6.28\times10^{-8}$). Finally, for the secondary outcome variables, additional analyses were performed using the same methodology.

Haplotype inference and LD

Haplotype inference was obtained using the rapid and accurate Shape-IT algorithm (Delaneau *et al.*, 2008, 2012). Then, for each SNP exhibiting a significant association, we looked for other SNPs in LD ($r^2>0.8$) in the HapMap population of Western European ancestry (CEU, HapMap data Release 24/phase II November 2008, on NCBI B36 assembly, dbSNP126; available at: http://www.hapmap.org) to identify the genes possibly involved with the associations. A SNP was assigned to a gene if it was located in the gene or in the 2-kb flanking regions (potential regulatory sequence); otherwise, it was considered intergenic. It is important to note that LD in HapMap population of Western European ancestry is very similar in our group of patients.

Bioinformatics exploration

To further explore the signals observed by the GWAS by using bioinformatics exploration we tried to look for modifications in mRNA expression levels (Yang *et al.*, 2010; Nica *et al.*, 2011; Dixon *et al.*, 2007; Zeller *et al.*, 2010), splicing (NetGene2, http://www.cbs.dtu.dk/services/NetGene2/), polyadenylation regions (polyAH, http://linux1.softberry.com/berry.phtml?topic=polyah&group=programs&subgroup=promoter and polyApred, http://www.imtech.res.in/raghava/polyapred/submission.html), transcription factor binding sites (SignalScan, http://www-bimas.cit.nih.gov/molbio/signal/, TESS, http://www.cbil.upenn.edu/cgi-bin/tess/tess?RQ=WELCOME, and TFSearch, http://www.cbrc.jp/research/db/TFSEARCH.html, derived from TRANSFAC database), and miRNA genes or miRNA targets (miRBAse, http://www.mirbase.org/, miRTarBase, http://mirtarbase.mbc.nctu.edu.tw/, MicroCosm Targets, http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Annexe 13. Article sur les variants du gène MC1R et rides du sommeil

MC1R major variants are a risk factor of sleep lines in Caucasian women.
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Article présenté en annexe 13

SHORT REPORT

MC1R major variants are a risk factor of sleep lines in Caucasian women

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Abstract

Background Sleep lines are caused by individual's sleeping positions and should be differentiated from expression wrinkles.

Objective The aim of the study was to investigate possible risk factors for sleep lines on a sizeable sample of middle-aged Caucasian women.

Methods This study involved a sample of 542 French middle-aged women (44 to 70 years old) from Paris area. Three standardized facial photographs (face and profiles) were examined independently by two dermatologists allowing the identification of sleep lines and the evaluation of the severity of several facial skin features. Possible impacts of MC1R gene polymorphisms were tested using logistic regression models.

Results Sixty women (11%) had facial sleep lines and showed generally more than one sleep line. The sleep lines were often located on the forehead, along the nose, on the cheeks and under the eyes, and more rarely on the chin. As expected, the sleep lines were associated with age, and the women with sleep lines showed also more severe signs of skin ageing. After adjustment on possible confounders, the presence of two major diminished function variants of the MC1R gene was identified as a strong risk factor for sleep lines [adjusted odds ratios (AOR) (95% CI): 8.25 (2.62–25.97)].

Discussion/Conclusion The data in the literature are scarce and this study is the first to be conducted on a sizeable sample of women. Our results suggest that genetic variations of MC1R are important determinants of the development of sleep lines.

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Conflict of interests

None.

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Introduction

Over time, damage on elastic fibres and collagen tend to accumulate resulting in deeper folds and wrinkles on the face. Between other etiologies for these damages, photoageing, earth's gravity and the repetition of expressions are known to be major aggravating factors. In 1987, Stegman following the observation of his own face, noticed particular creases affecting the lateral forehead that have been developed during sleep.[1] Consequently, he called them 'sleep creases'. In 1999, Fulton and Gaminchi expanded the clinical description on a small group of individuals and identified new sleep lines, such as oblique lines over the lateral orbicularis muscle, lines along the side of the nose and along the side of the chin, and therefore have preferred to call them 'sleep lines'.[2] In addition, these authors reported links between sleep lines and the location of underlying superficial musculo-aponeurotic system. More recently, Sarifakioglu *et al.*[3] published about this phenomenon, focusing on sleep lines on the forehead of men. Overall, the most notable finding of these studies was the demonstration that the sleep lines are different from wrinkles as sleep lines follow oblique directions, in comparison to

expression lines, as they are not secondary to muscle movements. Besides, they are initially slightly visible and transient, but they persist longer progressively and finally become noticeably visible and permanent.

We recently established that polymorphism in the melanocortin-1 receptor (MC1R) gene, besides its role on skin and hair colour, has a statistically significant impact on skin photoageing, and that carriers of two major diminished function variants show a significantly increased risk of severe photoageing compared with wild type individuals.[4] Therefore, we hypothesized that MC1R gene might be linked with other skin features, such as sleep lines.

To date, the studies on sleep lines have only been based on sparse clinical observations and poorly documented especially in women. Also, the impact of potential risk factors was never investigated. In this context, we conducted a study on a sizeable sample of middle-aged Caucasian women to investigate the potential links between sleep lines and behavioural, phenotypic and MC1R gene polymorphisms.

Material and methods

Material

Study design and population This cross-sectional study was carried out in the context of the SU.VI.MAX cohort (*SUpplémentation en VItamines et Minéraux Anti-oXydants* – antioxidant vitamin and mineral supplements), a longitudinal study, conducted on French middle–aged adult volunteers that focused on the relationships between nutrition and health through main chronic disorders prevalent in industrialized countries.[5,6] This study was conducted in fall/winter 2002–2003 on a sample of 570 middle-aged female volunteers (age range 44–70 years) who lived in Paris area.[4] To be included, volunteers had to be Caucasian and have no history of facial antiageing procedure such as filling agent's injection, laser intervention or plastic surgery. Moreover, women had to follow specific skin care instructions, especially no application of detergent or cosmetic product on the face for at least 12 hours before the study visit. Three standardized high resolution digital images (2008 × 3032 pixels) of the face were taken from each woman (one of the face, one of each profile) with a Kodak DCS 760 digital camera combined with a 105-mm camera lens (Kodak, Paris, France).

Of the 570 volunteers who agreed to participate in the study, 28 were excluded from the analysis because they did not fulfil at least one inclusion criterion: 18 had a history of recent antiageing invasive procedures and 10 were non-Caucasian. So, the final population was composed of 542 volunteers.

Assessment of general and phenotypic data Age (in years), height (in metres), weight (in kilogrammes), smoking habits (never, former, current), hormonal status [no menopausal, menopausal with hormonal replacement therapy (HRT), menopausal without HRT], were obtained on the day of the visit from a medical questionnaire during a standardized interview performed by trained interviewers. Natural hair colour at the age of 20 was ascertained and classified as following: red, blond, light brown, dark brown or black. Eye colour was recorded as being either brown/black, hazel, green or blue/grey. Skin colour was recorded as fair or dark. Skin phototype was recorded according to Fitzpatrick's classification, as follows: 'always burn, never tan': skin type I, 'always burn, then tan': skin type II, 'always tan, sometimes burn': skin type III, and 'always tan, never burn': skin type IV.[7] Body mass index (BMI in kg/m²) was calculated as the weight divided by the square of the height. Body mass index was categorized as underweight–normal (BMI < 25 kg/m²), overweight ($25 \leq$ BMI <30 kg/m²) or obese (BMI ≥ 30 kg/m²), according to the WHO recommendations.[8] Moreover, each participant was asked to complete a self-administered questionnaire relating to her sun exposure behaviour. The following item of the questionnaire (global self-assessment): 'How would you describe the intensity of your skin's exposure to the sun during your lifetime? none, mild, moderate or severe' was used to assess the intensity of lifetime sun exposure.

Assessment of severity of skin ageing features Each set of facial photographs was examined by a trained dermatologist to rate the severity of some skin ageing features using specific ordinal photographic scales: fine lines on the forehead (none-few/some/many), crow's feet (none-slight/moderate/severe), nasolabial folds (none-slight/moderate/severe) and glabellar frown (none-slight/moderate/severe).[9]

Assessment of the outcome variable: presence of sleep lines Afterwards, each set of facial photographs was examined independently by the same dermatologist and by another dermatologist to detect the presence of sleep lines. In case of disagreement, which occurred only three times, the set of photographs was examined again conjointly to reach an agreement. Then, a detailed description of the sleep lines detected was made, including location areas and the side of the face affected.

Detection of MC1R gene variants Blood samples were collected the day of the visit, lysed in NASBA lysis buffer (Organon Teknika BV, Boxtel, the Netherlands) and stored at −80°C until assay. The methods used for identification of the genes polymorphisms have been detailed elsewhere.[4]

Methods

Statistical analyses were performed using SAS® software release 9.1.3 (SAS Institute, Cary, NC, USA). A descriptive analysis of the population was first performed with Pearson's χ^2 independency test or Fisher's exact test when needed (SAS/STAT®,

FREQ procedure options CHISQ and EXACT FISHER). Then, to identify among the phenotypic and behavioural data, the variables associated with the presence of sleep lines, a series of logistic regression analyses adjusted for age were performed. Finally, all the variables individually associated with the presence of sleep lines were entered into a multivariate logistic regression model.[10] Interaction terms were also tested in the final model. The results are expressed as adjusted odds ratios (AOR) together with their 95% confidence interval (95% CI) estimates.

Table 1 Frequency (percentage) of women presenting at least one sleep line on each facial area (some women had sleep lines on more than one facial area)

Total number of women with sleep lines, *N* = 60
Women with sleep lines on the forehead, *n* = 30 (50%)
Women with sleep lines along the nose, *n* = 30 (47%)
Women with sleep lines on the cheek, *n* = 20 (33%)
Women with sleep lines on the area under the eyes, *n* = 18 (30%)
Women with sleep lines on the chin, *n* = 6 (10%)

Results

Among the 542 participants, sleep lines were detected on 60 (11%) women on five areas of the face: forehead, cheeks, chin, nose and under the eyes (Table 1). The majority of the women presented frequently more than one sleep line: 26 (43%) presented only one sleep line, 42% two sleep lines, 13% three sleep lines and one women presented four sleep lines. As expected, women with sleep lines had globally significantly more severe skin ageing features, excepted fine lines on forehead and glabellar frowns, than women with no sleep lines (Table 2).

The relationships between sleep lines and behavioural and phenotypic data are shown on Table 3. The presence of sleep lines is significantly linked with the age of the individuals, women with sleep lines being significantly older. Besides, a significant link was also found with MC1R genotype. The women with two major diminished function variants presented a higher risk to have sleep lines. No significant link was found with smoking habits, hormonal status and self-assessed lifetime sun exposure.

Discussion

The data concerning sleep lines in the literature are scarce, and to our knowledge, this study is the first realized on a sizeable sample of middle-aged Caucasian women. Our findings show that 11% of the women in our sample, aged between 44 and 70 years, had sleep lines, the majority of these women presenting more than one sleep line. The most common sleep lines in our sample were located on the forehead, the nose and the nasolabial folds, while

Table 2 Relationships between age-related skin features and presence of sleep lines (frequency and %)

Variable		Presence of sleep lines				Total		*P**
		No		Yes				
		N = 482		*N* = 60		*N* = 542		
Forehead fine lines	None	41	(8.5%)	3	(5.0%)	44	(8.1%)	0.44
	Some	228	(47.3%)	26	(43.3%)	254	(46.9%)	
	Many	213	(44.2%)	31	(51.7%)	244	(45.0%)	
Glabellar frown	Grade = 0	2	(0.4%)	0	(0.0%)	2	(0.4%)	0.19 F
	Grade = 1	31	(6.4%)	2	(3.4%)	33	(6.1%)	
	Grade = 2	29	(6.0%)	3	(5.0%)	32	(5.9%)	
	Grade = 3	115	(23.9%)	8	(13.3%)	123	(22.7%)	
	Grade = 4	108	(22.4%)	12	(20.0%)	120	(22.1%)	
	Grade = 5	197	(40.9%)	35	(58.3%)	232	(42.8%)	
Crow's feet	Grade = 0	1	(0.2%)	0	(0.0%)	1	(0.2%)	<.001 F
	Grade = 1	30	(6.2%)	1	(1.7%)	31	(5.7%)	
	Grade = 2	138	(28.6%)	3	(5.0%)	141	(26.0%)	
	Grade = 3	185	(38.5%)	18	(30.0%)	203	(37.5%)	
	Grade = 4	99	(20.5%)	24	(40.0%)	123	(22.7%)	
	Grade = 5	29	(6.0%)	14	(23.3%)	43	(7.9%)	
Nasolabial folds	Grade = 0	10	(2.1%)	0	(0.0%)	10	(1.9%)	<0.001
	Grade = 1	40	(8.3%)	3	(5.0%)	43	(7.9%)	
	Grade = 2	59	(12.2%)	1	(1.6%)	60	(11.1%)	
	Grade = 3	114	(23.7%)	9	(15.0%)	123	(22.7%)	
	Grade = 4	179	(37.1%)	25	(41.7%)	204	(37.6%)	
	Grade = 5	80	(16.6%)	22	(36.7%)	102	(18.8%)	

*P value of χ^2 test or of Fisher's exact test (F).

Table 3 Relationships between phenotypic data, behavioural data, genotypic data and presence of sleep lines. The adjusted odds ratios (AOR) are adjusted for age, excepted the age

Variables		Presence of sleep lines		Prob. of Wald test	AOR *	[95% CI] †
		No (n = 482)	Yes (n = 60)			
Age (in years)		57.4‡ ± 6.4	60.2 ± 6.4	0.0019	1.07	[1.03–1.12]
BMI classification	Normal	314§ (88%)	43 (12%)	0.26	1	
	Overweight	124 (89%)	15 (11%)		0.80	[0.43–1.51]
	Obese	44 (96%)	2 (4%)		0.32	[0.07–1.36]
Hormonal status	No menopausal	97 (95%)	5 (5%)	0.57	1	
	Menopausal with HRT	249 (89%)	32 (11%)		1.36	[0.44–4.20]
	Menopausal without HRT	136 (86%)	23 (14%)		1.73	[0.53–5.61]
Eye colour	Light	329 (89%)	42 (11%)	0.86	1.06	[0.59–1.91]
	Dark	148 (89%)	18 (11%)		1	
Hair colour	Red/blond/light	354 (88%)	48 (12%)	0.36	1.36	[0.70–2.67]
	Dark brown/Black	123 (91%)	12 (9%)		1	
Skin colour	Fair	363 (88%)	50 (12%)	0.15	1.70	[0.83–3.49]
	Dark	114 (92%)	10 (8%)		1	
Freckles	Present	195 (88%)	27 (12%)	0.45	1.24	[0.72–2.13]
	None	282 (90%)	33 (10%)		1	
Suntan intensity	None/slight/light	285 (88%)	38 (12%)	0.65	1.14	[0.65–2.00]
	Dark/very dark	192 (90%)	22 (10%)		1	
Sunburn event Frequency	Frequent/constant	131 (88%)	18 (12%)	0.42	1.28	[0.70–2.33]
	None/rare	346 (89%)	42 (11%)		1	
Skin phototype	I-II	234 (89%)	28 (11%)	0.91	0.97	[0.56–1.67]
	III-IV	248 (89%)	32 (11%)		1	
Lifetime sun exposure	None to mild	308 (88%)	42 (12%)	0.50	1	
	Moderate to severe	169 (90%)	18 (10%)		0.82	[0.45–1.48]
Smoking status	No smoker	271 (88%)	37 (12%)	0.90	1	
	Former smoker	157 (90%)	17 (10%)		0.88	[0.48–1.63]
	Current smoker	54 (90%)	6 (10%)		1.06	[0.42–2.71]
MC1R polymorphism	WT/WT	165 (89%)	20 (11%)	0.0011	1	
	Only minor variants	183 (90%)	20 (10%)		0.93	[0.48–1.80]
	One major variant	111 (92%)	10 (8%)		0.77	[0.35–1.73]
	Two major variants	8 (53%)	7 (47%)		8.25	[2.62–25.97]

*Adjusted Odds Ratio.
†95% confidence interval.
‡Mean ± Standard Deviation.
§Frequency and (%).
HRT = hormonal replacement therapy.

sleep lines on the chin were the least frequent. Besides, we found significant links between the presence of sleep lines and both chronological age and more severe age-related skin features. The exact patho-mechanisms underlying the development of sleep lines remain to be elucidated. However, dermal changes are known to be linked to collagen, elastic fibres and intercellular matrix damage, and are more prominent with age. Moreover, a number of phenotypic features are also known to influence the degree of skin ageing, notably skin colour and skin phototype.[11,12] Based on this evidence, it can be expected that the genetic background that influences the phenotype may take part in the process of development of sleep lines. Hence, recent findings have shed light on the role of MC1R polymorphism on skin colour, lentigines and the severity of facial photoageing, whereas studies investigating the impact of polymorphisms of other genes such as elastin, collagen, MMP1 and MMP9 on time-dependent skin changes are still expected.[4,13,14]

In conclusion, the onset and development of sleep lines are probably related to sleeping behaviour, as initially suggested by Stegman.[1] However, we clearly found a link between MC1R gene polymorphisms, i.e. the presence of two major diminished function variants, and the presence of sleep lines. This finding may shed light on the possible common pathways between skin photoageing and sleep lines.

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Approche épidémiologique du rôle des acides gras sur le vieillissement cutané dans le cadre de l'étude SU.VI.MAX

Résumé

L'éventuel effet photo-protecteur sur la peau des lipides a été jusqu'à présent peu examiné. L'objectif de cette thèse était d'étudier le lien entre l'apport alimentaire en acides gras mono-insaturés (AGMI) et en acides gras polyinsaturés (AGPI) n-3 et le photo-vieillissement cutané du visage chez une large population d'hommes et de femmes âgés entre 45 et 60 ans. Ces travaux ont mis en évidence un lien inverse entre les apports en en huile d'olive et la sévérité du photo-vieillissement. Lors de l'étude des AGPI n-3, un lien inverse a été mis en évidence entre la sévérité du photo-vieillissement et les apports en acide α-linolénique (ALA) des huiles végétales et des fruits et légumes chez les hommes. Chez les femmes un lien inverse a été trouvé avec les apports en acide eicosapentaénoïque (EPA) et une tendance avec les ALA des huiles végétales. Ces travaux soutiennent les recommandations en faveur d'un régime riche en huile d'olive et en AGPI n-3 comme celui du régime méditerranéen.

Mots-clés : Epidémiologie, photo-vieillissement cutané, acides gras, nutrition

Résumé en anglais

The possible effect of dietary lipids on the photoprotection of the skin has been few investigated so far. The aim of this thesis was to investigate the links between dietary monounsaturated fatty acids (MUFA) and n-3 polyunsaturated fatty acids (n-3 PUFAs) on facial skin photoaging in a large population of men and women aged between 45 and 60 years old. An inverse association was found between intakes of olive oil and the severity of photoaging. Concerning n-3 PUFAs, severe photoaging was inversely associated in men with intake of α-linolenic acid (ALA) from both vegetable oil and fruits & vegetable. In women, an inverse relationship was found with the intake of eicosapentaenoic acid and a tendency with ALA from vegetable oil. These findings support the recommendations for a diet rich in olive oil and n-3 PUFAs such as the Mediterranean diet.

Key-words: Epidemiology, cutaneous photoaging, fatty acids, diet