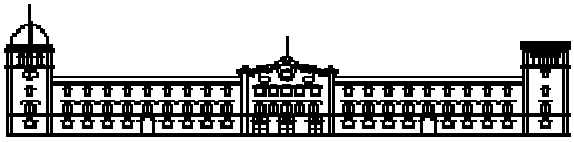
Fitoesterols i Salut Cardiovascular

Tesi presentada per Verònica Escurriol Martínez

Directors: Emili Ros Rahola i Montserrat Cofan Pujol



Tesi Doctoral

UNIVERSITAT DE BARCELONA

Facultat de Medicina

FITOESTEROLS I SALUT CARDIOVASCULAR

Tesi presentada per

Verònica Escurriol Martínez

Per optar al grau de

Doctor per la Universitat de Barcelona

Directors: Emili Ros Rahola i Montserrat Cofan Pujol

Tesi realitzada a la Unitat de Lípids,

Servei d'Endocrinologia i nutrició,

Hospital Clínic de Barcelona – IDIBAPS

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INFORME DELS DIRECTORS DE TESI

Barcelona, a 25 de maig del 2009.

Emili Ros Rahola, Doctor en Medicina i Consultor Sènior de l 'Unitat de Lípids, Servei d'Endocrinologia i Nutrició, Institut Clínic de Malalties Digestives i Metabòliques, i Montserrat Cofán Pujol, Doctora en Farmàcia i Investigadora de la Unitat de Lípids, Institut Clínic de Malalties Digestives i Metabòliques.

CERTIFIQUEN:

Que la tesi doctoral **FITOESTEROLS I SALUT CARDIOVASCULAR**, presentada per Verònica Escurriol Martínez per a optar al grau de Doctor per la Universitat de Barcelona ha estat realitzada sota la nostra direcció i compleix tots els requisits necessaris per a ser defensada davant el Tribunal d'avaluació corresponent.

Emili Ros Rahola **Monsterrat Cofan Pujol**

AGRAÏMENTS

La meva tesi va començar a finals del 2003 a l'IMIM on vaig tenir la sort de treballar amb l'equip del Jaume Marrugat. Allà vaig poder aprendre i compartir moments molt especials i entranyables amb Dani, Montse, Marta, Jaume, Roberto, Helmut, Maribel, Joan, Isaac, Susana i Sophie. Us envio una abraçada molt forta. I allà també vaig tenir la sort de conéixer a la Glòria, una molt bona amiga amb la qual compartim i aprenem plegades per la vida. Gràcies per estar aquí i per compartir la causalitat.

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...la vida no debe ser vista, debe ser vivida,

exprimida, rebañada, como un plato de comida,

racionada, que cuando se te acabe el tiempo no te falte nada...

Folie à trois

ABREVIACIONS

ABCA1	ATP-binding cassette A1
ABCG5 i G8	ATP-binding cassette G5 i G8
ACAT	Cholesterol O-acyl transferase
AGM	Àcids grassos monoinsaturats
AGP	Àcids grassos poliinsaturats
AGS	Àcids grassos saturats
CORA	Coronary Risk factors for Atherosclerosis in women study
EPIC	European Prospective Investigation into Cancer and Nutrition
FDA	Food and Drug Administration
HDL	Lipoproteïnes d'alta densitat
HMG CoA	Hidroximetil-glutaril coenzim A
IDF	International Diabetes Federation
IDL	Lipoproteïnes de densitat intermitja
LASA	Longitudinal Aging Study Amsterdam
LDL	Lipoproteïnes de baixa densitat
LXR	Liver X receptor
NCEP- ATP III	National Cholesterol Education Program Adult Treatment Panel III
NPC1L1	Niemann-Pick C1 Like 1
PROCAM	Munster Heart Study
REGICOR	Registre Gironí del Cor
rLDL	Receptor de LDL
VLDL	Lipoproteïnes de molt baixa densitat

1. INTRODUCCIÓ

1.1. Dieta i salut cardiovascular

La nostra salut està molt relacionada amb els hàbits alimentaris o la dieta habitual. Els aliments no són simples fonts d'energia, sinó que són un conjunt de molècules bioactives que poden interaccionar amb gens, proteïnes i molècules del nostre organisme. Per tant, el consum habitual d'una determinada dieta o aliments pot prevenir o provocar malalties. Les malalties multifactorials, com el càncer o la malaltia cardiovascular, en són un bon exemple. En els països occidentals, l'elevada prevalença de malaltia cardiovascular és atribuïda en gran mesura a l'estil de vida contemporani, que és sovint sedentari, i inclou una dieta rica en greix saturat i sucre, i baixa en àcids grassos poliinsaturats (AGP) omega3, fruites, vegetals i fibra (1).

L'interès principal d'aquest treball és l'estudi de la dieta habitual, i dels esterols vegetals o fitoesterols com a components específics de la mateixa, en relació a la salut cardiovascular en una població espanyola. Igual que d'altres països situats al voltant del mar Mediterrani, el nostre país s'ha caracteritzat per tenir unes taxes de malaltia cardiovascular notablement més baixes que les dels països del Centre i Nord d'Europa o Nord America. S'ha descrit un gradient nord-sud de taxes de mortalitat cardiovascular en l'Europa occidental, on la prevalença de factors de risc clàssics no s'associa a les taxes de mortalitat cardiovascular esperades (2;3). Les dades recollides en l'estudi Registre Gironí del Cor (REGICOR) mostren com, amb els mateixos factors de risc, la probabilitat de tenir un d'esdeveniment coronari és menor que la que es podria atribuir segons els criteris de les taules de Framingham (4). En la recerca dels factors que propicien aquesta protecció cardiovascular, en els últims anys s'ha considerat com a molt important la dieta Mediterrània. Troballes recents en estudis de cohort Europeus suggereixen que un elevat grau d'adherència a la dieta Mediterrània s'associa amb una reducció de la mortalitat (5-7).

Els aliments derivats de plantes són components importants de la dieta tradicional Mediterrània (8), que es caracteritza per una elevada ingesta de vegetals, fruites i oli d'oliva; una ingesta moderada de peix i alcohol, sobretot vi; i una baixa ingesta de productes làctics, carn i dolços (9). Les recomanacions generals per seguir una dieta saludable s'inspiren en aquesta dieta degut al seu elevat contingut en àcids grassos insaturats, calci, fibra i antioxidants naturals (10). L'evidència ecològica suggereix que, en comparació amb la dieta occidental, aquest patró alimentari està associat a concentracions més baixes de colesterol sèric (11), degut en part a una baixa ingesta d'àcids grassos saturats (AGS) i a l'abundància d'àcids grassos monoinsaturats (AGM) (12) i fibra (13). Hi ha altres factors que poden influenciar el colesterol del sèrum, entre ells els esterols vegetals o fitoesterols. Degut a que la relació entre la mortalitat de pacients amb malaltia cardiovascular i les concentracions de colesterol sèric és progressiva i contínua (14), les guies del National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) promouen canvis en l'estil de vida i els hàbits dietètics dirigits en gran part a reduir la colesterolèmia per així disminuir el risc cardiovascular (15). Ja que el colesterol de la sang és un dels principals factors de risc cardiovascular modificables, cal destacar els fitoesterols com uns nutrients particulars amb un notable potencial hipocolesterolemiant, donat que aquests nutrients afecten particularment l'absorció intestinal del colesterol, cal fer primer una breu revisió d'aquesta important via metabòlica per l'adquisició de colesterol per l'organisme.

1.2. Colesterol

1.2.1. Colesterol i salut cardiovascular

La relació entre les concentracions sèriques de colesterol i malaltia coronària és coneguda des de fa molts anys. El primer gran estudi clínic de prevenció cardiovascular dut a terme amb un fàrmac hipocolesterolemiant fa 20 anys (*Lipid Research Clinics Coronary Primary Prevention Trial*) va confirmar la reducció d'incidència de malaltia

cardíaca coronària en relació amb disminució de les xifres de colesterol (16). Des de llavors, nombrosos estudis epidemiològics i clínics han confirmat l'associació positiva entre el colesterol total i el colesterol transportat en lipoproteïnes de baixa densitat (LDL) (17) i negativa del colesterol transportat en lipoproteïnes d'alta densitat (HDL) (18) i el risc de desenvolupar arteriosclerosis i malaltia cardíaca coronària.

1.2.2. Metabolisme del colesterol

En els mamífers el colesterol pot ser obtingut a través de l'absorció del colesterol de la dieta (via exògena) o de la síntesi de novo en les cèl·lules de l'organisme (via endògena). Aquesta molècula és necessària per la síntesi d'hormones esteroides i àcids biliars, i és un component imprescindible de les membranes cel·lulars. Degut a la seva insolubilitat en medi aquós, per poder ser transportat pels fluids biològics, el colesterol s'uneix a fosfolípids i proteïnes formant les lipoproteïnes que són agregats polimoleculars esfèrics amb una capa externa hidrosoluble que conté fosfolípids, colesterol lliure i proteïnes de transport lipídic (apolipoproteïnes), i una part interna insoluble amb triglicèrids i èsters de colesterol (**Figura 1**). Podem distingir cinc grans grups de lipoproteïnes: quilomicrons, lipoproteïnes de molt baixa densitat (VLDL), lipoproteïnes de densitat intermitja (IDL), LDL i HDL, que tenen un tamany i una composició fitoquímica diferent. (**Figura 2**).

Figura 1. Composició d'una lipoproteïna

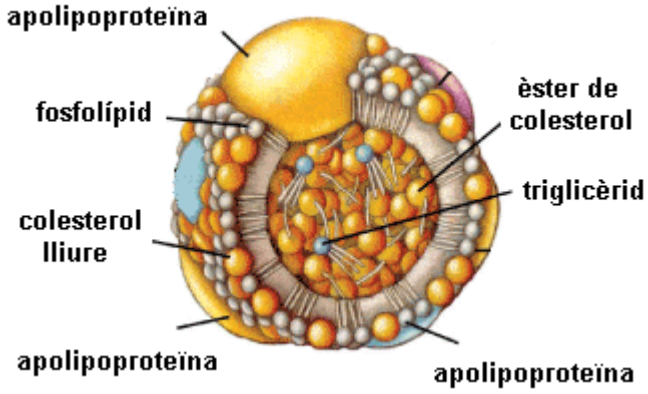
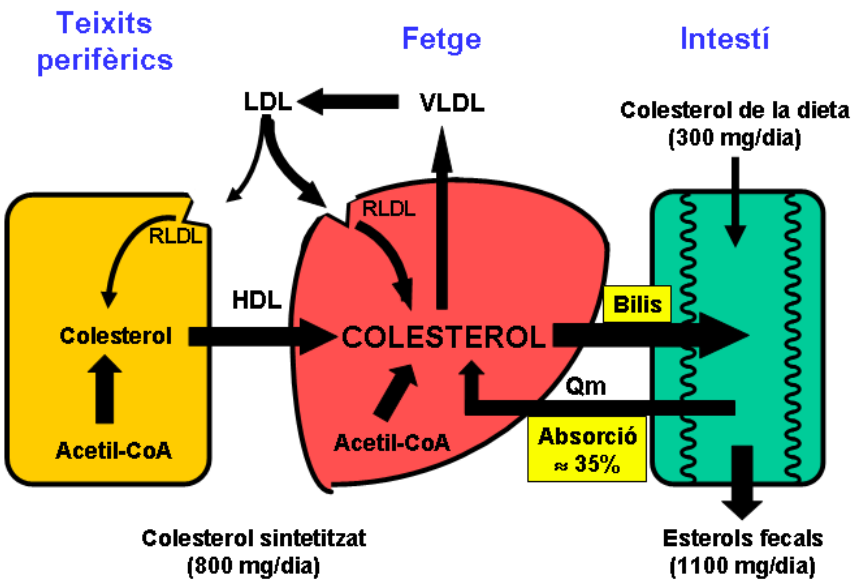


Figura 2. Composició de les lipoproteïnes

	Qm	VLDL	IDL	LDL	HDL
Diàmetre (nm)	500	43	27	26-27	6,5-9,5
Composició (% massa total)					
Proteïna	2	10	18	25	55
triglicèrids	85	50	26	10	4
Colesterol	4	20	34	37	17
Fosfolípid	9	20	22	20	24

L'homeòstasi del colesterol es manté equilibrant la síntesi endògena, l'absorció intestinal i la secreció d'àcids biliars i colesterol (19). Donat que els àcids biliars són reabsorbits eficientment i una part del colesterol biliar és reabsorbit a l'intestí, el balanç global del colesterol depèn de que les entrades (síntesi i dieta) s'equilibrin amb les pèrdues (excreció fecal) (**Figura 3**). El colesterol excretat amb la femta depèn de l'eficiència de l'absorció intestinal del colesterol biliar i dietètic. Per això la regulació de l'absorció intestinal del colesterol és de gran interès com a diana terapèutica per reduir les xifres de colesterol (20).

Figura 3. Homeòstasi global del colesterol a l'organisme.



1.2.2.1. Síntesi endògena del colesterol

Totes les cèl·lules de l'organisme tenen la capacitat de sintetitzar colesterol, però el fetge n'és l'òrgan central. Existeixen tres vies per adquirir el colesterol (**Figura 3**) (19):

1. Entrada de colesterol de la dieta a través dels remanents de quilomicrons.
2. Captació de les LDL que contenen colesterol de la circulació i teixits extrahepàtics mitjançant els receptors de LDL (rLDL), però també captació del colesterol en forma d'HDL i de remanents de VLDL per receptors específics.
3. Síntesi de l'esteroide a partir de l'acetil-CoA sota el control de l'enzim limitant 3-hidroximetil-glutaril coenzim A (HMG CoA) reductassa (la via inhibida per les estatines).

Donada la complexitat d'aquest tema i l'interès de la tesi en els fitoesterols que afecten principalment l'absorció intestinal del colesterol, no entrarem més en detall en aquest aspecte del metabolisme del colesterol.

1.2.2.2. Absorció intestinal del colesterol

L'absorció del colesterol és un procés complex, degut a la insolubilitat i hidrofobicitat d'aquesta molècula, que requereix diversos passos: emulsificació, hidròlisi de l'enllaç èster (quan està esterificat) per una hidrolassa pancreàtica específica, solubilització micel·lar, absorció en el jejú proximal, reesterificació en el citoplasma dels enterocits i transport a la limfa en quilomicrons (20;21). A part del colesterol dels aliments (uns 300 mg diaris en una dieta occidental), el colesterol intestinal també procedeix de dues fonts endògenes: la bilis, que contribueix a uns 1000 mg per dia, i la descamació de l'epiteli intestinal, que aporta una part molt petita (**Figura 3**).

El procés d'absorció del colesterol és particular degut a la seva relativa ineficiència, ja que en promig s'absorbeix només un 40%, podent variar entre el 30 i el 80% (22). El colesterol absorbit té com a destí final el fetge, que és el principal òrgan responsable de la producció i aclariment de les LDL (19;21); per tant, qualsevol variació en l'eficiència de

l'absorció intestinal d'aquest esteroide influeix en les concentracions sèriques del colesterol LDL. En humans, hi ha una correlació positiva entre les concentracions plasmàtiques de LDL i l'eficiència de l'absorció intestinal de colesterol. Malgrat això, les variacions del colesterol de la dieta sovint no estan associades amb canvis de les concentracions circulants de LDL i, per tant, s'han estudiat altres factors que influencien l'absorció del colesterol. Aquests inclouen factors dietètics, biliars, luminals, cel·lulars, genètics o farmacològics (20;23).

No entrarem en detall en tots aquests factors degut a la seva complexitat, però sí que comentarem un factor genètic al qual s'havia atribuït un important efecte sobre l'absorció del colesterol: el genotip de l'apoE (20;24). Aquesta apolipoproteïna té un al·lel comú (E3) i dos al·lels variants (E4 i E2), i participa en la captació en el fetge dels remanents dels quilomicrons que contenen colesterol d'origen intestinal mitjançant la interacció amb receptors hepàtics (25). S'havia suggerit que la possessió de l'al·lel E4 afavoria l'absorció intestinal del colesterol i l'al·lel E2 la reduïa, però recentment s'han aportat evidències en contra de l'associació del genotip de la apoE amb l'eficiència d'absorció intestinal de colesterol (26).

Entre els factors dietètics trobem els fitoesterols (tema principal d'aquest treball), que s'han relacionat amb una disminució de l'absorció intestinal de colesterol en estudis experimentals i clínics (27;28) i en els que ens centrarem en el següent apartat.

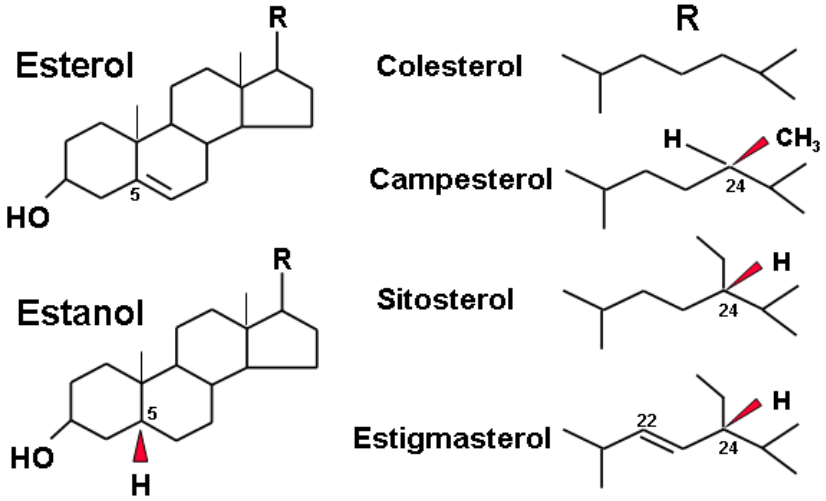
1.3. Esterols vegetals o fitoesterols

1.3.1. Definició, tipus i fonts

Els esterols que ingerim amb els aliments es composen de colesterol d'origen animal i esterols vegetals o fitoesterols. Els fitoesterols són els esterols de les plantes, amb una estructura similar i una funció anàloga a la del colesterol als vertebrats (29). En les plantes, s'ha descrit que aquestes molècules actuen com a components estructurals de les membranes vegetals i com intermediàries per sintetitzar cel·lulosa (30). Els esterols

vegetals són membres de la família dels triterpens i la seva estructura és similar a la del colesterol però inclou un grup metil o etil en el C-24. Dins del grup dels esterols vegetals trobem dues categories, els esterols que tenen un doble enllaç en posició 5 i els estanols, que no tenen aquest doble enllaç (31). S'han descrit més de dues-centes espècies d'esterols a les plantes, essent el sitosterol i el campesterol les més abundants (29) (**Figura 4**).

Figura 4. Formes moleculars del colesterol, esterols i estanols vegetals



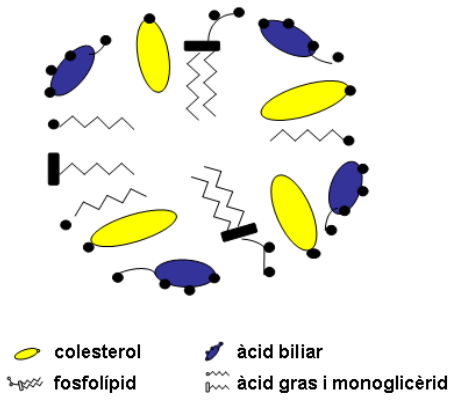
A diferència del colesterol, els fitoesterols no es poden sintetitzar a l'organisme humà, per tant, la seva adquisició ha de ser a través de la dieta. Són components importants dels cereals integrals, fruites seques, llavors i olis derivats i, per tant, d'una dieta rica en productes vegetals (29). La font més concentrada són els olis vegetals, com els de blat, girasol, soja i colza (contenen entre un 0.1 i un 1%) (28) però, amb les excepcions dels carbohidrats altament refinats i els productes animals, quasi tota la resta d'aliments contribueix a la ingesta de fitoesterols de la dieta. En la dieta occidental la ingesta diària de fitoesterols s'estima en 150-400 mg i és similar a la ingesta de colesterol, si bé pot ser superior en dietes vegetarianes (32).

1.3.2. Metabolisme dels fitoesterols

Malgrat que l'estructura química del colesterol i dels esterols vegetals és similar, el seu metabolisme difereix. Les concentracions sèriques dels fitoesterols són de l'ordre de 1000 vegades inferiors a les de colesterol i les d'estanols són encara més baixes (33). Això és degut a vàries raons. Com s'ha comentat, els fitoesterols no es poden sintetitzar a l'organisme i els hem d'adquirir amb la dieta. A més, la mucosa intestinal absorbeix fitoesterols amb menor eficiència que colesterol. A diferència del colesterol, els fitoesterols no són transformats en àcids biliars i, finalment, la seva excreció per la bilis és més ràpida que la del colesterol. Entrarem en més detall en alguns d'aquests conceptes tot seguit.

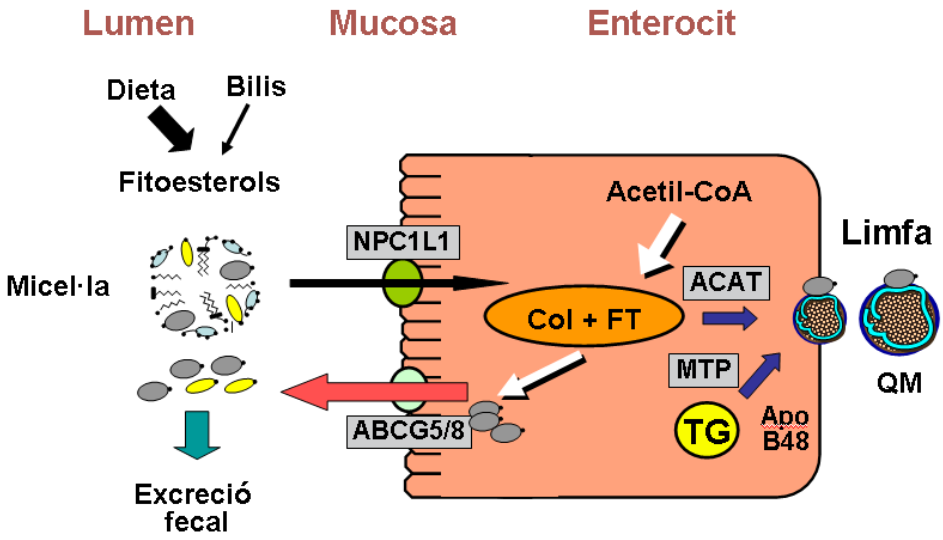
Per tal de poder-se absorbir a l'intestí, els esterols, que son molècules molt hidrofòbiques, han de ser solubilitzats, fet que s'aconsegueix integrant-los en estructures lipídiques anomenades micel·les (**Figura 5**), formades per àcids biliars, fosfolípids, monoglicèrids i àcids grassos, que actúen al lumen intestinal transportant lípids insolubles (colesterol, fitoesterols, vitamines liposolubles) de manera semblant a com ho fan les lipoproteïnes a la sang. La taxa d'absorció de fitoesterols (0.4%-3.5%) difereix de la del colesterol (35%-60%) i varia inversament amb la longitud de la cadena hidrocarbonada lateral característica de cadascun d'ells. En promig, el campesterol i el β-sitosterol s'absorbeixen 3 i 5 vegades menys que el colesterol, respectivament (34).

Figura 5. Estructura d'una micel·la mixta



L'absorció dels fitoesterols en l'intestí és fa a través de la proteïna transportadora *Niemann-Pick C1 Like 1* (NPC1L1) (35) (**Figura 6**). Aquest transportador s'inhibeix pel fàrmac ezetimiba i la reducció de l'absorció de colesterol s'associa també a una reducció de les xifres de fitoesterols (36). Però dins l'enterocit, un dels factors que més influencia l'absorció de fitoesterols és l'activitat dels transportadors *ATP-binding cassette G5 i G8* (ABCG5 i G8) que actuen en tàndem i permeten l'eflux d'esterols de les cèl·lules intestinals. Aquests transportadors eliminen colesterol i, sobre tot, fitoesterols per evitar la seva acumulació, retornant gran part dels fitoesterols al lumen intestinal per a la seva eliminació (37). De fet, la variabilitat genètica d'aquests transportadors contribueix a les xifres sèriques de fitoesterols (38) i es coneix que les mutacions d'aquests gens en situació d'homozigositat o heterozigositat composta causen la β-sitosterolèmia (39). Aquesta rara malaltia autosòmica recessiva es caracteritza per una hiperabsorció intestinal d'esterols, un augment de les concentracions de fitoesterols plasmàtics, xantomes i arteriosclerosi accelerada (40).

Figura 6. Mecanismes d'absorció i transport intestinal dels fitoesterols (I)



NPC1L1: Niemann-Pick C1 Like 1; ABCG5 i G8: ATP-binding cassette G5 i G8; Col: colesterol; FT: Fitoesterols; ACAT: cholesterol O-acyl transferase; MTP: microsomal triacylglycerol transfer protein; QM: quilomicró.

Un altre possible factor que pot explicar la baixa absorció dels fitoesterols és la *cholesterol O-acyl transferase* (ACAT), ja que les petites quantitats de fitoesterols que han aconseguit escapar a la devolució al lumen intestinal són incorporades als quilomicrons i transportades al fetge. Per poder-se incorporar als quilomicrons però, els esterols han d'estar esterificats. L'ACAT és la proteïna responsable d'esterificar-los i té una baixa afinitat pels fitoesterols, el que determina que la proporció de fitoesterols que es poden incorporar als quilomicrons sigui escassa (41). Un cop al fetge, els fitoesterols són excretats a la bilis mitjançant els mateixos transportadors ABCG5 i 8, aquest cop situats a la membrana canalicular dels hepatòcits (37). La taxa d'excreció és inversa a la d'absorció, de manera que el β-sitosterol s'excreta millor que el campesterol, i aquest, més ràpid que el colesterol (42). Els fitoesterols que han escapat de l'excreció biliar són segregats pels hepatòcits a la circulació en les VLDL i després transportats de nou al fetge per les LDL. Aquest mecanisme no ha estat comprovat directament però s'infereix de la seva distribució en les lipoproteïnes sèriques (43).

1.3.3. Efectes dels esterols vegetals sobre el metabolisme del colesterol: mecanisme d'acció

Existeix una gran variabilitat inter-individual en l'eficiència de l'absorció intestinal del colesterol. Malgrat i l'existència de diverses proteïnes de transport del colesterol i que llur variabilitat genètica pot influenciar-ne l'absorció (38;44), els factors dietètics també hi juguen un paper important i els fitoesterols en concret, són un dels factors dietètics que poden modular-la (20;23).

1.3.3.1. Efectes dels fitoesterols sobre el metabolisme del colesterol

L'efecte hipocolesterolèmic dels fitoesterols reduint tant les concentracions de colesterol total com les de colesterol LDL, sense afectar les concentracions de HDL o amb una tendència a augmentar-les, està ben establert mitjançant abundants evidències experimentals i d'estudis clínics, tant en població general com en individus

hipercolesterolèmics o diabètics (29;31;45-49). Les primeres observacions sobre l'efecte hipocolesterolèmic dels fitoesterols es van fer fa més de 50 anys quan es van alimentar pollastres amb llavors de soja i es va observar com disminuïen les concentracions plasmàtiques de colesterol (50). Com a mecanisme d'acció s'han proposat diferents possibilitats. Els esterols vegetals afecten l'absorció intestinal del colesterol, la seva síntesi i els sistemes d'eliminació (46).

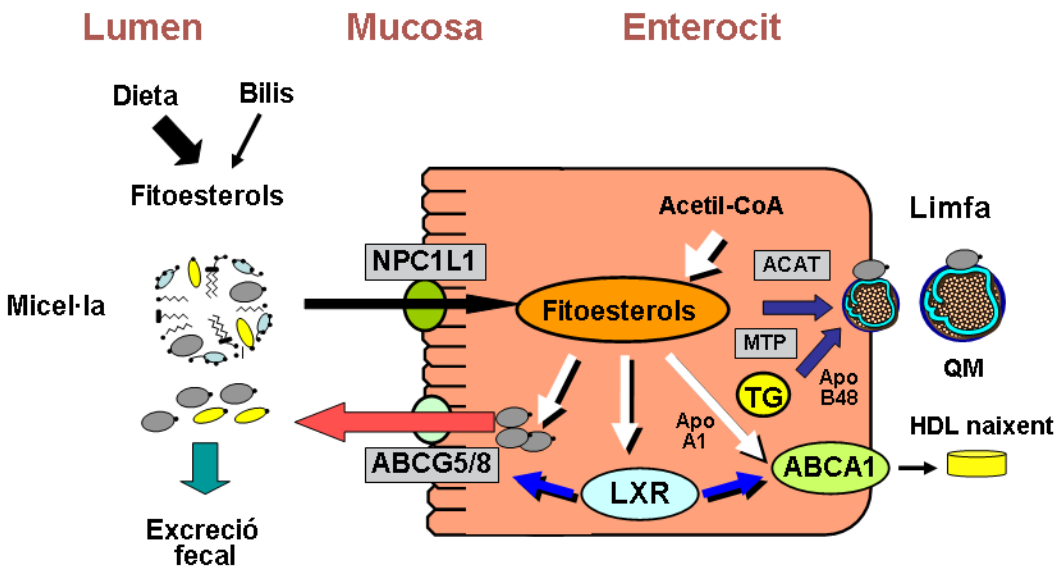
L'efecte de la inhibició de l'absorció intestinal del colesterol, tant del procedent de la dieta (uns 300 mg/dia) com del colesterol endògen procedent de la bilis (uns 1000 mg/dia), ha estat molt estudiat (51). Com ja s'ha comentat, per tal d'inhibir l'absorció intestinal de colesterol, els fitoesterols s'han d'integrar a les micel·les per tal de poder ser absorbits. L'estructura molecular dels fitoesterols els confereix més hidrofobicitat que el colesterol i, per tant, més afinitat per les micel·les intestinals. La disminució d'incorporació del colesterol en les micel·les per competició dels fitoesterols limita la quantitat de colesterol disponible per ser absorbit (31).

Altres estudis han suggerit que els fitoesterols no han de ser presents necessàriament al lumen intestinal junt amb el colesterol per inhibir la seva absorció i que exercirien efectes moleculars dins els enterocits i hepatòcits (52). En l'enterocit, els fitoesterols podrien reduir l'absorció de colesterol mitjançant la reducció de l'expressió del transportador NPC1L1, però aquest efecte no estaria mediat per canvis transcripcionals (53).

Un dels mecanismes per reduir la quantitat de colesterol absorbit és l'augment de l'expressió de transportadors implicats en l'excreció del colesterol. El *liver X receptor* (LXR), un receptor nuclear, és un sensor d'esterols que respon a l'excés de colesterol intracel·lular a través de l'activació o repressió de gens diana involucrats en l'homeòstasi del colesterol (54). Els fitoesterols actuen com a lligands del receptor LXR en experiments in vivo i in vitro. La seva activació i posterior augment transcripcional dels transportadors *ATP-binding cassette A1* (ABCA1) i ABCG5/G8 s'ha proposat com un dels mecanismes que participen en la reducció d'absorció intestinal de colesterol (55) (**Figura**

7). En cèl·lules Caco-2, un model cel·lular per l'estudi del metabolisme intestinal humà, s'ha trobat que una mescla de micel·les enriquides amb sitostanol és un inductor potent de l'expressió del transportador ABCA1 i els autors van concloure que els estanols (i possiblement els esterols) augmentaven l'excreció de colesterol al plasma (a les partícules HDL neixents) mitjançant l'ABCA1 (56). Però estudis amb ratolins deficients en ABCA1 i ABCG5/G8 no han observat una disminució d'absorció de colesterol en aquests animals (57;58), evidenciant que aquests transportadors no són dianes dels fitoesterols, al menys en ratolí (55).

Figura 7. Mecanismes d'absorció i transport intestinal dels fitoesterols (II)



NPC1L1: Niemann-Pick C1 Like 1; ABCG5 i G8: ATP-binding cassette G5 i G8; ACAT: cholesterol O-acyl transferase; MTP: microsomal triacylglycerol transfer protein; LXR: liver X receptor; ABCA1: ATP-binding cassette A1; QM: quilomicrons.

Un mecanisme proposat per explicar l'increment de la taxa d'excreció de colesterol és que els fitoesterols poden reduir l'esterificació intracel·lular del colesterol per afectació de l'activitat ACAT, una proteïna transmembrana present als enterocits que, com ja s'ha comentat, juga un paper important en l'absorció de colesterol, ja que l'esterifica perquè pugui ser utilitzat en la síntesi de quilomicrons. Els fitoesterols són un mal substrat per a aquesta proteïna i mitjançant la competició amb el colesterol, reduirien la quantitat de

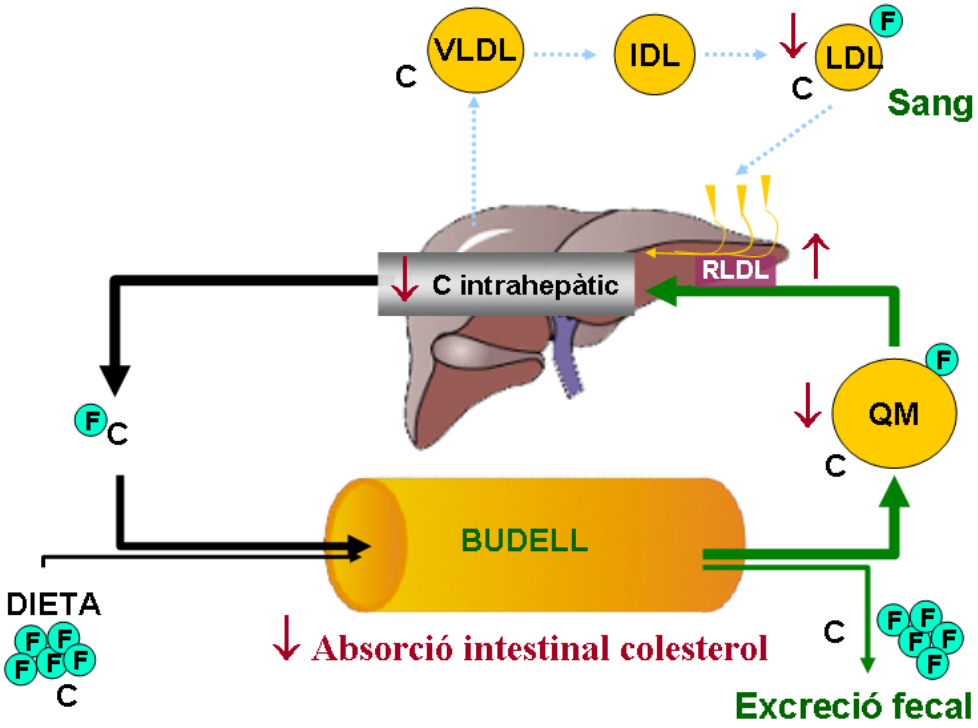
colesterol esterificat i, per tant, exportat al fetge amb els quilomicrons (41). Un estudi recent amb cèl·lules Caco-2 ha demostrat una reducció del contingut en colesterol esterificat i de la secreció de quilomicrons quan les cèl·lules es van incubar amb concentracions suprafisiològiques de fitoesterols (55). Els esterols no esterificats es secreten de nou al lumen intestinal pels transportadors ABCG5/8 (55). En el fetge, la competició dels fitoesterols amb l'ACAT i la subseqüent acumulació de colesterol no esterificat, junt amb un augment de l'expressió dels transportadors ABCG5 i 8, donaria lloc a un increment de l'excreció de colesterol biliar, que a la vegada limitaria l'absorció intestinal d'esterols (55;59).

Els fitoesterols però, poden influenciar el metabolisme del colesterol més enllà de la interferència amb l'absorció/excreció intestinal. Per exemple, una injecció subcutània setmanal de fitoesterols en hàmsters va reduir les xifres de colesterol en un 22% (60), assenyalant un efecte sistèmic. Un estudi en cèl·lules Caco-2 va demostrar una clara inhibició competitiva de la biosíntesi de colesterol quan aquestes cèl·lules s'incubaven amb concentracions fisiològiques d'estigmasterol o altres esterols que contenien un diè a C-22, però no amb β-sitosterol o campesterol (61). Malgrat i que l'estigmasterol és un component menor dels fitoesterols, els resultats d'aquest estudi poden tenir rellevància fisiològica, ja que el bloqueig de la biosíntesi del colesterol pot inhibir la proliferació cel·lular i la progressió del cicle cel·lular (62). En estudis *in vitro* en cèl·lules Caco-2 incubades amb β-sitosterol es va observar una disminució de l'expressió i massa de HMG-CoA reductassa i per tant, una reducció de la síntesi de colesterol (63). Encara i que la rellevància fisiològica *in vivo* no està investigada, els pacients sitosterolèmics mostraven una reducció de l'activitat de HMG-CoA reductasa ileal i de la síntesi de colesterol (64), suggerint que només grans quantitats de fiotesterols produirien aquests efectes *in vivo*. Per altra banda, la reducció d'absorció intestinal de colesterol produeix un augment de l'activitat HMG-CoA reductasa del fetge, i per tant, un increment en la síntesi de colesterol per tal de mantenir l'homeòstasi del colesterol a l'organisme (19).

1.3.3.2. Efectes dels fitoesterols sobre el metabolisme lipídic

La reducció de l'absorció de colesterol pels fitoesterols i la conseqüent disminució de la quantitat de colesterol que arriba al fetge té efectes compensatoris, augmentant la producció endògena de colesterol (65), però també l'expressió de receptors LDL (66), i això augmenta l'aclariment de LDL i IDL de la circulació, i reduïnt per tant la colesterolèmia. Degut a que les IDL són precursores de les LDL, també disminueix la producció de LDL, com s'ha demostrat mitjançant LDL radiomarcades (65) (**Figura 8**).

Figura 8. Metabolisme dels fitoesterols



C: colesterol; F: fitoesterol; QM: quilomicrons; RLDL: receptor de LDL.

1.3.3.3. Efectes dels fitoesterols sobre els carotenoides i vitamines liposolubles

La principal preocupació del consum de fitoesterols en dosis farmacològiques amb aliments funcionals és que s'associa a una reducció de les concentracions plasmàtiques de carotenoides, així com altres vitamines liposolubles (E, K i D). Els carotenoides, a més de ser precursors de la vitamina A, actuen com a neutralitzadors de radicals lliures, antioxidants i anticarcinogènics. Malgrat i que no s'han descrit reduccions fora dels rangs

fisiològics d'aquests components, és aconsellable acompanyar la ingesta d'aliments enriquits amb fitoesterols amb un major consum de fruites i verdures riques en carotenoides (de color verd fosc, groc i taronja) i tenir en compte que el seu consum podria ser un problema per persones amb un estat subòptim de vitamina A (67;68).

1.3.4. Aliments enriquits amb fitoesterols

El recent interès comercial sobre els esterols vegetals o fitoesterols és degut al seu efecte hipocolesterolemiant amb reducció selectiva del colesterol LDL, sense altres efectes adversos col·laterals. Des de mitjans dels anys 90 es comercialitzen en diferents països aliments enriquits amb fitoesterols pel control de la colesterolèmia. La inhibició de l'absorció de colesterol mitjançant dosis de grams de fitoesterols incorporats en varis aliments és una estratègia no farmacològica ben establerta per reduir el colesterol (69). L'administració de 1,5 a 2,5 g diaris de fitoesterols disminueix el colesterol LDL un promig d'un 10% (46), mentre que dosis majors no s'associen a un efecte superior (70). Aquest efecte reductor és el mateix si s'ingereix una quantitat determinada de fitoesterols una o vàries vegades al dia (69).

Un metanàlisi recent de 41 estudis d'intervenció sobre l'eficàcia i la seguretat de la suplementació de la dieta amb esterols vegetals va arribar a la conclusió de que el seu us en el context d'una dieta saludable és ideal per reduir el colesterol LDL en persones amb risc elevat de desenvolupar malaltia cardiovascular (68). Actualment es poden trobar al mercat diversos productes enriquits amb fitoesterols. En un inici eren aliments grassos com margarina, maionesa, formatge per untar, llet o iogurt. Recentment s'han incorporat en aliments baixos en greix com begudes làctiques i iogurts desnatats, sucs, pa, cereals, xocolata o productes càrnics (67).

1.3.4.1. Seguretat del consum de fitoesterols

La seguretat alimentària es basa en assegurar la qualitat dels aliments. El mètode que s'utilitza és l'anàlisi del risc d'un determinat aliment, és a dir, la probabilitat i gravetat d'un efecte advers per a la salut (67).

Els fitoesterols compten amb el referendo europeu sobre la seva seguretat quan es van avaluar com a nous aliments. La utilització de productes enriquits amb fitoesterols ha estat aprovada per vàries agències reguladores, incloent el Comitè Científic per Aliments de la Unió Europea (71) i la *Food and Drug Administration* (FDA) dels EEUU (72). La FDA ha autoritzat que s'inclogui la relació preventiva de la malaltia cardiovascular dels fitoesterols a etiquetats i al·legacions de salut des de l'any 2000 (67). El NCEP ATP III recomana desde el 2001 els aliments funcionals enriquits amb fitoesterols com una part de l'estratègia de prevenció primària i secundària de les malalties cardiovasculars (73). Altres societats com la Societat Espanyola de Cardiologia (74) consideren els fitoesterols com una opció dietètica addicional pel maneig de la hipercolesterolèmia.

El consum de fitoesterols es considera segur segons els estudis en models animals, sense efectes toxicològics rellevants. En humans s'ha establert que una dosi diària de 1,5 a 3 g diaris de fitoesterols disminueix les concentracions sanguínies de colesterol LDL un 10-15% en diverses poblacions, edats i condicions, inclosos nens i persones sota tractament farmacològic hipocolesterolemiant sense descriure's cap efecte tòxic (67). L'augment de l'excreció fecal de colesterol i els seus metabolits que produeix la ingesta de fitoesterols en dosis farmacològiques incrementa la seva concentració en l'intestí, però aquest fet no s'ha relacionat amb cap efecte sobre càncer de colon (68). Ja que el consum de més de 3 g al dia no redueix més el colesterol i pot disminuir els nivells de betacarotè en sang, el Comitè Científic de l'Alimentació Humana va recomanar no augmentar aquesta quantitat i la Comissió Europea va limitar la utilització d'aquests compostos en aliments suplementats en quantitats no superiors a 3 g. En l'etiqueta d'aquests productes s'ha d'indicar clarament que contenen esterols i les conveniències del seu us (71).

1.3.4.2. Recomanacions a grups específics de la població pel consum d'aliments amb esterols vegetals

En un principi, aquests productes haurien de reservar-se als individus que necessitin reduir les concentracions de colesterol LDL degudes a hipercolesterolèmia o, sota control mèdic, en cas d'estar sotmesos a un programa de prevenció secundària després d'haver patit un episodi clínic de malaltia cardiovascular (75). Però també poden beneficiar-se'n individus moderadament hipercolesterolèmics per no haver de seguir una teràpia amb fàrmacs o per reduir la dosi d'aquests. De fet, l'efecte d'una teràpia conjunta de fitoesterols o fitoestanols amb estatines és additiu, ja que llavors s'inhibeix tant la síntesi com l'absorció de colesterol. També s'ha observat que en individus amb hiperlipoproteïnèmies del tipus IIa o IIb (on es troben elevades les concentracions de LDL o de LDL i VLDL, respectivament), l'efecte de la combinació de fibrats i fitoesterols produeix efectes additius sobre el colesterol LDL (67).

Els grups poblacionals que haurien de tenir cautela amb el consum d'aliments enriquits en fitoesterols són els nens i les dones embarassades. Els possibles efectes adversos dels fitoesterols en la disminució de les concentracions plasmàtiques de betacarotè i potser altres vitamines podrien representar un problema en nens si s'utilitzen durant un període llarg de temps. En tot cas, s'ha demostrat la seva eficàcia i seguretat en nens amb hipercolesterolèmia familiar heterozigota, tant per evitar la utilització d'estatines quan encara no han madurat sexualment com per reduir-ne la dosi si es considera que són canditats al tractament farmacològic (76). No hi ha estudis realitzats en dones embarassades i per tant, no es saben les conseqüències del consum de fitoesterols a llarg termini en aquest grup poblacional (75).

Un grup poblacional d'especial atenció són aquells subjectes que pateixen una sitosterolèmia, que en cap cas son candidats a rebre fitoesterols (67).

1.3.5. Fitoesterols de la dieta habitual

L'efecte dels fitoesterols d'aliments naturals sobre l'absorció intestinal i les xifres sèriques de colesterol és un tema poc estudiat (31). Estudis realitzats amb aliments rics en fitoesterols (oli de blat de moro i magdalenes de germen de blat) que contenien uns 150 mg de fitoesterols van mostrar que hi havia una inhibició aguda de l'absorció intestinal de colesterol del 40%, que revertia quan s'administraven els mateixos aliments després d'haver-los extret els fitoesterols (28;77). Un estudi amb pacients amb ileostomia i alimentats amb fitoesterols van mostrar com el contingut en fitoesterols de la dieta estava inversament relacionat amb l'absorció de colesterol (78). Un altre estudi, on s'avaluava l'absorció de colesterol després de menjars de prova suplementats amb 200, 300 o 500 mg de fitoesterols en solució micel·lar, va trobar que es reduia l'absorció del colesterol fins i tot a les dosis més baixes (79). Per tant, en quantitats relativament baixes com les que es troben en alguns aliments naturals, els fitoesterols poden reduir l'absorció de colesterol. De fet, la ingesta de fitoesterols amb la dieta habitual es va relacionar inversament amb les concentracions de colesterol total i LDL en dues cohorts poblacionals (80;81), suggerint que els fitoesterols consumits amb la dieta habitual són bioactius.

1.4. Fitoesterols i salut cardiovascular

L'augment de la ingesta de fitoesterols amb una dieta enriquida naturalment (82-84) o mitjançant aliments suplementats (33) incrementa les concentracions d'aquests compostos en sèrum. La ingesta de 2 g/dia de fitoesterols fa que augmentin de 2 a 3 vegades les seves concentracions sèriques (33) i, malgrat i estar dins dels límits fisiològics, aquest augment de la fitosterolèmia ha estat àmpliament discutit en relació a la seguretat d'aquests compostos (68).

Com ja s'ha comentat, la malaltia recessiva sitosterolèmia es caracteritza per una hiperabsorció de fitoesterols, xifres circulants elevades i aterosclerosi accelerada, amb

dipòsit de fitoesterols a les plaques d'ateroma (39). Per tant, s'ha inferit que els augments de fitoesterols circulants també podrien ser aterogènics en individus no sitosterolèmics. Aquest tema ha estat molt debatut i ha generat una gran controversia (8;40;68;69;85-88).

1.4.1. Fitoesterols i malaltia cardiovascular: controvèrsia

La concentració sèrica de fitoesterols es relaciona amb l'absorció intestinal de colesterol (89). L'augment de fitoesterols sèrics pot indicar una hiperabsorció de colesterol que representi un augment hereditari del risc, tal com han suggerit alguns estudis. Glueck et al. (90) van ser els primers en relacionar xifres de fitoesterols sèrics incrementades amb un augment de risc cardiovascular en 595 subjectes hipercolesterolèmics, concloent que els fitotesterols eren un factor de risc per malaltia coronària arterial independentment del colesterol sèric en subjectes amb edat inferior a 55 anys. El grup de Miettinen (91;92) ha estudiat aquesta relació en dones postmenopàusiques en les quals afirmen que el metabolisme del colesterol i dels fitoesterols està alterat quan hi ha malaltia cardíaca coronària. En un primer estudi amb 29 dones postmenopàusiques amb malaltia coronària arterial i 20 controls aparellats per edat, la presència de malaltia coronària es va relacionar amb una síntesi disminuïda i una eliminació ineficient de colesterol en base a les xifres d'escualè ajustat per colesterol (91). En un segon estudi realitzat en 47 dones postmenopàusiques amb malaltia arterial coronària i 62 controls, malgrat i que es va trobar una eficiència d'absorció similar, els quocients de fitoesterols eren més elevats en els casos que en els controls (92). El grup de von Bergmann (93) va analitzar la relació entre una història familiar positiva de malaltia coronària amb les concentracions de fitoesterols sèrics en 53 pacients sense medicació hipolipemiant que van ser admesos per cirurgia de derivació coronaria. No hi havia diferències en les xifres de lípids, però els controls tenien concentracions de fitoesterols més baixes que els que tenien una història familiar positiva, suggerint que diferències en el metabolisme dels fitoesterols contribueixen al risc associat amb història familiar. Miettinen et al (94) van estudiar 868

pacients que seguien un tractament hipolipemiant amb simvastatina, en els quals es va identificar un subgrup amb síntesi de colesterol disminuïda i absorció augmentada (per tant, amb concentracions elevades de fitoesterols), que tenien un major número d'episodis coronaris recurrents malgrat i les xifres disminuïdes de colesterol en sang degut al tractament. Finalment, resultats de l'estudi prospectiu Munster Heart Study (PROCAM) (95) van mostrar que pacients amb infart de miocardi o mort cardíaca sobtada tenien concentracions més elevades de fitoesterols en sang. Aquells que estaven en el tercil superior de risc coronari segons l'algoritme PROCAM, tenien un risc augmentat de 3 vegades si les xifres de sitoesterol eren superiors a 0,21 mg/dl.

En canvi, altres estudis recents han mostrat una associació nul·la (96;97) o inversa (98) entre les concentracions elevades de fitoesterols i el risc de patir un episodi de malaltia cardiovascular. En un estudi de casos i controls dintre de l'estudi prospectiu European Prospective Investigation into Cancer and Nutrition (EPIC) - Norfolk es va analitzar la relació entre els fitoesterols plasmàtics i el risc de patir malaltia coronària en 373 casos i 758 controls. El quocient sitosterol/colesterol era més baix en els casos que en els controls, però en l'anàlisi multivariat no es va trobar una associació entre les concentracions de fitosterols sèrics i el risc de patir un episodi coronari (96). Els mateixos resultats es van trobar en l'estudi prospectiu Coronary Risk factors for Atherosclerosis in women study (CORA), on es van analitzar 186 casos i 231 controls de dones pre i postmenopàusiques (97). En canvi, quan es va analitzar aquesta associació en una cohort de 1242 subjectes amb edats superiors als 65 anys participants del Longitudinal Aging Study Amsterdam (LASA), es va observar que les concentracions (i els quocients) de fitoesterols eren més baixes en els casos i que les concentracions de sitosterol estaven associades a una reducció del risc de patir malaltia cardiovascular (71).

Per altra banda, la sitosterolèmia es caracteritza per desenvolupar una aterosclerosi accelerada. La relació entre els fitoesterols i les plaques d'ateroma s'ha analitzat en estudis experimentals i clínics. Weingartner et al. van realitzar un estudi en ratolins *knock*

out d'ApoE ateroscleròtics als quals se'ls va suplementar amb una dieta amb 2% de fitoesterols o se'ls va administrar ezetimiba, un inhibidor de l'absorció. Malgrat que es va veure un efecte hipolipemiant semblant, la formació de plaques ateroscleròtiques va ser el doble en els ratolins suplementats amb fitoesterols que als que van rebre ezetimiba (99). En un estudi clínic recent del grup de von Bergmann (99), les concentracions de fitoesterols en sang i vàlvula aòrtica de 10 pacients amb estenosi aòrtica que van consumir margarina suplementada amb fitoesterols eren majors en comparació a 72 pacients controls. Miettinen et al. (100). també van estudiar aquest fenomen en teixit arterial de 25 pacients que van ser sotmesos a endarterectomia carotídia. Es va mesurar el contingut en fitoesterol de les plaques i es va observar que era tan més elevat quant més alts eren els fitoesterols en sèrum. El tractament amb estatines es va relacionar amb xifres incrementades de fitoesterols en sèrum i en les plaques d'ateroma (100). Per una altra banda, un estudi de 2542 persones de mitjana edat no va trobar cap associació entre les concentracions de fitoesterols i l'aterosclerosi coronària mesurada mitjançant tomografia computeritzada del cor (101). En la mateixa publicació es descriu com ratolins femella salvatges o hipercolesterolèmiques amb concentracions plasmàtiques de fitoesterols augmentades més de vint vegades (degut a la inactivació dels transportadors ABCG5/G8) es van alimentar amb dieta normal o alta en colesterol durant 7 mesos i no es van veure diferències en l'àrea de lesió aòrtica. Així doncs, la controvèrsia entre les concentracions augmentades de fiotesterols en sang i el risc cardiovascular és un tema debatut en la comunitat científica (8;40;68;69;85-88).

1.4.2. Fitoesterols i síndrome metabòlica

Les concentracions plasmàtiques de campesterol o sitosterol i els seus quocients amb el colesterol són un reflex de l'eficiència de l'absorció intestinal de colesterol (102), mentre que les de lathosterol, un precursor del colesterol, són un bon índex de la síntesi endògena de l'esteroide (89). Existeix una relació recíproca entre síntesi i absorció de colesterol, de manera que les persones que sintetitzen poc tendeixen a absorbir molt i

viceversa (103), la qual cosa és coherent amb el precís control homeostàtic del metabolisme del colesterol. És ben conegut que l'obesitat s'associa a un augment de la síntesi de colesterol (104), i això s'ha confirmat en estudis recents del grup de Miettinen (105). Aquests autors també han mostrat que la síndrome metabòlica, la diabetis i la resistència a la insulina es caracteritzen per una síntesis augmentada de colesterol (determinada per l'increment de les xifres plasmàtiques de lathosterol) junt amb una menor absorció de l'esteroide (determinada por unes concentracions plasmàtiques disminuïdes de fitoesterols) (106-108). Assman et al. (109) van estudiar les concentracions dels quocients de cholestanol, campesterol i sitosterol (com a índex d'absorció de colesterol) i les de lathosterol (com a indicadores de la síntesi de colesterol) en 324 homes i 168 dones de l'estudi PROCAM amb l'objectiu d'estudiar la relació amb la síndrome metabòlica i els seus components. Malgrat que no es va veure una relació dels esterols no-colesterol amb la severitat de la síndrome metabòlica, sí que es va observar una relació negativa dels fitosterols amb l'índex de massa corporal i els triglicèrids, i del lathosterol amb els nivells de colesterol HDL i positiva amb l'índex de massa corporal. En la mateixa línia, els individus amb síndrome metabòlica tendien a tenir concentracions disminuïdes de campesterol/colesterol comparat amb els controls (110).

Per tant, existeix una contradicció entre el presumpte risc cardiovascular de les concentracions moderadament elevades de fitoesterols i el que la situació oposada (concentracions baixes de fitoesterols) es trobi en la sindrome metabòlica, una entitat d'elevat risc cardiovascular.

1.5. Dieta, fitoesterols i factors socials

En l'estudi de la dieta és important tenir en compte els hàbits dietètics i analitzar els factors que els influencien. És ben conegut que els hàbits dietètics juguen un paper clau en l'estat de la salut i que un dieta de baixa qualitat està associada amb una salut pitjor.

Aquests factors interaccionen amb la disponibilitat i el preu del menjar, influenciant doncs la qualitat de la dieta habitual (111).

Com s'ha comentat anteriorment, hi ha un gradient nord-sud de taxes de mortalitat cardiovascular en l'Europa occidental, on la prevalença de factors de risc clàssics no resulta en les taxes esperades (2). En alguns països del nord d'Europa s'han descrit diferències entre individus amb diferent nivell socioeconòmic (112), essent els grups més desavantatjats els que tenen una dieta més pobre i pitjor salut en comparació amb les classes més benestants (113).

En la recerca dels factors que propicien aquesta millor supervivència dels països situats al voltant del mar Mediterrani, la dieta ha estat un dels factors més estudiats. Un elevat grau d'adherència a la dieta Mediterrània s'ha associat amb una reducció de la mortalitat (5;6). Espanya està situada al Mediterrani i estudis realitzats en aquesta regió suggereixen una elevada adherència a aquest patró dietètic. La cohort espanyola de l'estudi EPIC tenia la major ingesta de fruites i verdures en comparació a la resta de països europeus que participen en aquest estudi (114). Un recent meta-anàlisi sobre el nivell socioeconòmic i la ingesta de fruites i verdures a 7 països europeus va desvetllar que les persones amb una ingesta més elevada d'aquests aliments saludables pertanyien a classes socioeconòmiques elevades (112). Degut a que el consum de fitoesterols amb la dieta habitual s'ha relacionat amb una millora del perfil lipídic en dues cohorts (80;81), l'estudi de la influència dels factors socials i econòmics que influencien el consum d'aliments rics en fitoesterols pot ajudar a dilucidar condicionants dels hàbits dietètics d'una determinada regió, i per tant, de la seva salut.

2. JUSTIFICACIÓ I OBJECTIUS

La dieta és un important determinant de la salut i la seva rellevància és evident degut al paper preventiu o causal que pot representar en el desenvolupament de determinades malalties (5;6). Els fitoesterols són components essencials d'una dieta rica en vegetals com la dieta Mediterrània (29). La ingesta d'aquests compostos augmenta les seves concentracions en sèrum (82-84), reflex del consum d'una dieta habitual saludable. Però les xifres incrementades de fitoesterols en sang també s'han relacionat amb un augment del risc de patir malaltia cardiovascular en alguns estudis, tot i que la bibliografia és contradictòria (8;40;68;69;85-88).

Per altra banda, la síndrome metabòlica és una agrupació de factors de risc que té com a base l'obesitat abdominal i la resistència a la insulina associada, i cursa amb al menys 3 dels següents components: obesitat abdominal, hipertensió arterial, hipertrigliceridèmia, colesterol HDL baix i hipergucèmia en dejó o diabetis, i comporta un risc molt elevat de desenvolupar malaltia cardiovascular (115-117). Aquesta síndrome s'associa a una disminució de l'absorció d'esterols i a un increment de la síntesi de colesterol, és a dir, a xifres disminuïdes de fitoesterols en sang (106). Aquesta aparent contradicció suggereix la necessitat d'una nova interpretació de la concentració en sang dels fitoesterols per poder entendre el seu paper dins el metabolisme del colesterol i la seva relació amb la salut.

L'estudi de la dieta habitual i dels hàbits dietètics és complex. En països del Nord d'Europa i Nord-Amèrica, la qualitat de la dieta està relacionada amb les desigualtats socioeconòmiques (112;113). A Espanya, el patró de dieta Mediterrània està prou establert (118), però el paper dels fitoesterols en relació al nivell socioeconòmic no ha estat avaluat.

Els treballs de recerca de la present tesi estan orientats a ampliar el coneixement de la implicació dels fitoesterols de la dieta i el sèrum sobre el perfil lipídic, els factors de risc

cardiovascular i la malaltia cardiaca coronària, així com a investigar els factors socials que influencien el consum d'una dieta rica en fitoesterols.

2.1. Article 1: "Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet".

Diversos treballs han mostrat com la ingesta de fitoesterols a dosis farmacològiques redueixen l'absorció de colesterol (69). Altres estudis han evidenciat un efecte hipocolesterolemiant dels fitoesterols ingerits amb la dieta habitual. Experiments en pacients amb ileostomia van mostrar que el contingut en fitoesterols de la dieta estava inversament relacionat amb l'absorció de colesterol (78). Estudis amb oli de blat de moro o germen de blat (28;77) van mostrar una reducció del 40% en l'absorció del colesterol que va revertir quan els mateixos aliments es van donar després d'haver-ne eliminat els fitoesterols. Recentment, dos grans estudis de cohort van observar una relació inversa entre la'ingesta de fitoesterols i la colesterolèmia (80;81).

La **hipòtesi** del present estudi és que un increment en la ingesta de fitoesterols amb la dieta habitual, és a dir, sense aliments suplementats, exercirà un efecte hipocolesterolemiant mesurable.

L'**objectiu** d'aquest estudi és avaluar l'efecte hipocolesterolemiant d'un augment de la ingesta de fitoesterols amb la dieta en un subgrup d'individus de l'estudi de prevenció cardiovascular primària PREvención con DIeta MEDiterránea (PREDIMED), al cap d'un any d'intervenció dietètica.

2.2. Article 2: "Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort ".

Defectes genètics i mutacions en els transportadors ABCG5/G8 produeixen la malaltia coneguda com sitosterolèmia (39), que es caracteritza per tenir concentracions elevades de fitoesterols en sang i desenvolupar aterosclerosis accelerada.

En els últims anys s'han publicat estudis contradictoris que afirmen que els fitoesterols sèrics són un factor de risc cardiovascular (90;91;93;95), no ho són (96;97) o bé són protectors (98). La qüestió sobre si les xifres elevades de fitoesterols plasmàtics en individus no sitosterolèmics podrien ser un factor de risc cardiovascular s'ha debatut extensament (8;40;68;69;85-88).

El consum de fitoesterols amb la dieta, entre altres factors, determina les seves concentracions sèriques (85). L'estudi de les xifres de fitoesterols en sang en relació a la malaltia cardiovascular en una població que consumeix una dieta rica en productes vegetals com l'espanyola (118) és d'especial rellevància.

La **hipòtesi** d'aquest treball és que els fitoesterols són marcadors de la ingesta d'aliments saludables i concentracions elevades en plasma no s'associen a un augment de risc de patir malaltia cardiaca coronària.

L'**objectiu** del present estudi va ser avaluar, en un estudi de caos i controls en la cohort espanyola de l'estudi EPIC, l'associació entre les concentracions de fitoesterols del plasma i la incidència de malaltia cardíaca coronària després de 10 anys de seguiment.

2.3. Article 3: "Plasma phytosterols are inversely associated with the metabolic syndrome and its components".

El manteniment de la homeòstasi del metabolisme del colesterol inclou la regulació recíproca entre la síntesi i l'absorció del colesterol (103). Les xifres de esterols no-colesterol i els seus quocients amb colesterol sèric són un reflex de l'eficiència d'aquests processos (89;102).

L'obesitat, la síndrome metabòlica ,la diabetis i la resistència a la insulina s'associen a un augment de la síntesi de colesterol, per tant, a xifres elevades de lathosterol, i a una menor absorció intestinal de colesterol, determinada per concentracions circulants disminuïdes de fitoesterols (104-108).

Així doncs, existeix una contradicció entre el presumpte risc cardiovascular de les concentracions moderadament elevades de fitoesterols i el que la situació oposada (concentracions baixes de fitoesterols) es trobi en la sindrome metabòlica, una entitat d'elevat risc cardiovascular.

La **hipòtesi** d'aquest estudi és que les concentracions elevades de fitoesterols sèrics es relacionen amb un perfil cardiometabòlic més saludable.

L'**objectiu** del present treball va ser investigar les associacions entre els esterols no-colesterol plasmàtics i els components de la síndrome metabòlica, inclòs la pròpia síndrome, en una població de pacients dislipèmics i en subjectes sans de la població espanyola de l'estudi EPIC.

2.4. Article 4: "Plant sterol intake and education level in the Spanish EPIC cohort".

Els hàbits dietètics juguen un paper clau en l'estat de la salut i un dieta de baixa qualitat està associada a una salut pitjor (113). En l'estudi de la relació de la dieta amb la salut no podem descuidar que existeixen factors com el gènere, l'ètnia i la posició socioeconòmica que interaccionen amb la disponibilitat i preu del menjar i que, per tant, juguen un paper important en la qualitat de la dieta consumida (111). En països del nord d'Europa s'ha descrit que els individus amb menor nivell socioeconòmic tenen una dieta més pobre i pitjor salut que els subjectes més benestants (112;113).

A Espanya es consumeix una dieta aproximada al patró de dieta Mediterrània (118-120), rica en productes vegetals, i per tant, rica en fitoesterols. Aquesta dieta s'ha associat a diversos efectes beneficiosos en factors de risc cardiovascular i mortalitat (5;6;119). L'estudi de la dieta habitual (i el consum de fitoesterols com a marcadors de dieta saludable) en relació a la posició socioeconòmica és rellevant en aquest context.

La **hipòtesi** d'aquest estudi és que el gènere i la classe social influenciaran el consum de fitoesterols de la dieta en una població amb una ingesta abundant d'aquests components.

L'**objectiu** d'aquest treball és investigar l'associació entre el nivell assolit d'educació, com a mesura de la posició socioeconòmica (121), el gènere i la ingesta de nutrients (amb especial atenció als fitoesterols), mitjançant un anàlisi transversal de la cohort espanyola de l'estudi EPIC.

3. CÒPIES DELS ARTICLES ORIGINALS

3.1. Article 1: “Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet”

3.2. Article 2: “Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort ”

3.3. Article 3: “Plasma phytosterols are inversely associated with the metabolic syndrome and its components”

3.4. Article 4: “Plant sterol intake and education level in the Spanish EPIC cohort”

3.1. Article 1

Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet.

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ORIGINAL CONTRIBUTION

Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet

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Abstract
Background Phytosterols in natural foods are thought to inhibit cholesterol absorption. The Mediterranean diet is rich in phytosterol-containing plant foods.
Aim of the study To assess whether increasing phytosterol intake from natural foods was associated with a cholesterol-lowering effect in a substudy of a randomized trial of nutritional intervention with Mediterranean diets for primary cardiovascular prevention (PREDIMED study).
Methods One hundred and six high cardiovascular risk subjects assigned to two Mediterranean diets supplemented with virgin olive oil (VOO) or nuts, which are phytosterol-rich foods, or advice on a low-fat diet. Outcomes were 1-year changes in nutrient intake and serum levels of lipids and non-cholesterol sterols.
Results Average phytosterol intake increased by 76, 158 and 15 mg/day in participants assigned VOO, nuts and low-fat diets, respectively. Compared to participants in the low-fat diet group, changes in outcome variables were observed only in those in the Mediterranean diet with nuts group, with increases in intake of fibre, polyunsaturated fatty acids and phytosterols ($P < 0.020$, all) and significant ($P < 0.05$) reductions of LDL-cholesterol (0.27 mmol/l or 8.3%) and the LDL/HDL-cholesterol ratio (0.29 mmol/l or 11.5%). Variations in saturated fat, cholesterol or fibre intake were unrelated to LDL-cholesterol changes. In the whole group, changes in serum sitosterol-to-cholesterol, which reflect those of dietary phytosterol intake and absorption, correlated inversely to LDL-cholesterol changes ($r = -0.256$; $P = 0.008$). In multivariate analyses, baseline LDL-cholesterol, increases in serum sitosterol ratios and statin use were independently associated with LDL-cholesterol reductions.
Conclusions Small amounts of phytosterols in natural foods appear to be bioactive in cholesterol lowering.

Keywords Phytosterols · Mediterranean diet · Nuts · Olive oil · Cholesterol

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Introduction

Coronary heart disease (CHD) is the main cause of death and morbidity in industrialized countries. Mediterranean

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countries have a low CHD mortality rate compared with Northern Europe or the US [33], which has been ascribed in part to dietary habits [20]. Recent findings from large cohort studies [15, 32] and controlled feeding trials, as recently reviewed [7], suggest that adherence to the Mediterranean dietary pattern is associated with reduced CHD mortality and beneficial effects on cardiovascular disease biomarkers, respectively.

The traditional Mediterranean diet is characterized by a high intake of vegetables, fruits and olive oil; a moderate intake of fish and alcohol, mostly wine; and a low intake of dairy products, meat and sweets [18, 35]. Ecological evidence suggested that this dietary pattern was associated with lower serum cholesterol than the so-called Western diet, with higher intakes of meat, dairy products and sweets [13]. This was confirmed by a feeding trial in which Mediterranean and Western diets were switched [8], and may be explained in part by a lower dietary content of saturated fatty acids (SFA) and a higher content of monounsaturated fatty acids (MUFA) [4] and fibre [2] in the traditional Mediterranean diet. Other factors in the diet apart from fat and fibre could influence serum cholesterol. These may include bioactive plant sterols.

Plant sterols or phytosterols are important components of a vegetable-based diet, being particularly abundant in cereals, nuts, seeds and oils derived from them [23]. They are structurally related to cholesterol, but have bulkier and more hydrophobic molecules, which confer them a higher affinity for intestinal micelles than cholesterol. Consequently, cholesterol is displaced from micelles and the amount available for absorption is limited [25]. Inhibition of cholesterol absorption by gram doses of phytosterols incorporated into various foods is a well-established non-pharmacological strategy for cholesterol lowering [29]. An important question is whether plant sterols in natural vegetable foods or oils are also bioactive and might help lower serum cholesterol. At least there is convincing evidence that they reduce cholesterol absorption [6, 26, 27]. Thus, feeding experiments in patients with an ileostomy showed that the phytosterol content of the diet was highly and inversely correlated to cholesterol absorption [6]. In addition, single meal studies with phytosterol-rich corn oil and wheat germ muffins showed a $\approx$ 40% reduction of cholesterol absorption that reverted to baseline when the same foods were given after being depleted of phytosterols [26, 27]. Recently, dietary phytosterol intake was inversely related to serum cholesterol in large population studies [1, 14], suggesting that they are indeed bioactive in the usual diet.

The aim of the present study of a subsample of participants in the PREDIMED study [7], a large feeding trial of primary cardiovascular prevention, was to assess whether increasing phytosterol intake from natural foods in an enhanced Mediterranean diet was associated with cholesterol lowering.

Methods

Study population

The PREDIMED study is a parallel group, multi-centre, randomized and controlled 5-year clinical trial. Eligibility of participants has been described elsewhere [7, 19]. Participants were randomized to three groups, two Mediterranean diets, supplemented with either virgin olive oil (VOO) or mixed nuts, or advice on a low-fat diet. At baseline and after intervention for 1 year, participants filled a 137-item validated food frequency questionnaire (FFQ) [17] and underwent anthropometric measurements and venipuncture.

This exploratory analysis includes 114 participants chosen at random from the lists of two recruiting centres (Hospital Clínic, Barcelona and Hospital Sant Joan, Reus) among those who provided FFQs and blood samples at baseline and 1 year and were not using plant sterol supplements. Recruitment took place between October 2004 and December 2005. All participants gave informed consent to a protocol approved by the local review boards.

Diets

Trained dieticians delivered the dietary intervention, which was based on quarterly individual and group education meetings. The dieticians gave advice to follow either the recommended Mediterranean diets or the low-fat diet and provided written material with elaborate descriptions of target foods and seasonal shopping lists, meal plans and cooking recipes. The general procedures and dietary recommendations for the three groups have been described [7, 19]. The participants allocated to the Mediterranean diets also received free provisions of VOO (1 l/week) or mixed nuts (30 g/day, as 15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts). Participants in the olive oil group were recommended to consume a minimum of 50 ml of the supplemental olive oil per day. The extra olive oil was provided to improve adherence, account for family needs and allow sufficient oil for deep frying.

Food intake during the preceding year was derived from the FFQs. The consumption of energy and nutrients was calculated from Spanish food composition tables. Intake of total phytosterols and their measured components in the diet at baseline and after intervention for 1 year was estimated from a database of Spanish foods [12]. The phytosterol content of the olive oil and nuts used in the study was analysed in a reference laboratory, as described [7].

The mean values of six measurements of total phytosterols in random samples of VOO, walnuts, almonds and hazelnuts were 156, 199, 224 and 175 mg/100 g, respectively. The average sitosterol content ranged from 79% in almonds to 96% in olive oil, while the average campesterol content was <6% in all foods.

Laboratory determinations

Coded samples of serum were shipped to a central laboratory and stored at −80 °C until assay. Cholesterol and triglycerides were measured by enzymatic procedures, and HDL-cholesterol was determined after precipitation with phosphotungstic acid and magnesium chloride. The concentration of LDL-cholesterol was calculated by the Friedewald equation.

Serum non-cholesterol sterols (campesterol, sitosterol and lathosterol) were determined by gas chromatography using a modification of the method of Heinemann et al. [10]. The ratios to cholesterol of the plant sterols, campesterol and sitosterol, are accepted as surrogate markers for the efficiency of cholesterol absorption, while the cholesterol precursor lathosterol is a reliable index of cholesterol synthesis [22]. Epicoprostanol (2 μg) was added to serum (0.1 ml) as internal standard. After alkaline hydrolysis, extraction, and derivatization to trimethylsilyl ethers, sterols were quantified on a 30-m nonpolar capillary column (TRB-Esterol; Teknokroma, Barcelona) with a gas chromatography apparatus (Autosystem^{TM}, Perkin Elmer, Norwalk, CT, USA). Inter- and intra-assay coefficient variations were 5.0 and 3.2% for lathosterol, 1.9 and 1.6% for campesterol and 2.0 and 1.8% for sitosterol, respectively.

Statistical analyses

Mean values and SD were used to describe continuous variables. For analysis of laboratory variables, the average of two measurements was used as the final value. Values with a skewed distribution were transformed to their natural logarithm for analyses. Analysis of variance (ANOVA) or Chi-square tests, as appropriate, were used for comparisons of baseline variables among the three groups. We examined 1-year changes in energy, nutrient intake, serum lipids and non-cholesterol sterols by ANOVA with adjustment for age, sex and baseline body weight. Centre had no effect on outcomes and was excluded from further analyses. Pearson correlation coefficients were used to assess relationships between continuous variables. Multiple linear regression analysis was used to build a predictive model of LDL-cholesterol change. All statistical tests were two tailed and the significance level was set at $P < 0.05$. Analyses were performed using SPSS, version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Of the initially chosen 114 participants, data from eight subjects were not included in the final analysis because five of them had a change in statin use during the study, which might interfere with sterol values, and three subjects provided FFQ data which were out of range for total energy intake (500–3,500 kcal/day for women and 800–4,000 kcal/day for men).

The clinical characteristics of the 106 participants are presented in Table 1. By study design, the sample was composed of older, mostly overweight subjects with a sizeable burden of cardiovascular risk factors, which were reasonably balanced among the three arms. Likewise, there were no among-group differences in serum non-cholesterol sterol-to-cholesterol ratios.

As shown in Table 2, baseline food, energy and nutrient intake were similar among groups. The self-selected participant's dietary habits reasonably conformed to the traditional Mediterranean food pattern in several aspects, as they recorded high intakes of olive oil, cereals, vegetables, fruits and fish; moderate intakes of legumes, nuts and alcoholic beverages and low intakes of industrial bakery products and sweets. They deviated from the traditional Med diet, however, because of high intakes of meat and dairy products. The baseline diet was high in fibre, due to high intakes of vegetables and fruits, and also in total fat and MUFA, which can be attributed in part to customary use of olive oil. For the same reason, the phytosterol content of the baseline diet was rather high.

The main dietary changes recorded at 1 year were high increases in VOO and nut consumption in the corresponding Mediterranean diet groups provided with these foods. As derived from the FFQs, olive oil intake only increased marginally by 11 g/day in the group supplemented with VOO because participants just exchanged their usual olive oil, mainly refined olive oil (15 g/day reduction), for the virgin variety supplied (24 g/day increase). Nut intake significantly increased by 29 g/day in participants in the Mediterranean diet with nuts. Participants in all groups increased consumption of fruits and vegetables by an average of 77 and 33 g/day, respectively, and marginally decreased meat consumption by an average 8 g/day, while maintaining a low level of alcohol intake of $\approx$ 13 g/day. Mean body weight changes at 1 year were −0.81 kg in the VOO group, −0.41 kg in the nuts group and 0.34 kg in the low-fat diet group ($P = 0.223$).

Changes in energy and nutrient intake adjusted for age, sex and baseline body weight were usually minor (Table 3). At 1 year, participants in the Mediterranean diet with VOO increased intake of total phytosterols and sitosterol, but not campesterol, while the group assigned the Mediterranean diet with nuts increased intake of total

Table 1 Baseline characteristics of participants

	Mediterranean diet with VOO ($n = 35$)	Mediterranean diet with nuts ($n = 37$)	Recommended low-fat diet ($n = 34$)	P value[a]
Age (years)	66.1 ± 6.2	65.1 ± 6.3	67.6 ± 6.6	0.238
Men	18 (51.4)	24 (64.9)	20 (58.8)	0.512
Family history of CHD	8 (23.5)	7 (18.9)	11 (32.4)	0.415
Current smokers	7 (20.0)	14 (37.8)	11 (32.4)	0.243
BMI (kg/m^2)	28.0 ± 3.1	28.3 ± 2.8	29.4 ± 3.9	0.171
Type 2 diabetes mellitus	16 (45.7)	19 (51.4)	17 (50.0)	0.884
Hypertension	31 (88.6)	30 (81.1)	30 (88.2)	0.587
Dyslipidemia	28 (80.0)	26 (70.3)	24 (70.6)	0.575
Statin treatment	11 (31.4)	14 (37.8)	9 (26.5)	0.588
Other lipid-lowering agents	4 (11.4)	2 (5.4)	2 (5.9)	0.567
Serum lipids (mmol/l)				
Total cholesterol	5.51 ± 0.83	5.24 ± 0.87	5.21 ± 0.79	0.243
LDL-cholesterol	3.54 ± 0.66	3.24 ± 0.79	3.15 ± 0.64	0.061
HDL-cholesterol	1.41 ± 0.33	1.34 ± 0.33	1.39 ± 0.31	0.691
Triglycerides[b]	1.08 ± 1.52	1.39 ± 1.51	1.35 ± 1.64	0.128
LDL/HDL ratio	2.62 ± 0.66	2.52 ± 0.72	2.30 ± 0.56	0.124
Triglycerides/HDL ratio[b]	1.91 ± 1.69	2.29 ± 1.75	2.40 ± 1.91	0.217
Serum non-cholesterol sterols-to-cholesterol (μmol/mmol)				
Lathosterol	1.38 ± 0.60	1.49 ± 0.64	1.45 ± 0.89	0.815
Campesterol	2.66 ± 1.46	2.68 ± 1.45	2.67 ± 1.58	0.997
Sitosterol	2.30 ± 1.26	2.27 ± 1.20	2.22 ± 1.20	0.961

Values are number (%) or mean ± SD

VOO virgin olive oil, *CHD* coronary heart disease

[a] P value by Chi-square test or ANOVA

[b] Data computed as logarithm and expressed as anti-logarithm

energy, fibre, energy from total fat, PUFA and total plant sterol and their components, with a reciprocal decrease in carbohydrate intake. Cholesterol intake decreased in the low-fat diet group. Compared to the low-fat diet group, there were no differences in nutrient changes in the Mediterranean diet group with VOO, while significant increases in intake of fibre, PUFA and phytosterols were observed in the Mediterranean diet with nuts group. Figure 1 shows the contribution of the main plant-derived food groups to phytosterol intake at 1 year in each intervention arm. In all diet groups, the main source was oils (essentially, olive oil), followed by cereals and then vegetables and fruits to the same extent, while the contribution of legumes and nuts was minor, except for the group supplemented with nuts, in which nuts were second to oils as the main contributors to phytosterol intake.

Table 4 shows the effects of diets on outcome variables adjusted for age, sex and baseline body weight. The Mediterranean diets with VOO or nuts were associated with significant reductions from baseline in LDL-cholesterol (−4.2 and −6.8%, respectively) and the LDL/HDL-cholesterol ratio (−6.1 and −9.5%, respectively), while only participants in the nut-supplemented group showed an increase in HDL-cholesterol (5.2%). The treatment effect by comparison with the low-fat diet group was significant ($P < 0.05$) only for reduction in LDL-cholesterol (0.27 mmol/l or 8.3%) and the LDL/HDL-cholesterol ratio (0.29 mmol/l or 11.5%) in the Mediterranean diet with nuts group. In the group supplemented with nuts, the 1-year LDL-cholesterol responses were non-significantly higher in statin users compared with nonusers (−7.8% vs. −4.2%, respectively, $P = 0.25$). There were no effects of diets on concentrations of triglycerides, triglyceride/HDL ratios, lathosterol or campesterol, while sitosterol increased, albeit non-significantly, in the two Mediterranean diet groups.

Changes of serum lipids were significantly and negatively correlated (e.g., bigger reductions) to baseline levels, with $r = -0.339$ ($P < 0.001$) for total cholesterol, $r = -0.408$ ($P < 0.001$) for LDL-cholesterol and $r = -0.322$ ($P = 0.001$) for triglycerides. Changes in total and LDL-cholesterol were unrelated to those of dietary plant sterols, SFA, fibre or cholesterol intakes (all r values < 0.16;

Table 2 Key foods, energy and nutrients of self-selected prestudy diets in the three intervention groups

	Mediterranean diet with VOO ($n = 35$)	Mediterranean diet with nuts ($n = 37$)	Low-fat diet ($n = 34$)	P value[a]
Foods (g/day)				
Olive oil	39 ± 18	36 ± 15	41 ± 20	0.485
Cereals	229 ± 130	197 ± 71	201 ± 94	0.378
Vegetables	332 ± 135	295 ± 102	307 ± 107	0.382
Fruits	217 ± 115	242 ± 120	242 ± 124	0.603
Legumes	16.7 ± 13.0	13.0 ± 6.0	14.5 ± 9.0	0.274
Nuts	11.4 ± 11.6	14.2 ± 16.6	15.0 ± 16.4	0.585
Fish or seafood	85 ± 53	81 ± 37	74 ± 24	0.470
Meat and meat products	93 ± 35	105 ± 36	115 ± 45	0.396
Industrial bakery products	27.5 ± 36	25.9 ± 48.7	27.0 ± 55.3	0.989
Dairy products	302 ± 190	254 ± 173	214 ± 130	0.074
Alcohol	12.5 ± 15.4	14.7 ± 17.0	14.9 ± 15.0	0.780
Nutrients				
Total energy (kcal/day)	2161 ± 630	2143 ± 525	2128 ± 626	0.725
Energy from protein (%)	16.8 ± 2.5	16.6 ± 2.7	16.6 ± 2.9	0.922
Energy from carbohydrate (%)	43.8 ± 6.0	41.5 ± 6.8	40.9 ± 7.2	0.201
Fibre (g/day)	29.7 ± 11.0	26.2 ± 7.8	26.4 ± 7.3	0.663
Energy from total fat (%)	36.6 ± 5.5	37.6 ± 6.3	38.6 ± 7.2	0.475
Saturated fatty acids (%)	9.8 ± 2.3	10.5 ± 2.2	10.2 ± 2.2	0.493
Monounsaturated fatty acids (%)	18.1 ± 3.5	17.7 ± 4.0	19.2 ± 4.3	0.329
Polyunsaturated fatty acids (%)	5.7 ± 1.8	6.4 ± 2.1	6.4 ± 2.1	0.305
Cholesterol (mg/day)	343 ± 108	365 ± 104	355 ± 101	0.717
Total plant sterols (mg/day)	351 ± 94	342 ± 90	373 ± 129	0.352
Sitosterol (mg/day)	222 ± 57	212 ± 51	230 ± 75	0.406
Campesterol (mg/day)	34.3 ± 12.4	31.5 ± 10.1	32.8 ± 9.9	0.263
Other sterols (mg/day)[b]	94 ± 34	99 ± 35	110 ± 57	0.309

Data as mean ± SD

[a] P value by ANOVA

[b] Includes stigmasterol, stigmastanol and unspecified plant sterols

$P > 0.1$). However, changes of serum sitosterol ratios correlated negatively with those of serum total cholesterol ($r = -0.285$; $P = 0.003$) and LDL-cholesterol ($r = -0.256$, respectively; $P = 0.008$).

Finally, we used a stepwise multiple regression analysis to build a predictive model of the lipid response, with LDL-cholesterol change as dependent variable and sex, age, diabetes status, statin use, baseline BMI and levels of LDL-cholesterol and non-cholesterol sterol ratios, and 1-year changes in weight, intake of SFA, fibre, dietary cholesterol and plant sterols, and non-cholesterol sterol ratios as independent variables. Baseline LDL-cholesterol [regression coefficient (B) = −0.236; $P < 0.001$], change in serum sitosterol-to-cholesterol ratios ($B = -0.158$; $P = 0.011$) and statin use ($B = -0.203$; $P = 0.032$) were independently associated with LDL-cholesterol changes (adjusted $R^2 = 0.22$).

Discussion

In this study, asymptomatic older persons at high-cardiovascular risk who improved their diet after nutritional education and supplementation with VOO or mixed nuts during a 1-year period showed improved lipid profiles in comparison with those recommended a low-fat diet. The reductions in total cholesterol and LDL-cholesterol were unrelated to changes in dietary SFA, cholesterol or fibre intake, but they were associated with dietary enrichment with plant sterols, as indirectly estimated by increases in serum sitosterol ratios.

Observed changes in nutrient intake were not of great magnitude. Increased nut intake was mirrored by significant increases in healthy nutrients more than increased VOO intake, because participants in this group merely exchanged their usual refined oil for the virgin oil supplied.

Table 3 Changes in energy and nutrient intake

	Mean changes from baseline at 12 months (95% CI)			Mediterranean diet with olive oil vs. low-fat diet		Mediterranean diet with nuts vs. low-fat diet	
	Mediterranean diet with VOO ($n = 35$)	Mediterranean diet with nuts ($n = 37$)	Low-fat diet ($n = 34$)	Mean (95% CI) between group difference	*P* value	Mean (95% CI) between group difference	*P* value
Total energy (kcal/day)	−66 (−239 to 106)	181 (11 to 351)	−24 (−213 to 165)	−43 (−358 to 273)	1.00	204 (−109 to 519)	0.34
Energy from protein (%)	0.42 (−0.41 to 1.25)	−0.24 (−1.06 to 0.58)	−0.28 (−1.19 to 0.63)	0.70 (−0.82 to 2.22)	0.79	0.05 (−1.47 to 1.56)	1.00
Energy from carbohydrate (%)	−0.76 (−2.98 to 1.47)	−2.79 (−4.99 to −0.60)	1.15 (−1.29 to 3.59)	−1.91 (−5.97 to 2.16)	0.77	−3.94 (−7.98 to 0.11)	0.06
Fibre (g/day)	2.34 (−0.61 to 5.28)	6.22 (3.31 to 9.12)	−0.03 (−3.26 to 3.20)	2.37 (−3.01 to 7.74)	0.86	6.25 (0.89 to 11.60)	0.016
Energy from total fat (%)	1.29 (−1.09 to 3.66)	3.53 (1.19 to 5.87)	0.21 (−2.40 to 2.81)	1.08 (−3.26 to 5.42)	1.00	3.32 (−1.00 to 7.64)	0.19
SFA (%)	−0.64 (−1.39 to 0.11)	−0.67 (−1.41 to 0.07)	−0.25 (−1.07 to 0.57)	−0.39 (−1.76 to 0.98)	1.00	−0.42 (−1.78 to 0.94)	1.00
MUFA (%)	1.17 (−0.45 to 2.78)	1.49 (−0.10 to 3.09)	−0.01 (−1.78 to 1.76)	1.18 (−1.77 to 4.13)	0.99	1.51 (−1.43 to 4.44)	0.64
PUFA (%)	0.18 (−0.54 to 0.90)	2.70 (1.99 to 3.41)	0.25 (−0.54 to 1.04)	−0.07 (−1.38 to 1.25)	1.00	2.46 (1.14 to 3.77)	<0.001
Cholesterol (mg/day)	−26 (−61 to 9)	−25 (−59 to 10)	−60 (−98 to −21)	33 (−30 to 97)	0.61	35 (−28 to 98)	0.54
Total plant sterols (mg/day)	76 (26 to 126)	158 (110 to 205)	15 (−35 to 64)	62 (−25 to 148)	0.26	143 (58 to 228)	<0.001
Sitosterol (mg/day)	36 (8 to 63)	94 (68 to 121)	2 (−26 to 29)	34 (−14 to 82)	0.27	93 (46 to 139)	<0.001
Campesterol (mg/day)	0.82 (−3.02 to 4.66)	5.37 (1.74 to 9.00)	−1.56 (−5.36 to 2.23)	2.38 (−4.26 to 9.03)	1.00	6.93 (0.45 to 13.42)	0.032
Other sterols (mg/day)[a]	36 (19 to 54)	50 (33 to 66)	16 (−1 to 33)	20 (−10 to 50)	0.31	34 (4 to 63)	0.020

Changes are mean values (95% CI) and are calculated as 1-year value minus baseline value; adjusted for age, sex and baseline body weight

[a] Includes stigmasterol, stigmastanol and unspecified plant sterols

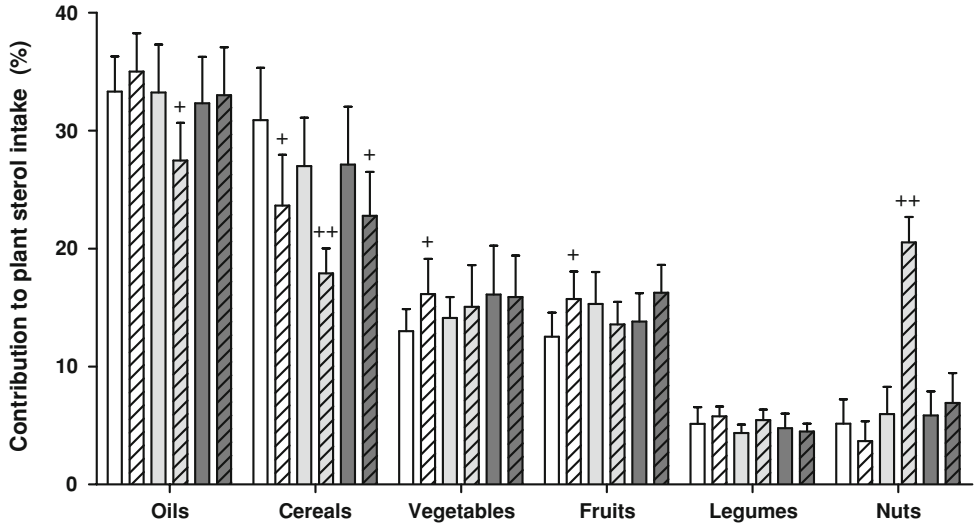

Fig. 1 Contribution of plant-based food groups to estimated daily intake per person of plant sterols in the three intervention groups of the PREDIMED participants at baseline and at 1 year intervention. Values are mean (95% IC). $^{+}P < 0.05$, $^{++}P < 0.001$ vs. baseline value. *White bar* indicates Med diet + VOO baseline, *white bar with striped lines* indicates Med diet + VOO 1-year, *light grey bar* indicates Med diet + nuts baseline, *light grey bar with striped line* indicates Med diet + nuts 1-year, *dark grey bar* indicates Med diet + low-fat baseline and *dark grey bar with striped line* indicates Med diet + low-fat 1-year

Participants in the low-fat diet group showed little changes in nutrient intake because they followed a similar diet as that self-selected at the onset of the study. Noticeably, plant sterol intakes increased only in the VOO and nut groups, by 22 and 46%, respectively.

In spite of relatively small overall changes in macronutrient intake, beneficial changes in lipid profiles occurred only in the Mediterranean diet groups, particularly in the group given supplemental nuts, confirming the results of the 3-month assessment of a larger PREDIMED cohort [7]. The increase in total fat and unsaturated fatty acid consumption in the mixed nuts group might be relevant to the observed HDL-cholesterol increase, as reported after nut-enriched diets [9].

Feeding experiments in humans indicate that plant sterols in natural foods reduce intestinal cholesterol absorption [6, 26, 27] and two large population studies showed that phytosterol intake was inversely related to serum cholesterol [1, 14], suggesting that they are indeed bioactive in the usual diet. Our main research question was whether this bioactivity was operational to lower serum cholesterol in free-living persons who increased consumption of plant sterol-rich foods. The present results support an association between increasing dietary phytosterol intakes and LDL-cholesterol lowering. Plant sterol intake increased in the two Mediterranean diet groups, to a higher extent in the group with nuts. While both groups showed decreases of LDL-cholesterol from baseline, only the change after the nut-supplemented diet was significantly different from that observed in the low-fat diet. The cholesterol-lowering efficacy of nut intake in feeding trials has often been higher than that predicted on the basis of fatty acid exchange [9], and the phytosterol content of nuts might be responsible in part for this effect [30]. Our findings are consistent with this hypothesis, as the small changes in intake of SFA or fibre, two important dietary determinants of serum cholesterol levels, were unrelated to those of serum LDL-cholesterol. While the latter were similarly unrelated to changes of dietary phytosterol intake, they correlated inversely with those of serum sitosterol ratios, a reliable index of intestinal sterol absorption [3, 22]. Inherent inaccuracies of the subjective FFQ technique to estimate the intake of nutrients, especially of those with high intra- and inter-individual variability such as plant sterols [25], in face of objective determinations of serum levels may explain these discrepancies. At any rate, daily plant sterol intake increased by an average of 158 mg in the Mediterranean diet with nuts group. Based on studies with phytosterol-enriched foods, the contribution to cholesterol lowering from these small doses in natural foods seems unexpected. However, a higher effect from naturally occurring plant sterols fits with the curvilinear relationship between phytosterol intake and serum cholesterol derived from clinical studies [29]. Recent studies have shown that treatment of hypercholesterolemic subjects with healthy diets enriched with high-fibre foods and pharmacological doses of phytosterols, together with nuts in an isoenergetic diet [11] or specific phytochemicals within the context of a weight-losing diet [16] can have marked beneficial effects on the lipid profile.

The effects of diet on serum non-cholesterol sterols deserve some consideration. Even though plant sterol

Table 4 Changes in serum lipids and non-cholesterol sterols

	Mean changes from baseline at 12 months (95% CI)			Mediterranean diet with olive oil vs. low-fat diet		Mediterranean diet with nuts vs. low-fat diet	
	Mediterranean diet with VOO ($n = 35$)	Mediterranean diet with nuts ($n = 37$)	Low-fat diet ($n = 34$)	Mean (95% CI) between group difference	*P* value	Mean (95% CI) between group difference	*P* value
Lipids (mmol/l)							
Total cholesterol	−0.15 (−0.31, 0.00)	−0.11 (−0.26, 0.04)	0.04 (−0.12, 0.20)	−0.19 (−0.47 to 0.08)	0.26	−0.15 (−0.42 to 0.12)	0.51
LDL-cholesterol	−0.15 (−0.29, −0.00)	−0.22 (−0.36, −0.08)	0.05 (−0.10, 0.20)	−0.20 (−0.46 to 0.06)	0.20	−0.27 (−0.53 to −0.01)	0.036
HDL-cholesterol	0.02 (−0.03, 0.07)	0.07 (0.02, 0.11)	0.00 (−0.05, 0.05)	0.02 (−0.07 to 0.11)	1.00	0.06 (−0.02 to 0.15)	0.24
Triglycerides[a]	−1.06 (−1.18, 1.04)	1.07 (−1.03, 1.18)	−1.05 (−1.06, 1.06)	−1.01 (−1.21 to 1.18)	1.00	1.12 (−1.07 to 1.34)	0.37
LDL/HDL ratio	−0.16 (−0.29, −0.02)	−0.24 (−0.37, −0.11)	0.05 (−0.10, 0.19)	0.20 (−0.44 to 0.04)	0.13	−0.29 (−0.52 to −0.05)	0.014
Triglyceride/HDL ratio[a]	−1.08 (−1.21, 1.04)	1.03 (−1.08, 1.15)	−1.05 (−1.19, 1.06)	−1.27 (−1.67, 1.03)	0.09	1.02 (−1.28, 1.32)	0.91
Non-cholesterol sterols/cholesterol (μM/mM)							
Lathosterol	0.05 (−0.15, 0.25)	−0.04 (−0.23, 0.16)	0.07 (−0.14, 0.27)	−0.02 (−0.37 to 0.34)	1.00	0.10 (−0.45 to 0.25)	1.00
Campesterol	0.04 (−0.31, 0.39)	−0.05 (−0.39, 0.29)	−0.06 (−0.41, 0.30)	0.10 (−0.52 to 0.71)	1.00	0.01 (−0.60 to 0.62)	1.00
Sitosterol	0.22 (−0.02, 0.47)	0.22 (−0.02, 0.45)	0.06 (−0.19, 0.31)	0.16 (−0.27 to 0.59)	1.00	0.15 (−0.27 to 0.58)	1.00

Changes are mean values (95% CI) and are calculated as 1-year value minus baseline value; adjusted for age, sex and baseline body weight

[a] Data computed as logarithm and expressed as anti-logarithm

absorption is poor, it is enhanced by increased amounts in the intestinal lumen, resulting in increased serum phytosterol concentrations, as documented in feeding trials of natural phytosterol-rich foods [24, 31, 34]. The fact that in our study only sitosterol levels increased in comparison with campesterol can be explained by the relative abundance of sitosterol in Mediterranean foods competing with campesterol for absorption [3].

Inverse correlations were observed between changes in serum sitosterol and those of total and LDL-cholesterol. In addition, the LDL-cholesterol response was independently associated with serum sitosterol increases at multivariate analysis. For these reasons, the presumed atherogenicity of increased serum phytosterols [3, 28] must be questioned when dealing with populations consuming diets rich in vegetables, nuts and plant-derived oils, such as the Mediterranean diet.

The fact that participants undergoing statin treatment showed a trend towards more pronounced LDL-cholesterol reductions than untreated ones also supports a phytosterol effect, because decreased cholesterol synthesis by statins is compensated by increased intestinal absorption of sterols [21], and in this situation dietary plant sterols should be more effective to lower blood cholesterol. Indeed, as recently shown by de Jong et al. [5], there is a synergistic cholesterol-lowering effect of plant sterol supplement consumption and statins, with effectiveness maintained on the long term.

Our study has limitations, such as the small number of participants in each group, which might reduce statistical power and the assessment of nutrient intake based on subjective self-reports. The nutritional education on the low-fat diet was less intense than the intervention in the two Mediterranean diet groups is an added limitation. In fact, fat intake at 1 year was similar to baseline in the group assigned the low-fat diet. This was partly because of the study design, but also because participants belonged to a Mediterranean culture, where people have a long-lasting preference for using olive oil. Therefore, the differences in outcomes between the Mediterranean diet groups and the low-fat diet group might be attributed mainly to the supplemental foods provided. Strengths of the study are that it reproduces real-life conditions with home-prepared foods and that plant sterol intake was determined from composition tables constructed with Spanish foods [12].

In conclusion, the results of this study suggest that part of the beneficial effects of the enhanced Mediterranean diets on the lipid profile is due to increased consumption of plant sterols, which might be bioactive even in small amounts in their natural food matrices. More work is warranted to confirm that intake of natural phytosterol-rich foods has a cholesterol-lowering effect in free-living populations.

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3.2. Article 2

Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort.

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Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort

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Running title: Plant sterols and coronary heart disease

Abstract

Aims: To evaluate the association of plasma phytosterols with coronary heart disease (CHD) in a population with a high phytosterol intake.

Methods and results: Baseline CHD risk factors, phytosterol intake and plasma noncholesterol sterol levels were evaluated in participants of a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) Spanish cohort who developed CHD (n=306) and matched controls (n=592) who remained free of CHD after a 10-year follow-up. Sitosterol-to-cholesterol ratios increased across tertiles of phytosterol intake (P=0.026). The HDL-cholesterol level increased and adiposity measures, cholesterol/HDL ratios, and levels of glucose, triglycerides and lathosterol, a cholesterol synthesis marker, decreased across plasma sitosterol tertiles (P<0.02; all). Compared to controls, cases had non-significantly lower median levels of phytosterol intake and plasma sitosterol. The multivariable-adjusted odds ratio for CHD across the lowest to the highest plasma sitosterol tertile was 0.59 (95% confidence interval, 0.37 to 0.95). Associations were weaker for plasma campesterol. Apolipoprotein E genotype was unrelated to CHD risk or plasma phytosterols.

Conclusion: Plasma sitosterol levels appear to be associated with a lower CHD risk in the EPIC-Spain cohort. Results also suggest that higher dietary phytosterol intake and higher plasma phytosterols are markers of a lower cardiometabolic risk.

Keywords: phytosterols, lathosterol, cholesterol, coronary heart disease, EPIC study.

Introduction

Dietary sterols consist of animal-derived cholesterol and plant-derived noncholesterol sterols or phytosterols. Phytosterols are important components of a vegetable-based diet and are particularly abundant in whole grains, nuts, seeds and oils derived from them. The principal molecular forms are sitosterol and campesterol (1). These compounds are structurally related to cholesterol, but have bulkier and more hydrophobic molecules, which confer them a higher affinity for intestinal micelles than has cholesterol. Consequently, cholesterol is displaced from micelles and the amount available for absorption is limited. The phytosterol content of usual diets is similar to that of cholesterol (150 to 450 mg/day), but their intestinal absorption is much less efficient [see review (2)]. Because of low absorption and rapid biliary elimination, physiological plasma concentrations of phytosterols are in the order of 10^{-3} those of cholesterol. Their ratios to cholesterol are accepted as surrogate markers for the efficiency of cholesterol absorption, while those of the cholesterol precursor lathosterol are a reliable index of cholesterol synthesis (3).

The lower absorption of phytosterols compared to cholesterol is attributable to active resecretion back into the intestinal lumen, a process which is mediated by the half-transporters ABCG5 and ABCG8. Genetic defects in these transporters (4) cause sitosterolemia, a rare autosomal recessive disorder characterized by intestinal sterol hyperabsorption, raised plasma phytosterol levels, xanthomas and accelerated atherosclerosis. Because of the presumed pathogenic role of elevated plasma phytosterols in sitosterolemia, the question whether high levels of circulating phytosterols might also be atherogenic in non-sitosterolemic individuals has been much debated (5,6). This is a substantial concern, given that inhibition of cholesterol absorption by gram doses of phytosterols incorporated into various foods is widely used as a non pharmacological strategy for cholesterol lowering, but is associated with increased serum phytosterol concentrations (6). Results of epidemiological studies have suggested either a

direct association between plasma phytosterols and CHD risk (7-10) or a null (11,12) and even inverse association (13).

A relevant yet unexplored issue is the effect on CHD risk of naturally-occurring phytosterols in the usual diet. Two large cross-sectional studies have shown a weak inverse association between dietary phytosterol intake and plasma total and LDL-cholesterol levels (14,15). Participants in the Spanish cohort of EPIC, a large prospective study in Europe (16), have a higher consumption of phytosterol-rich vegetables and fruits than most European countries and the US (17), confirming their adherence to the Mediterranean dietary pattern. We hypothesized that plasma phytosterol concentrations are markers of a healthy diet and are associated with a decreased risk for CHD, instead of an increased risk, in the Spanish EPIC cohort. To address this issue, we examined the association among dietary phytosterol intake, plasma levels of phytosterols, cardiometabolic risk factors and the risk of future CHD.

Methods

Study Design. We performed a nested case-control study among participants of the Spanish EPIC study (17). Subjects were recruited as part of a 10-country collaborative study designed to investigate dietary and other determinants of cancer (16). The population of the Spanish branch of EPIC included 41 440 individuals. Participants were healthy men and women volunteers, principally blood donors, aged between 30 and 69 years at enrolment between October 1992 and July 1996 in five regions, three in Northern Spain (Asturias, Navarra and Gipuzkoa) and two in Southern Spain (Murcia and Granada). In face-to face interviews, each participant was administered questionnaires to collect information on lifestyle factors, including food consumption and smoking, and a complete medical history, including a prior diagnosis of hypertension, hyperlipidemia or diabetes and medication use. Anthropometric measurements [height and weight, with calculation of body mass index (BMI) in kg/m^2, and

waist circumference] were obtained by using standardized procedures and a blood sample was taken. Close to 60% blood samples were collected after an overnight fast. To ascertain the vital status, annual record linkages were carried out with the national databases of the Instituto Nacional de Estadística, Spain. For this analysis, the follow-up for vital status was complete until 31 December 2004; the mean follow-up period was ≈10 y. All subjects provided written informed consent to a protocol approved by local ethical review boards.

Case Ascertainment. Cases were defined as participants who had a definite fatal or nonfatal myocardial infarction or angina requiring a revascularization procedure. Participants who at recruitment had a prior diagnosis of CHD that was validated thereafter were excluded from further analyses (N=193). For the identification of potential cases, a record linkage between the EPIC database and local hospital discharge registers was performed. A Population Myocardial Infarction Register available in three participating regions (Navarra, Guipuzkoa and Murcia) was also used. At censoring, 468 definite cases of incident fatal and nonfatal myocardial infarction and 141 cases of angina were identified.

Participants. Of the 609 confirmed CHD cases, approximately one half (n=315) were randomly selected for inclusion in the present analysis. Using an incidence density method (18), two controls, randomly selected among subjects in the cohort still at risk for CHD at the time of diagnosis of each case (namely, subjects that had not suffered a CHD event at the time their matched case-pair had an event), were matched to each case by center, sex, age (within 5 years), and time of enrolment (within 3 months). If needed, the same subjects could serve as a control more than once. Because we analysed incident (new) cases, any individual who suffered a CHD event during the 10-year follow-up period was no longer eligible to be a control.

Dietary Information. Information on usual food intake over the year before enrolment was collected by a validated computerized diet history questionnaire (19,20). Energy and nutrient

intakes were estimated using a conversion table in a computerized database especially compiled for the EPIC study in Spain. Intake of total phytosterols and their main components was estimated from the database of Spanish foods developed by Jiménez-Escrig et al. (21)

Laboratory Measurements. Coded samples of plasma and blood cells (buffy coat) were shipped to a central laboratory and stored at -80°C until assay. Plasma glucose was measured by the glucose-oxidase method in fasting samples. Cholesterol and triglycerides were determined by enzymatic procedures; triglycerides were measured only in fasting samples. HDL-cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride. The concentration of LDL-cholesterol was calculated as total cholesterol minus HDL-cholesterol minus triglycerides/5 when triglyceride levels were ≤3.36 mmol/L in fasting samples, and by the homogeneous method of Daiichi Pure Chemicals Company (N-geneous® LDL, Genzyme Diagnostics, Cambridge, MA) when triglyceride levels were >3.36 mmol/L and in nonfasting specimens. The determinations were made in an ADVIA 1800 chemical analyzer (Siemens Healthcare Diagnostics, Madrid, Spain). Genomic DNA was obtained to determine the apolipoprotein E (apoE) genotype (22).

Serum noncholesterol sterol levels were determined by gas chromatography using a modification of the method of Heinemann et al. (23). Epicoprostanol (2 μg) was added to serum (0.1 mL) as internal standard. After alkaline hydrolysis, extraction, and derivatization to trimethylsilyl ethers, sterols were quantified on a 30-m nonpolar capillary column (TRB-Esterol, Teknokroma, Barcelona) equipped with flame ionization detection in a Perkin Elmer GC AutosystemTM (Perkin Elmer, Norwalk, CT) apparatus. Each run quantified lathosterol, campesterol and sitosterol. Noncholesterol sterols are expressed as ratios to cholesterol (μg/mg cholesterol). Inter- and intra-assay coefficient variations were 5.0% and 3.2% for lathosterol, 1.9% and 1.6% for campesterol, and 2.0% and 1.8% for sitosterol, respectively.

Statistical Analyses. Qualitative variables are expressed as number (%). Data for continuous variables are presented as mean±SD. Phytosterol intakes and plasma levels of triglycerides and noncholesterol sterols had a skewed distribution and are presented as medians and interquartile ranges. Participants with more than 3 SDs from the mean of daily total energy intake were considered to have implausible dietary data and excluded from further analysis. We categorized control subjects by tertiles of both dietary phytosterol intake and plasma phytosterol-to-cholesterol ratios and used ANOVA, chi-square and Kruskall-Wallis statistics, as appropriate, to calculate tests for trend for cardiovascular risk factors. Pearson correlation coefficients were constructed to test for relationships between intake of phytosterols and other dietary variables. Because phytosterol intake was strongly related to total energy consumption and showed age and gender differences, age-, gender-, and energy-adjusted phytosterol values were used when examining associations with other dietary variables or plasma lipid values. Unpaired t-tests, chi-square tests or the Mann-Whitney test, as appropriate, were used for comparisons of variables between cases and controls. These statistical tests were 2-tailed and significance was set at $P<0.05$. Analyses were performed using SPSS, version 15.0 (SPSS Inc., Chicago, IL).

Odds ratios (OR) and 95% confidence intervals (CI) for CHD risk by plasma phytosterol ratios were calculated by conditional logistic regression using the PHREG procedure (SAS statistical software, v. 9, SAS Institute, Cary, NC), stratified by the case–control set. Risk estimates were computed as 'crude' (adjustment for matching variables only); after adjustment by cardiovascular risk factors and apoE genotype; and after additional adjustment by intake of energy and nutrients, including plant sterols. We did not adjust for statin use because of negligible numbers of subjects under statin treatment at recruitment (7 of 306 cases and 8 of 596 controls).

Results

From the total number of 315 CHD cases 9 were excluded, 1 due to implausible energy intake and 8 because plasma sterol determination failed due to insufficient or spoilt plasma samples; corresponding exclusions among the 630 control subjects were 3 and 35.

Associations of dietary phytosterols in control subjects. The median total phytosterol intake of the whole cohort (n=899) was 301 mg/d and was higher in men (326 mg/d, range 82 to 851) than in women (223 mg/d, range 26 to 501) (P<0.001). **Table 1** shows that phytosterol intake in control subjects was associated directly with both the male sex and total energy intake and inversely with age, but was unrelated to plasma lipid levels after adjusting by sex, age and energy intake. Plasma campesterol- and sitosterol-to-cholesterol ratios increased across tertiles of phytosterol intake, but only the sitosterol increase was significant (P=0.026). Sex-, age- and energy-adjusted phytosterol intake was directly correlated (P<0.001) with intake of fruits (r=0.473), legumes (r=0.320), cereals (r=0.231), fiber (r=0.603), vegetable protein (r=0.432), and polyunsaturated fatty acids (r=0.268) and inversely correlated (P<0.001) with intake of animal protein (r=–0.188), saturated fatty acids (r=–0.293) and cholesterol (r=–0.239). Intake of measurable phytosterol subclasses showed similar associations (data not shown).

Plasma phytosterols and risk factors in control subjects. Table 2 shows the distribution of cardiovascular risk factors, plasma noncholesterol sterol ratios and dietary phytosterol intake across tertiles of plasma sitosterol-to-cholesterol ratios in the control group. Plasma sitosterol tertiles were associated directly with HDL-cholesterol levels, plasma campesterol-to-cholesterol ratios, and phytosterol intake and inversely with BMI, waist circumference, plasma glucose and triglyceride levels, cholesterol/HDL ratios and lathosterol-to-cholesterol ratios. The total cholesterol level increased nonsignificantly with increasing plasma sitosterol.

Tertiles of plasma campesterol-to-cholesterol ratios showed similar associations (data not shown).

Characteristics of CHD cases and matched controls. The baseline characteristics of assessable CHD cases (n=306) and controls (n=592) are shown in **Table 3**. Matching secured that sex and age were comparable between cases and controls. Predictably, participants with incident CHD during follow-up had higher waist circumference and BMI and were more likely to smoke and have obesity, diabetes, hypertension and hyperlipidemia than controls. Also, total cholesterol, LDL-cholesterol and triglyceride levels were higher and HDL-cholesterol was lower in cases than controls. ApoE genotype frequency distribution and plasma noncholesterol sterol ratios were similar between the two groups. Intakes of total phytosterols and sitosterol, the main dietary noncholesterol sterol, were nonsignificantly lower in cases compared to controls. There were no case-control differences in energy or nutrient intake (data not shown).

Plasma phytosterols and CHD risk. Table 4 shows the unadjusted and adjusted ORs for future CHD by tertiles of plasma sitosterol- and campesterol-to-cholesterol ratios explored via conditional regression. Because this analysis requires strict case-control matching, 300 cases and 600 controls (39 used twice) were used by the model. The multivariable-adjusted risk for CHD was 41% lower in the highest plasma sitosterol-to-cholesterol tertile compared with the lowest tertile. The plasma campesterol ratios were not associated with the risk of CHD.

Discussion

The results of this study in the prospective Spanish EPIC cohort suggest that elevated levels of plasma phytosterols are not associated with an increased risk of incident CHD. Instead the upper tertile of cholesterol-adjusted plasma sitosterol, the main dietary phytosterol, was inversely related to CHD risk after controlling for various confounders. Furthermore, phytosterol intake with natural foods, a measure of healthy dietary choices, and HDL-cholesterol increased and adiposity measures, cholesterol/HDL ratios, and levels of fasting glucose, triglycerides and lathosterol, a cholesterol precursor, decreased across plasma sitosterol tertiles. Our results suggest that plasma phytosterols are a marker of a healthy diet and a lower cardiometabolic risk and are thus associated with a reduced risk for CHD.

The controversy on whether raised plasma phytosterols are a CHD risk factor in non-sitosterolemic individuals has been fed by the inconsistent findings of epidemiological studies (7-13). Other contradictory evidences have been presented recently. An investigation from the Dallas Heart Study showed no relationship between plasma phytosterols and a surrogate marker for CHD, namely coronary calcium scores measured by electron beam computed tomography (24). Miettinen et al. (25) reported an association between raised plasma phytosterol levels and increased phytosterol content of surgically removed carotid plaques, while Weingärtner et al. (26) showed that consumption of phytosterol-enriched margarine correlated with increased plasma concentrations and tissue deposition in aortic valves obtained at surgery in patients with aortic stenosis. Finally, Silbernagel et al. (27) showed a weak association of plasma phytosterol ratios with increased severity of angiographically assessed coronary artery disease. Our results agree with those of recent studies (11-13) in suggesting a lack of association and even a protective role of elevated plasma phytosterol concentrations on risk of future CHD.

The median phytosterol intake (301 mg/d) in the EPIC-Spain cohort was similar to that previously reported from another Spanish population (21) and from the EPIC-Norfolk study (14). However, it was slightly higher than that reported from a Swedish population study (15) and nearly 3 times higher than that described in the CORA study from Germany (12), which casts doubt on the reliability of the phytosterol database used in that study. As previously reported (14,15), the phytosterol content in the usual diet of EPIC-Spain participants correlated directly with consumption of healthy foods and nutrients, such as fruits and seeds, vegetable protein, fiber, and polyunsaturated fatty acids and inversely with intake of unhealthy diet components, such as animal protein, saturated fatty acids and cholesterol. Importantly, cholesterol-adjusted plasma sitosterol levels increased with increasing phytosterol intake. In the absence of supplementation with phytosterol-enriched foods, not commercially available in Spain at the time the EPIC cohort was recruited in 1992-1996, this finding confirms that plasma phytosterol levels are a marker of high phytosterol intake with the usual diet, which in turn reflects healthy food choices.

Besides diet, other factors may influence plasma phytosterol levels [see review (5)]. The apoE genotype has been studied in this regard because circulating phytosterols are markers for cholesterol absorption and apoE plays a major role in lipid transport, but findings have been inconsistent (5). In the present study, apoE variants were unrelated to plasma phytosterols. We did not investigate variability at the ABCG5/8 gene loci, a potent heritable factor influencing phytosterol levels (28). An additional factor and an important one determining circulating levels of noncholesterol sterols is adiposity. Recent work by Miettinen and colleagues (29-32) has shown that insulin resistance states, such as obesity, the metabolic syndrome and type 2 diabetes, are associated with increased cholesterol synthesis (i.e., high plasma lathosterol-to-cholesterol ratios) and reduced cholesterol absorption (i.e., low plasma sitosterol-to-cholesterol ratios). When obese diabetic subjects underwent weight

reduction, their cholesterol synthesis decreased while absorption increased (32). Indeed, there are evidences that a synergy exists whereby changes in cholesterol synthesis result in an opposite response of cholesterol absorption to maintain cholesterol homeostasis (33). Concurring with these concepts, our results show that adiposity, fasting glucose, triglycerides and the cholesterol synthesis marker lathosterol decreased across tertiles of plasma sitosterol ratios. As measures of the efficiency of sterol absorption, plasma sitosterol ratios were associated with nonsignificant increases of the total cholesterol level. However, because HDL-cholesterol increased proportionately to a greater extent, the cholesterol/HDL ratio was lower. Thus increased plasma phytosterols signaled leaner individuals with a lower overall cardiometabolic risk.

An additional factor that influences plasma phytosterol-to-cholesterol ratios is statin treatment (5,33). This is of little concern for the present study, given that statins were sparingly used at the time of recruitment of the cohort. Finally, the plasma sitosterol and campesterol ratios to cholesterol were in the same physiological range as reported in previous studies (7-13), strengthening the reliability of our data.

This study has limitations. Only a single measure of dietary exposure and risk factor status at baseline was available, therefore changes in diet and other lifestyle variables that might have influenced CHD risk throughout the ≈10-y follow-up were not accounted for. It could be argued that the findings are specific for the Mediterranean cohort studied, but the similarity of plasma noncholesterol levels with those reported in epidemiologic studies from the US (7,24), Finland (8), Germany (9,10,12), the UK (11) and The Netherlands (13) suggests that they may be generalized to other populations.

In conclusion, circulating phytosterols are unrelated to the risk of incident CHD in the prospective cohort of the Spanish EPIC study. Elevated plasma phytosterols are associated with higher dietary phytosterol intake, which reflects healthy food choices in the habitual diet.

Furthermore, they are indicative of an efficient intestinal cholesterol absorption, which only marginally raises total cholesterol levels but is strongly and inversely related to features of the metabolic syndrome. Plasma concentrations of the main phytosterols, sitosterol and campesterol, appear to be markers of a lower cardiometabolic risk. The present results suggest that moderately elevated plasma sitosterol, but not campesterol, might signal individuals with a reduced risk for CHD. However, taking into consideration all the epidemiological studies dealing with this topic (7-13,24-28), we cannot still answer the important question of whether circulating phytosterols are proatherogenic, antiatherogenic or neutral.

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Conflicts of interest: none declared.

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TABLE 1. Distribution of Plasma Lipids and Noncholesterol Sterol Ratios by Tertiles of Total Plant Sterol Intake in Control Subjects.

Characteristics	**Tertile 1**	**Tertile 2**	**Tertile 3**	***P* value***
Plant sterol intake, mg/d	**≤258.7**	**258.8-359.9**	**≥360.0**	
N	198	197	197	
Sex, male (%)	111 (56)	167 (85)	182 (92)	<0.001
Age, years	55.5±7.6	53.6±6.8	53.3±7.1	0.006
Total energy intake, KJ/d	8317±2515	10179±2564	12581±2912	<0.001
Plasma lipids, mmol/L				
Total cholesterol				
Unadjusted	5.67±0.85	5.67±0.91	5.67±0.91	0.996
Adjusted†	5.67	5.67	5.67	0.812
LDL-cholesterol				
Unadjusted	3.63±0.80	3.65±0.86	3.73±0.83	0.371
Adjusted†	3.63	3.65	3.73	0.320
HDL-cholesterol				
Unadjusted	1.45±0.36	1.37±0.34	1.32±0.34	<0.001
Adjusted†	1.45	1.37	1.32	0.083
Cholesterol/HDL ratio				
Unadjusted	4.12±1.12	4.40±1.25	4.58±1.42	0.002
Adjusted†	4.12	4.40	4.58	0.065
Triglycerides, mmol/L‡	1.09 [0.83-1.47]	1.23 [0.83-1.64]	1.13 [0.87-1.64]	0.384
Plasma noncholesterol sterol-to-cholesterol ratios, μmol/mmol				
Lathosterol	1.56 [1.10-2.09]	1.63 [1.33-2.18]	1.68 [1.31-2.16]	0.308
Campesterol	1.43 [1.17-2.03]	1.58 [1.25-1.93]	1.73 [1.26-2.11]	0.139
Sitosterol	1.27 [0.96-1.76]	1.36 [1.10-1.86]	1.54 [1.18-1.87]	0.026

Values are mean±SD or number (percent). Triglycerides and noncholesterol sterol ratios are medians [interquartile ranges].

**P* for linear trend calculated by ANOVA, chi-square or Kruskall-Wallis statistics, as appropriate.

†Adjusted by sex, age and energy intake.

‡Measured in 361 fasting samples.

TABLE 2. Distribution of Cardiovascular Risk Factors and Noncholesterol Sterols by Tertiles of Plasma Sitosterol-to-Cholesterol Ratios in Control Subjects.

Characteristics	**Tertile 1**	**Tertile 2**	**Tertile 3**	***P****
Sitosterol-to-cholesterol ratio, µg/mg	**≤1.16**	**1.17-1.63**	**≥1.64**	
N	198	196	198	
Age, years	60.9±7.1	60.6±7.7	59.9±7.7	0.368
Male sex, n (%)	149 (75.3)	160 (81.6)	151 (76.3)	0.263
Body mass index, kg/m^2	30.1±4.2	28.4±3.1	27.5±3.2	<0.001
Waist circumference, cm	102.2±10.1	97.7±9.2	94.9±9.7	<0.001
Hypertension, n (%)	47 (23.9)	54 (27.6)	43 (21.7)	0.394
Fasting glucose, mmol/L†	5.11±1.94	4.56±0.94	4.56±0.94	0.002
Plasma lipids, mmol/L				
Total cholesterol	5.67±0.91	5.59±0.83	5.78±0.93	0.087
LDL-cholesterol	3.65±0.80	3.60±0.73	3.76±0.93	0.170
HDL-cholesterol	1.30±0.31	1.35±0.31	1.48±0.39	<0.001
Triglycerides mg/dl†	1.32 [0.94-2.09]	1.12 [0.83-1.44]	1.03 [0.81-1.37]	<0.001
Cholesterol/HDL ratio	4.56±1.23	4.32±1.16	4.21±1.41	0.019
ApoE genotype, n (%)‡				
ApoE2	15 (8.7)	14 (8.2)	14 (7.7)	
ApoE3	130 (75.1)	122 (71.8)	141 (77.5)	
ApoE4	28 (16.2)	34 (20.0)	27 (14.8)	0.743
Plasma noncholesterol sterol-to-cholesterol ratios, µmol/mmol				
Lathosterol	2.00 [1.55-2.41]	1.59 [1.27-2.07]	1.46 [1.09-1.87]	<0.001
Campesterol	1.09 [0.94-1.27]	1.53 [1.33-1.77]	2.18 [1.84-2.62]	<0.001
Total plant sterol intake, mg/d	287 [217-363]	328 [238-407]	317 [242-401]	0.023
Sitosterol	184 [132-225]	204 [149-252]	201 [154-255]	0.016
Campesterol	28 [20-35]	34 [24-43]	31 [25-40]	0.003

**P* value for linear trend calculated by ANOVA, chi-square or Kruskall-Wallis statistics, as appropriate.

†Measured in 361 fasting samples.

‡Determined in 532 participants and classified as ApoE2 (E2/2+E2/3), ApoE3 (E3/3) or ApoE4 (E3/4+E4/4), with exclusion of 7 subjects with the E2/4 genotype.

TABLE 3. Baseline Characteristics of CHD Cases and Matched Control Subjects.

Variables	Cases (N=306)	Controls (N=592)	*P* value*
Age, y	54.3±7.4	54.1±7.2	0.765
Men, n (%)	241 (78.8)	461 (77.9)	0.727
Smoking, N (%)			
Never	93 (30.4)	251 (42.4)	
Past	65 (21.2)	143 (24.2)	
Current	148 (48.4)	198 (33.4)	<0.001
Body mass index, kg/m^2	29.5±3.7	28.7±3.7	0.001
Waist circumference, cm	100.4±9.8	98.3±10.1	0.003
Obese, n (%)	124 (40.5)	179 (30.2)	0.002
Hypertension, n (%)	110 (35.9)	144 (24.3)	<0.001
Type 2 diabetes, n (%)	41 (13.4)	41 (6.9)	0.001
Hyperlipidemia, n (%)	126 (41.3)	150 (25.3)	<0.001
Fasting glucose, mmol/L†	4.78±1.44	4.39±1.28	0.001
Total cholesterol, mmol/L	6.11±0.96	5.70±0.88	<0.001
LDL-cholesterol, mmol/L	4.07±0.91	3.68±0.83	<0.001
HDL-cholesterol, mmol/L	1.27±0.39	1.37±0.36	<0.001
Triglycerides, mmol/L†	1.41 [1.06-2.04]	1.13 [0.84-1.59]	<0.001
Cholesterol/HDL ratio	5.16±1.50	4.36±1.28	<0.001
ApoE genotype, n (%)‡			
ApoE2	18 (6.6)	43 (8.2)	
ApoE3	205 (75.1)	393 (74.9)	
ApoE4	50 (18.3)	89 (17.0)	0.673
Total dietary plant sterols, mg/d	299 [236-386]	321 [246-403]	0.089
Campesterol, mg/d	30 [23-38]	31 [23-40]	0.371
Sitosterol, mg/d	178 [143-228]	192 [148-241]	0.055
Plasma noncholesterol sterol-to-cholesterol ratios, μmol/mmol			
Lathosterol	1.53 [1.23-2.05]	1.56 [1.14-2.05]	0.697
Campesterol	1.49 [1.19-1.94]	1.54 [1.19-1.98]	0.382
Sitosterol	1.30 [1.00-1.70]	1.37 [1.03-1.78]	0.105

Values are mean±SD or number (percent). Triglycerides and noncholesterol sterol ratios are medians [interquartile ranges].
*Unpaired t-test, chi-square test or Man-Whitney test, as appropriate.
†Measured in 550 fasting samples (189 cases and 361 controls).
‡Determined in 808 participants and classified as ApoE2 (E2/2+E2/3), ApoE3 (E3/3) or ApoE4 (E3/4+E4/4), with exclusion of 10 subjects with the E2/4 genotype.

TABLE 4. Odds Ratios (95% confidence intervals) for Incident CHD across Tertiles of Plasma Phytosterol to Cholesterol ratios.

	Cases	Controls	Unadjusted OR	Adjusted OR	
	No. (%)	No. (%)		Model*	Model†
Sitosterol-to-cholesterol ratio					
≤1.14	109 (36.3)	169 (30.1)	1	1	1
1.15-1.59	103 (34.3)	186 (33.2)	0.846 (0.596-1.202)	0.918 (0.587-1.435)	0.938 (0.588-1.495)
≥1.60	88 (29.3)	206 (36.7)	0.651 (0.456-0.927)	0.618 (0.391-0.977)	0.590 (0.365-0.953)
	P for trend		0.017	0.034	0.027
Campesterol-to-cholesterol ratio					
≤1.29	103 (34.3)	175 (31.2)	1	1	1
1.30-1.78	104 (34.7)	182 (32.4)	0.957 (0.674-1.357)	1.115 (0.722-1.722)	1.079 (0.683-1.706)
≥1.79	93 (31.0)	204 (36.4)	0.743 (0.520-1.062)	0.897 (0.575-1.401)	0.787 (0.487-1.273)
	P for trend		0.099	0.617	0.321

*Adjusted by smoking, BMI, diabetes, hypertension, total cholesterol, HDL-cholesterol, and apoE genotype.

†Additionally adjusted by intake of energy, protein, fiber, saturated, mono and polyunsaturated fatty acids, alcohol, cholesterol, and plant sterols.

3.3. Article 3

Plasma phytosterols are inversely associated with the metabolic syndrome and its components

Cofán M*, Escurriol V*, García-Otín AL, Moreno-Iribas C, Larrañaga N, Sánchez MJ, Tormo MJ, Redondo ML, González CA, Corella D, Pocoví M, Civeira F, Ros E.

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Key Words:	Cholesterol Metabolism, Metabolic Syndrome, Cardiovascular Risk Factors, Adiposity, Lipids and Lipoproteins

Cross-sectional association of plasma markers of cholesterol homeostasis with the metabolic syndrome and its components

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ABSTRACT

OBJECTIVE—Increased plasma phytosterols, which reflect increased cholesterol absorption, have been related to an increased risk of atherosclerosis. However, obesity, diabetes and the metabolic syndrome (MetS), with an inherent high risk for cardiovascular diseases, have been associated with reduced cholesterol absorption. We investigated associations between plasma noncholesterol sterols and MetS components.

RESEARCH DESIGN AND METHODS—With a cross-sectional design, we related MetS components to plasma noncholesterol sterols-to-cholesterol ratios measured by gas chromatography in 674 dyslipidemic patients and in 361 healthy subjects participating in a prospective cohort study.

RESULTS—Plasma phytosterols-to-cholesterol ratios were inversely associated with all components of the MetS and the syndrome proper. In the dyslipidemic group, multivariable analyses showed that a 1-SD increase in sitosterol-to-cholesterol ratio was associated with a reduced risk for any MetS feature, ranging from 0.57 (95% CI, 0.45 to 0.71) for visceral adiposity to 0.82 (95% CI, 0.69 to 0.98) for high blood pressure. The risk of having MetS was nearly halved, with ORs of 0.49 (95% CI, 0.38 to 0.64) or 0.56 (95% CI, 0.44-0.70), depending on the definition. Results were opposed for plasma lathosterol, a marker of cholesterol synthesis. Except for high blood pressure, the findings were reproduced in the healthy cohort. ApoE genotype was unrelated to plasma noncholesterol sterols.

CONCLUSIONS—In both dyslipidemic and healthy populations, the MetS is associated with increased plasma lathosterol, a cholesterol synthesis marker, and decreased plasma sitosterol, a marker of cholesterol absorption. Thus, elevated plasma phytosterols concentrations are related to an overall lower cardiometabolic risk.

Key words: Cholesterol Metabolism. Metabolic syndrome. Cardiovascular risk factors. Adiposity. Lipids and Lipoproteins

Introduction

Dietary sterols consist of animal-derived cholesterol and plant sterols or phytosterols. The principal molecular forms of phytosterols are sitosterol and campesterol (1). These compounds are structurally related to cholesterol, but have bulkier and more hydrophobic molecules, which confer them a higher affinity for intestinal micelles than has cholesterol. Consequently, cholesterol is displaced from micelles and the amount available for absorption is limited. The phytosterol content of usual diets is similar to that of cholesterol, but their intestinal absorption is much less efficient [see review (2)]. Because of low absorption and rapid biliary elimination, physiological plasma concentrations of phytosterols are in the order of 10^{-3} those of cholesterol.

The lower absorption of phytosterols compared to cholesterol is attributable to active resecretion back into the intestinal lumen, a process which is mediated by the half-transporters ABCG5 and ABCG8. Genetic defects in these transporters (3) cause sitosterolemia, a rare autosomal recessive disorder characterized by intestinal sterol hyperabsorption, raised plasma phytosterol levels, xanthomas and accelerated atherosclerosis. Because of the presumed pathogenic role of elevated plasma phytosterols in sitosterolemia, the question whether high levels of circulating phytosterols might also be atherogenic in non-sitosterolemic individuals has been much debated (4,5).

The plasma ratios of phytosterols to cholesterol are accepted as surrogate markers for the efficiency of intestinal cholesterol absorption, while those of the cholesterol precursor lathosterol are a reliable index of cholesterol synthesis (6). A reciprocal relationship exists between cholesterol synthesis and absorption, whereby individuals who synthesize little cholesterol tend to absorb more, while those disclosing high cholesterol synthesis show low absorption rates (7), thus these interrelated regulatory mechanisms contribute to tightly controlling cholesterol homeostasis. It has been known for some time that obesity is

associated with markedly increased cholesterol synthesis (8). More recently, Miettinen and colleagues have shown that obesity and related metabolic disturbances, such as type 2 diabetes, insulin resistance and the metabolic syndrome (MetS), are characterized not only by increased cholesterol synthesis (as determined by raised plasma levels of lathosterol) but also by low cholesterol absorption (as determined by decreased plasma phytosterols levels) (9-12). Other authors have reported similar findings in patients with the MetS (13).

The presumed enhanced cardiovascular risk of moderately elevated plasma phytosterols levels (4, 5) is counterintuitive with the notion that the opposite situation, namely low plasma phytosterol levels, occurs in the MetS, a cluster of risk factors carrying a high cardiovascular risk (14). Former studies showing an association of obesity-related metabolic disturbances with low circulating phytosterols levels (9-13) involved small numbers of study subjects. We therefore assessed whether the association between the MetS and the low cholesterol absorption/high cholesterol synthesis trait existed in two independent groups of subjects well phenotyped for cardiovascular risk factors, a group of 674 Lipid Clinics patients and a sample of 361 healthy persons drawn from the Spanish population cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC), a large prospective study in Europe (15).

Research design and methods

Subjects

This is a cross-sectional study of two samples, a series of 674 asymptomatic adults with primary lipid disorders attending two Lipid Clinics in Spain and a group of 361 healthy individuals from the Spanish EPIC cohort. The dyslipidemic subjects were consecutively evaluated at Lipid Clinics in Hospital Clínic of Barcelona (n=360) and Hospital Miguel Servet of Zaragoza (n=314) from June 2004 to September 2008. Secondary causes of

dyslipidemia, including renal and hepatic disease, untreated hypothyroidism, alcohol abuse, and treatment with drugs influencing lipid metabolism were excluded in all subjects by clinical history and blood tests. The 361 healthy subjects were controls from a nested case-control study of coronary heart disease (CHD) within the Spanish EPIC cohort, which includes healthy men and women volunteers aged between 30 and 69 years at enrolment between October 1992 and July 1996 in five Spanish regions, three in Northern Spain (Asturias, Navarra and Gipuzkoa) and two in Southern Spain (Murcia and Granada).

All subjects provided informed consent to a protocol approved by the corresponding Institutional Ethical Review Boards.

Clinical and laboratory characteristics

Dyslipidemic group. We assessed all subjects for clinical history, medication use, demographic characteristics, and standard cardiovascular risk factors. History of smoking was established if the subject reported smoking ever ≥3 cigarettes per day for at least 5 years. Height and weight were measured with the subjects without shoes wearing light clothes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. We measured waist circumference midway between the lowest rib and the iliac crest using an anthropometric tape. Blood pressure was measured in duplicate with a validated semi-automatic oscillometer (Omron HEM-705CP, The Netherlands). Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg or current use of antihypertensive medication. Type 2 diabetes was diagnosed by fasting plasma glucose ≥7.0 mmol/l (126 mg/dl), 2–h plasma glucose ≥11.1 mmol/l (200 mg/dl) during an oral glucose tolerance test or treatment with antidiabetic agents.

After at least 4 weeks without hypolipidemic drug treatment and no intake of phytosterol-supplemented foods, fasting blood was obtained by venipuncture and drawn into plain and K3EDTA tubes. Serum and plasma samples were separated by mild centrifugation and used

immediately for glucose and lipid analyses or stored at -80ºC for later determination of noncholesterol sterols levels. Genomic DNA was also obtained and stored at -80ºC. Blood glucose was measured by the glucose-oxidase method. Cholesterol and triglycerides were determined by standard enzymatic methods. HDL cholesterol was measured by a precipitation method. LDL cholesterol was calculated as total cholesterol – HDL cholesterol – triglycerides/5, except in samples with triglycerides ≥4.48 mmol/l (400 mg/dl), when it was measured in the d=1.063 g/mL fraction separated by density gradient ultracentrifugation. *EPIC cohort.* Information on socio-demographic characteristics; lifestyle factors, including lifetime history of tobacco use; medical history, including a prior diagnosis of hypertension, hyperlipidemia, or diabetes; anthropometric measurements, including waist circumference; and fasting blood samples were obtained by standard methods at enrolment, as described (15). Blood pressure measurements were not obtained, thus the data on this MetS feature is limited to self-reported hypertension. Determinations of plasma glucose and lipid profiles were performed in the corresponding frozen samples by the methods described, with the exception of LDL cholesterol when the triglyceride concentration was ≥4.48 mmol/l (400 mg/dl), which was measured by the homogeneous method of Daiichi Pure Chemicals Company (N-geneous® LDL, Genzyme Diagnostics, Cambridge, MA).

Analysis of noncholesterol sterols

Plasma noncholesterol sterols (lathosterol, campesterol and sitosterol) levels were determined by gas chromatography using a modification of the method of Heinemann and colleagues (16). Epicoprostanol (2 µg) was added to serum (0.1 ml) as internal standard. After alkaline hydrolysis, extraction, and derivatization to trimethylsilyl ethers, sterols were quantified on a 30-m nonpolar capillary column (TRB-Esterol, Teknokroma, Barcelona, Spain) equipped with flame ionization detection in a Perkin Elmer GC AutosystemTM (Perkin Elmer, Norwalk, CT, USA) apparatus. Each run quantified lathosterol, campesterol and sitosterol.

Noncholesterol sterols are expressed as ratios to cholesterol (µmol/mmol cholesterol). Inter- and intra-assay CVs were 5.0% and 3.2% for lathosterol, 1.9% and 1.6% for campesterol, and 2.0% and 1.8% for sitosterol, respectively.

Apolipoprotein E genotyping

The apolipoprotein E (apoE) genotype was determined in genomic DNA by using the method of Hixson and Vernier (17). To test associations with other covariates, E4/3 and E4/4 genotypes were grouped (E4), E3/3 were considered a single group (E3), and E3/2 and E2/2 were grouped (E2), while E4/2 subjects were excluded.

Definition of metabolic syndrome

Data on waist circumference, systolic and diastolic blood pressure (or diagnosis of hypertension), fasting blood glucose (or diagnosis of diabetes), fasting triglycerides and HDL cholesterol levels were used to classify subjects as having the metabolic syndrome by two common definitions, as proposed by the updated National Cholesterol Education Program Adult Treatment Panel (ATP) III (15) and the International Diabetes Federation (IDF) (18).

Statistical analyses

Data for continuous variables are presented as mean ± SD or medians (interquartile ranges) depending on normality distribution. Categorical variables are shown as number (percent). Gender differences in risk factor levels were evaluated by the unpaired t-test, Mann-Whitney or chi-square tests, as appropriate. Differences in noncholesterol sterol levels by apoE genotype were assessed by analysis of variance. Odds Ratios (OR) and 95% confidence intervals (CI) for MetS and its components by plasma noncholesterol sterol ratios transform to Z scores were calculated by logistic regression. All models were adjusted by sex, age, and smoking. The interaction of noncholesterol sterols and sex was not statistically significant for MetS and therefore was not introduced in the multivariable models. The statistical tests were

2-tailed and significance was set at P<0.05. Analyses were performed using SPSS, version 15.0 (SPSS Inc., Chicago, IL).

Results

The dyslipidemic group was made up of 391 men and 283 women, with mean age 47±13 years, mean body mass index (BMI) 26.32±4.00 kg/m^2, and a wide range of serum total cholesterol levels (4.40 to 16.27 mmol/L). The EPIC cohort consisted of 279 men and 82 women, with mean age 54 ± 8 years, mean BMI 28.57 ± 3.82 kg/m^2, and total cholesterol levels ranging from 3.29 to 9.19 mmol/L. Table 1 shows the clinical characteristics, adiposity measures, cardiovascular risk factors, and noncholesterol ratios of the two study groups by sex. In the dyslipidemic group, women were older and leaner and had lower blood pressure and higher sitosterol-to-cholesterol ratio than men. In the EPIC cohort, women had self-reported hypertension more frequently than men. In both groups, men had a higher proportion of smokers, waist circumference, triglycerides, cholesterol/HDL ratios, and lathosterol ratios and lower HDL cholesterol than women. The disparity in the rates of smoking among men in the two groups can be attributed in part to the time difference of nearly 12 years in data acquisition and to dyslipidemic men having received strong advice against smoking.

Predictably from the characteristics of the study groups, dyslipidemic patients had a greater proportion of MetS features than EPIC participants, except for visceral obesity by any definition, which was lower in both sexes in the dyslipidemic group and may be attributed in part to the younger age of participants and their having often received dietary treatment for excess body weight (Table 2). By either the ATP III or IDF definition, dyslipidemic men and women had nearly twice the MetS rates of their EPIC group counterparts. Men had a greater prevalence of MetS than women in the dyslipidemic group, but rates were similar in the EPIC cohort. Noncholesterol sterols were unrelated to apoE genotypes in either group (Table 3).

In multivariable analyses (Figure 1), a 1-SD increase in sitosterol-to-cholesterol ratio was associated with a reduced OR for any MetS feature, ranging from 0.57 (95% CI, 0.45 to 0.71) for visceral adiposity to 0.82 (95% CI, 0.69 to 0.98) for high blood pressure in the dyslipidemic group. The risk of having MetS was nearly halved, with ORs of 0.49 (95% CI, 0.38 to 0.64) for the ATP III definition and 0.56 (95% CI, 0.44-0.70) by IDF criteria. The results were similar when considering 1-SD increases in campesterol-to-cholesterol ratio (data not shown). The ORs for a 1-SD increase in lathosterol-to-cholesterol ratio followed almost exactly the opposite pattern, in such a way that the risk of having the MetS proper was nearly doubled by the two definitions. When performing the same analyses in the EPIC sample (Figure 2), the results were similar except for the nonsignificant changes in risk of having high blood pressure, but it must be borne in mind that these results refer only to self-reported treated hypertension. Considering such a conservative definition of the MetS, 1-SD increases in sitosterol ratios more than halved the risk, while 1-SD increases in lathosterol ratios increased risk between 1.5 and 2 times.

Conclusions

The results of this cross-sectional study in 674 asymptomatic, untreated dyslipidemic patients show that increasing plasma levels of lathosterol (a cholesterol synthesis marker) and decreasing levels of sitosterol and campesterol (cholesterol absorption markers) are associated with all MetS components (visceral obesity, high blood pressure, raised concentrations of glucose and triglycerides, low HDL cholesterol) and the syndrome proper, as defined by ATP III or IDF criteria. These findings, which have been reproduced in an altogether different group of 361 healthy participants in the prospective Spanish EPIC cohort, strongly suggest that raised plasma phytosterols levels within physiological ranges are markers of a lower cardiometabolic risk.

The present results confirm the profound influence that obesity, type 2 diabetes and the MetS have on cholesterol homeostasis, as described by Miettinen and colleagues (9-12) and other authors (13) in small studies. Miettinen's group also studied 268 hypercholesterolemic CHD patients and found markers of high cholesterol synthesis/low absorption in those with overweight, high triglycerides and low HDL cholesterol (19) and subsequently confirmed these findings in another group of 263 asymptomatic subjects with mild hypercholesterolemia (20). The results of a CHD case-control study with 1131 participants in the EPIC-Norfolk study also showed that plasma phytosterols ratios were correlated inversely to BMI and triglycerides and directly to HDL cholesterol (21). However, none of these studies (19-21) focused on the MetS as a whole. Recently, Assmann and colleagues (22) reported graded relationships between plasma noncholesterol sterols ratios and BMI (in lieu of waist circumference), triglycerides, HDL cholesterol and the number of MetS components in a sample of 492 subjects from the Prospective Cardiovascular Münster (PROCAM) study and, although no data on blood pressure or fasting glucose were reported, the associations followed trends similar to those observed in our study.

The results of multivariable analyses (Figure 1) show that each 1-SD increase in the plasma sitosterol-to-cholesterol ratio is associated with a lower risk of any MetS feature, with risk reductions ranging from 43% for visceral obesity to 18% for high blood pressure. The reduction in risk of disclosing the full syndrome was 44% or 51%, depending on the definition. That the strongest effect of increasing sitosterol ratios was on visceral obesity is not surprising, given that adiposity appears to be the driving force for development of MetS and related conditions (14). The lower risk of high blood pressure with increasing phytosterol ratios has not been described before. Unfortunately, we could not reproduce this finding in the EPIC participants because no blood pressure measurements were available. The reciprocal associations with MetS components of increasing lathosterol ratios, indicative of enhanced

cholesterol synthesis, concur with the known synergy whereby changes in cholesterol absorption result in an opposite response of cholesterol synthesis to maintain cholesterol homeostasis (7). The strong effect of adiposity reducing cholesterol absorption and, consequently, plasma phytosterol levels appears to be reversible with weight loss, as shown by changes in plasma noncholesterol sterol ratios in obese patients with type 2 diabetes (10).

The probable mechanism whereby excessive adiposity and attendant high cholesterol synthesis reduce cholesterol absorption is by stimulation of the nuclear receptor LXR, a sterol sensor that responds to excess cellular cholesterol through activation or repression of target genes involved in cholesterol homeostasis (23). Thus, overexpression of ABCG5/8 transporters by LXR activation will both promote biliary cholesterol excretion and limit intestinal sterol absorption (24). Conversely, low cholesterol synthesis in lean individuals might repress LXR and its target genes ABCG5/8, thus allowing a higher mass of intestinal sterols into intestinal cells for delivery to chylomicrons. As recently shown, high cholesterol traffic within enterocytes induces intestinal ABCA1-mediated cholesterol efflux (25) and could thus be relevant to the increased HDL cholesterol level associated with enhanced cholesterol absorption.

Besides adiposity, other important factors may influence plasma phytosterols levels (4,7). The apoE genotype has been studied in this regard because circulating phytosterols are markers for cholesterol absorption and apoE plays a major role in lipid transport, but findings have been inconsistent (4,7). In the present study, apoE variants were unrelated to plasma phytosterols (Table 3). We did not investigate variability at the ABCG5/8 gene loci, a potent heritable factor influencing phytosterol levels (20). An additional factor that influences phytosterols levels is statin treatment, via the profound decrease in circulating cholesterol (the denominator in the ratio) and a compensatory increase in intestinal absorption of cholesterol and its markers (the numerator) (4,7). This is of little concern for the present study, given that

blood samples were obtained off hypolipidemic drug treatment in dyslipidemic patients and statins were sparingly used at the time of recruitment of the EPIC cohort in 1992-1996. Finally, consumption of phytosterol-enriched products raises plasma phytosterol levels (5) but care was taken to prohibit these supplements before obtaining blood samples in the dyslipidemic group and, furthermore, they were not commercially available in Spain when EPIC participants were enrolled.

Our study has limitations. The population sample was made up of controls matched to CHD cases from a nested case-control study within the Spanish EPIC cohort. Because of that, the proportion of men is higher than the proportion of women and the results have to be interpreted in this specific context, although all ORs were adjusted by sex. Other potentially confounding factors for MetS, such dietary variables, physical activity, and menopausal status were not adjusted for. Also, the data about hypertension were self-reported in the EPIC group, which makes this MetS variable unreliable and the MetS definition conservative. However, all other MetS components showed associations with plasma noncholesterol sterols similar than those observed in the main dyslipidemic group, arguing in favor of the validity of the findings. The study also has strengths, such as a high statistical power and accurate phenotyping of participants for cardiometabolic risk factors. Finally, the plasma noncholesterol-to-cholesterol ratios were in the same physiological range as reported in previous studies (9-13,19-22), strengthening the reliability of our data.

In conclusion, increased plasma phytosterols within physiological ranges signal leaner individuals with a lower prevalence of the MetS and all its components. The lower cardiometabolic risk associated with higher plasma phystosterols should translate into a reduced risk rather than an increased risk for CHD.

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Table 1—*Clinical and biochemical characteristics of study groups by sex*

	Dyslipidemic group		EPIC cohort	
Variables	Men (n=391)	Women (n=283)	Men (n=279)	Women (n=82)
Age, years	46 ± 12	50 ± 14†	54 ± 7	55 ± 8
Ever smoked, n (%)	140 (35.8)	57 (20.1)†	196 (70.3)	16 (19.5)†
BMI (kg/m^2)	27.13 ± 3.26	25.17 ± 4.62†	28.49 ± 3.70	28.86 ± 4.21
Waist circumference (cm)	97 ± 9	86 ± 13†	100 ± 9	91 ± 10†
Systolic blood pressure	130 ± 21	124 ± 21*	-	-
Diastolic blood pressure	79 ± 13	76 ± 13*	-	-
Hypertension, n (%)	88 (22.5)	54 (19.1)	35 (12.5)	18 (22.0)*
Diabetes mellitus, n (%)	23 (5.9)	8 (2.8)	19 (6.8)	6 (7.3)
Glucose (mmol/L)	5.36 ± 1.01	5.11 ± 8.0	4.75 ± 1.46	4.67 ± 1.02
Total cholesterol (mmol/L)	7.46 ± 1.86	7.80 ± 1.68	5.65 ± 0.91	5.59 ± 0.84
LDL cholesterol (mmol/L)	5.31 ± 1.74	5.59 ± 1.68	3.69 ± 0.87	3.50 ± 0.79
HDL cholesterol (mmol/L)	1.17 ± 0.28	1.58 ± 0.44†	1.31 ± 0.31	1.55 ± 0.32†
Triglycerides (mmol/L)	1.62 (1.12 – 2.70)	1.08 (0.77 – 1.61)*	1.20 (0.89 - 1.66)	1.02 (0.72 - 1.30)*
Cholesterol /HDL ratio	6.40 ± 6.55	4.93 ± 3.82†	4.55 ± 1.33	3.77 ± 1.03†
Lathosterol/cholesterol (µmol/mmol)	1.68 ± 0.76	1.31 ± 0.63†	1.82 ± 0.77	1.60 ± 0.65*
Campesterol/cholesterol (µmol/mmol)	2.08 ± 1.06	2.23 ± 1.20	1.68 ± 0.62	1.82 ± 1.00
Sitosterol/cholesterol (µmol/mmol)	1.79 ± 0.89	1.99 ± 1.11*	1.53 ± 0.60	1.58 ± 0.90

Values are mean ± SD except for triglycerides (medians and interquartile ranges).

*P<0.05, †P<0.001 versus men by unpaired t-test.

Table 2—*Frequency of metabolic syndrome components in the two study groups*

	Dyslipidemic group		EPIC cohort	
	Men	Women	Men	Women
N	391	283	279	82
Metabolic syndrome components				
Visceral obesity by ATP III criteria	104 (26.6)	110 (38.9)†	109 (39.1)	48 (58.5)‡
Visceral obesity by IDF criteria	225 (57.5)	168 (59.4)	213 (76.3)	67 (81.7)
Elevated triglycerides	187 (47.8)	68 (24.0)‡	69 (24.7)	14 (17.1)
Low HDL cholesterol	127 (32.5)	86 (30.4)	50 (17.9)	17 (20.7)
High blood pressure*	218 (55.8)	125 (44.2)†	35 (12.5)	18 (22.0)†
High fasting glucose/diabetes	117 (29.9)	57 (20.1)†	42 (15.1)	8 (9.8)
Metabolic syndrome				
ATP III definition	126 (32.2)	64 (22.6)†	30 (10.8)	11 (13.4)
IDF definition	149 (38.1)	74 (26.1)†	44 (15.8)	12 (14.6)

Values are n (%).

*Only self-reported data on treated hypertension was available for EPIC participants.

†$P<0.05$, ‡$P<0.001$ versus men by chi-square test.

Table 3 — *Noncholesterol sterol ratios by apolipoprotein E genotype in the two study groups

	Dyslipidemic patients			EPIC participants		
ApoE genotype	E2	E3	E4	E2	E3	E4
N	47	432	150	27	235	55
Lathosterol/cholesterol (µmol/mmol)	1.67 ± 0.58	1.51 ± 0.76	1.55 ± 0.72	1.77 ± 0.62	1.78 ± 0.73	1.75 ± 0.72
Campesterol/cholesterol (µmol/mmol)	2.19 ± 1.33	2.12 ± 1.07	2.30 ± 1.24	1.72 ± 0.77	1.72 ± 0.76	1.69 ± 0.63
Sitosterol/cholesterol (µmol/mmol)	1.86 ± 1.08	1.85 ± 0.99	1.98 ± 1.01	1.56 ± 0.76	1.56 ± 0.70	1.49 ± 0.58

Values are mean ± SD and are adjusted by age, sex and smoking.

*ApoE genotypes were available in 642 dyslipidemic patients and 324 EPIC participants.

Genotypes shown are E2 (E2/2 plus E2/3), E3 (E3/3) and E4 (E4/3 plus E4/4),

with exclusion of E4/2 subjects (13 in the dyslipidemic group and 7 in the EPIC cohort).

No significant differences in noncholesterol sterol ratios by apoE genotype in either group by ANOVA.

Figure Legends

Figure 1. Odds for MetS components (ATP III criteria) and the syndrome proper (ATP III and IDF definitions) for a 1-SD increase in plasma sitosterol-to-cholesterol and lathosterol-to-cholesterol ratios in 674 dyslipidemic subjects.

Data were adjusted for sex, age, and smoking and are presented as point estimates with 95% CIs.

Figure 2. Odds for MetS components (ATP III criteria) and the syndrome proper (ATP III and IDF definitions) for a 1-SD increase in plasma sitosterol-to-cholesterol and lathosterol-to-cholesterol ratios in 361 healthy subjects from the Spanish EPIC cohort.

Data presentation as in Figure 1.

High blood pressure defined as self-reported treated hypertension.

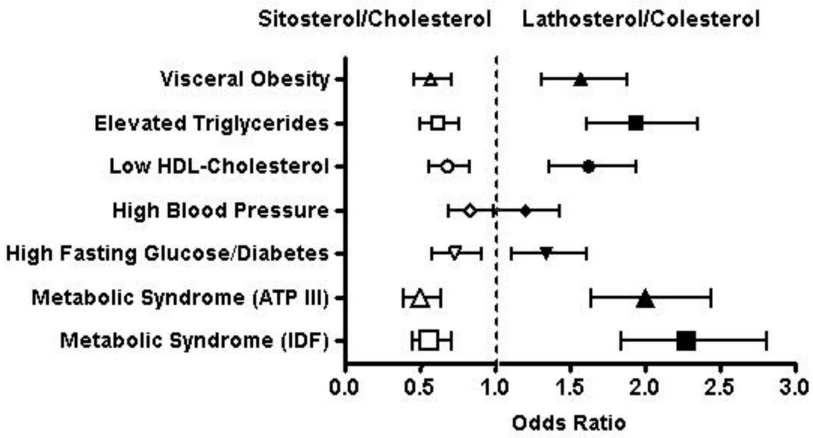

Figure 1. Odds for MetS components (ATP III criteria) and the syndrome proper (ATP III and IDF definitions) for a 1-SD increase in plasma sitosterol-to-cholesterol and lathosterol-to-cholesterol ratios in 674 dyslipidemic subjects. Data were adjusted for sex, age, and smoking and are presented as point estimates with 95% CIs.
120x80mm (600 x 600 DPI)

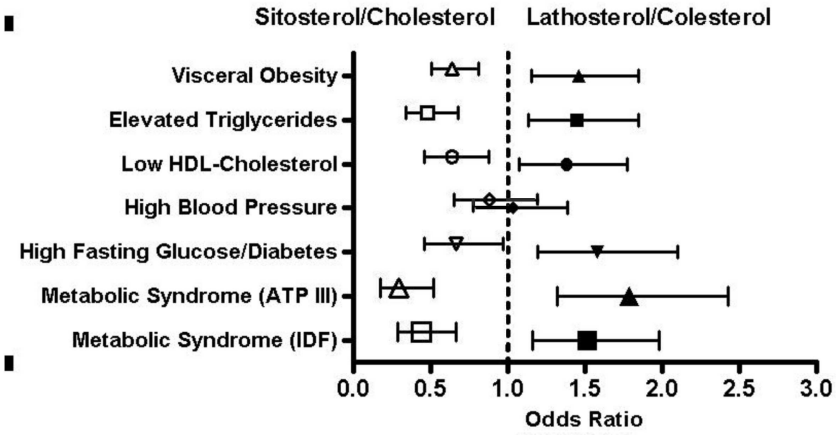

Figure 2. Odds for MetS components (ATP III criteria) and the syndrome proper (ATP III and IDF definitions) for a 1-SD increase in plasma sitosterol-to-cholesterol and lathosterol-to-cholesterol ratios in 361 healthy subjects from the Spanish EPIC cohort. Data presentation as in Figure 1. High blood pressure defined as self-reported treated hypertension.
120x80mm (600 x 600 DPI)

3.4. Article 4

Plant sterol intake and education level in the Spanish EPIC cohort.

Escurriol V, Marí-Dell'olmo M, Rohlfs I, Borrell C, Chirlaque MD, Buckland G, Rodriguez L, Sánchez MJ, Amiano P, Egüés N, Ros E.

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Research letter

Plant sterol intake and education level in the Spanish EPIC cohort

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Abstract

Objective: A cross-sectional study was conducted in the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC) cohort to investigate the association among education level, as a measurement of socioeconomic position, gender, and dietary nutrient intake, focusing on plant sterols, in a Mediterranean population.

Methods: A sample of 25 615 women and 15 552 men (29–69 y old) from the Spanish EPIC cohort was recruited in 1992–1996. Nutrient and plant sterol intakes were estimated using a validated dietary history questionnaire and Spanish food composition tables.

Results and conclusion: Few differences in nutrient or plant sterol consumption existed between men and women with different education levels. Age and energy-adjusted linear regression models of plant sterol intake showed a small increase in subjects with lower education and higher consumption in men than in women. Homogeneity of healthy dietary habits across different socioeconomic groups in this population reflects a wide availability of characteristic Mediterranean foods at the time of recruitment. However, current changes in food supply and the increasing cost of healthy foods may lead to socioeconomic inequalities in Spain parallel to those taking place in other European populations.

Keywords: Plant sterol; Education level; Socioeconomic; Gender; Mediterranean

Introduction

Dietary habits play an important role in health status. A poor-quality diet has been associated with adverse health outcomes. The quality of the diet is affected by factors such as gender, ethnicity, and socioeconomic position in interaction with the availability and price of food [1]. Adherence to the Mediterranean diet has been associated with decreased coronary heart disease, cancer, and all-cause mortality [2]. The traditional Mediterranean diet is characterized by a high content of fiber and monounsaturated fatty acids. Within the context of the whole dietary pattern, these nutrients favorably influence cardiovascular risk factors [3]. Plant sterols are essential components of a healthy vegetable-based diet, and their

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consumption has been inversely related to serum cholesterol levels in two large population-based cohorts [4,5]. These compounds are particularly abundant in cereals, nuts, seeds, and oils derived from them [6]. Recently, a plant sterol database of common Spanish foods has become available [7], thus allowing evaluation of plant sterol consumption in local population groups. The Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC), a large prospective study in Europe, was found to have the highest intake of fruits and vegetables in Europe [8], indicating a good adherence to important components of the Mediterranean dietary pattern.

Data from participants in the Italian branch of EPIC revealed that unhealthy dietary habits were inversely correlated with educational level, especially in the south of Italy [9]. However, at the time of recruitment, participants in the Spanish EPIC cohort followed a fairly uniform food pattern across different educational levels and geographic areas [10]. The present study aimed to investigate the relation among the level of education, as a measurement of socioeconomic position [11], gender disparities, and plant sterol intake, as a marker of healthy food choices, in a cross-sectional analysis of the Spanish EPIC cohort.

Materials and methods

A cross-sectional investigation of participants in the Spanish EPIC study was undertaken. The study included 41 446 subjects (25 812 women and 15 634 men), who were 29 to 69 y of age at the time of enrollment from 1992 to 1996 and were recruited from three northern regions (Asturias, Guipuzkoa, and Navarra) and two southern regions (Granada and Murcia). Participants were healthy volunteers, mainly recruited from blood donor lists, who completed general and dietary history questionnaires. All subjects provided written informed consent to a protocol approved by local review boards.

Energy and nutrient intakes were estimated from a validated dietary history questionnaire using a validated conversion table [12]. Consumption of plant sterols was estimated using a Spanish database [7].

The primary endpoint was dietary sterol intake. Other variables were age, gender, geographic location, and socioeconomic position, assessed from educational level [11] (categorized as no schooling, primary, secondary, or university), and the intake of total energy, fiber, cholesterol, and macronutrients.

Continuous variables are expressed as mean ± standard deviation or mean and 95% confidence interval (CI). Analyses of variance statistics with Bonferroni's post hoc tests ($P < 0.05$) were used for comparing means of continuous variables across educational levels by gender. Multiple linear regression analyses were used to build gender-specific–adjusted models assessing the association between plant sterol intake and socioeconomic status. To compare sterol intake between men and women, the entire population was analyzed as the fully adjusted model and also adjusted by gender. Analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

Results

From the total number of 41 446 subjects, 279 were excluded due to missing data on schooling. Men consumed more plant sterols than women, with means ± standard deviations of 337.9 ± 115.5 and 250.4 ± 92.1 mg/d, respectively ($P < 0.001$). Tables 1 and 2 present nutrient and dietary sterol intakes and educational levels in men and women, respectively. A lower educational level was associated with older age in men and women. In men, a gradient of decreasing intake of total energy, fiber, and total plant sterols and increasing intake of protein, total fat, saturated fatty acids, and monounsaturated fatty acids was observed for increasing educational level. No clear trends in intake of carbohydrate, polyunsaturated fatty acids, and cholesterol existed across educational levels. In women, a decrease in carbohydrate and an increase in total fat, saturated fatty acid, and monounsaturated fatty acid intake were observed for higher educational levels. Intake of total plant sterols decreased only in women with the highest education. However, gender-specific nutrient changes across socioeconomic positions were minor.

Adjusted linear regression model of total plant sterol intake (Table 3) showed decreased consumption in subjects with a higher educational level in men and women. In the adjusted model (model 2), men and women without formal education consumed more total plant sterols than those with a university education (8.7 mg/d, CI 3.9–13.5, and 13.1 mg/d, CI 9.7–16.4, respectively). Men consumed 13.9 mg/d (CI 11.9–15.8) more plant sterols than women when the entire population was analyzed and gender was introduced in the regression analysis (data not shown).

Discussion

In this cross-sectional study of nutrient and plant sterol intake in relation to educational level in a large Mediterranean cohort of the Spanish EPIC study, small differences were observed among groups with different educational levels. Men had a higher intake of plant sterols than women, which largely accounted for a higher energy intake, and subjects with a low educational level had a modestly higher intake than those with higher

Table 1
Age and intake of energy, nutrients, and dietary plant sterols by educational level in the Spanish EPIC cohort of men*

	No schooling	Primary	Secondary	University
No. of subjects	4158	5834	3240	2320
Age (y)	54.2 ± 6.8	50.3 ± 6.8†	48.5 ± 6.7‡	48.4 ± 6.9‖¶#
Total energy (kJ/d)	11 346 ± 3165	11 595 ± 3102†	11 227 ± 2943§	10 529 ± 2798‖¶#
Energy from protein (%)	17.5 ± 2.6	17.5 ± 2.5	17.6 ± 2.5	17.7 ± 2.6‖
Energy from carbohydrate (%)	39.0 ± 7.4	38.4 ± 7.4†	38.0 ± 7.3‡	39.0 ± 7.5¶#
Fiber (g/d)	28.9 ± 9.3	28.8 ± 9.7	27.5 ± 9.1§	27.2 ± 9.7‖¶
Energy from total fat (%)	35.4 ± 5.9	36.0 ± 6.0†	36.7 ± 5.9‡§	37.2 ± 5.9‖¶#
SFA (%)	10.4 ± 2.7	10.7 ± 2.7†	10.9 ± 2.8‡§	11.3 ± 2.9‖¶#
MUFA (%)	15.4 ± 3.7	15.5 ± 3.7	16.0 ± 3.6‡§	16.3 ± 3.4‖¶#
PUFA (%)	6.0 ± 2.5	6.1 ± 2.5	6.00 ± 2.4§	5.8 ± 2.1‖¶#
Cholesterol (mg/d)	444 ± 178	467 ± 175†	453 ± 168§	410 ± 153‖¶#
Total plant sterols (mg/)d	343 ± 115	349 ± 118†	336 ± 113§	309 ± 106‖¶#

EPIC, European Prospective Investigation into Cancer and Nutrition; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Values are means ± SDs. By analysis of variance, all P values were <0.001 for each gender, except energy from protein ($P = 0.013$ for men and $P > 0.1$ for women).

† $P < 0.05$, primary versus no schooling group.

‡ $P < 0.05$, secondary versus no schooling group.

§ $P < 0.05$, secondary versus primary schooling group.

‖ $P < 0.05$, university versus no schooling group.

¶ $P < 0.05$, university versus primary schooling group.

# $P < 0.05$, university versus secondary schooling group.

education. All plant sterols came from natural sources because supplemented foods were not available in Spain at the time of the survey. Dietary plant sterols are markers of healthy food choices [6], and prior food intake data from this cohort showed a weak inverse association between educational level and consumption of such food items [10]. Contrary to evidence from northern European countries [13], our results suggest that, at the time of the survey (1992–1996), inequalities in education were not an obstacle to a healthy dietary pattern in Spain.

As recently reported [7], the main contributors to the intake of plant sterols in the Spanish diet are vegetable oils, cereals, fruits, pulses, and vegetables. Traditionally, these foods have been widely available and affordable in Spain; hence, their consumption has been customarily high in persons of a low socioeconomic position. The average values

Table 2
Age and intake of energy, nutrients, and dietary plant sterols by educational level in the Spanish EPIC cohort of women*

	No schooling	Primary	Secondary	University
No. of subjects	10 146	10 187	2837	2445
Age (y)	51.7 ± 7.8	46.9 ± 7.6†	43.3 ± 7.3‡§	44.0 ± 7.6‖¶#
Total energy (kJ/d)	7963 ± 2409	8295 ± 2457†	8255 ± 2406‡	8338 ± 2462‖#
Energy from protein (%)	18.4 ± 3.1	18.5 ± 3.0	18.5 ± 3.0	18.5 ± 3.1
Energy from carbohydrate (%)	43.5 ± 6.8	42.0 ± 7.0†	40.9 ± 7.1‡§	40.6 ± 6.7‖¶
Fiber (g/d)	23.1 ± 8.1	22.9 ± 8.0	22.1 ± 8.1‡§	22.6 ± 7.9‖
Energy from total fat (%)	36.8 ± 6.0	37.9 ± 6.1†	38.6 ± 5.8‡§	38.9 ± 5.7‖¶
SFA (%)	11.4 ± 3.1	11.6 ± 3.0†	12.1 ± 3.1‡§	12.4 ± 3.2‖¶#
MUFA (%)	15.5 ± 3.6	15.6 ± 3.7†	16.1 ± 3.5‡§	16.6 ± 3.3‖¶#
PUFA (%)	5.9 ± 2.3	6.3 ± 2.5†	6.0 ± 2.3§	5.7 ± 2.0‖¶#
Cholesterol (mg/d)	318 ± 132	349 ± 137†	342 ± 131‡	339 ± 133‖#
Total plant sterols (mg/d)	246 ± 91	259 ± 94†	250 ± 90§	241 ± 86¶#

EPIC, European Prospective Investigation into Cancer and Nutrition; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Values are means ± SDs. By analysis of variance, all P values were <0.001 for each gender, except energy from protein ($P = 0.013$ for men and $P > 0.1$ for women).

† $P < 0.05$, primary versus no schooling group.

‡ $P < 0.05$, secondary versus no schooling group.

§ $P < 0.05$, secondary versus primary schooling group.

‖ $P < 0.05$, university versus no schooling group.

¶ $P < 0.05$, university versus primary schooling group.

# $P < 0.05$, university versus secondary schooling group.

Table 3
Linear regression models of total plant sterol intake (milligrams per day) across educational levels in 15 552 men and 25 615 women from the Spanish EPIC cohort*

Plant sterol intake	No schooling		Primary		Secondary		R^2
	RC	95% CI	RC	95% CI	RC	95% CI	
Men							
Model 1	30.9	25.1–36.7	40.6	35.0–46.1	26.8	20.6–32.8	0.013
Model 2	8.7	3.9–13.5	7.6	3.1–12.1	2.4	−2.6 to 7.3	0.372
Women							
Model 1	2.7	−1.4 to 6.7	18.1	14.1–22.2	9.1	4.1–14.0	0.007
Model 2	13.1	9.7–16.4	11.7	8.5–14.9	4.8	0.9–8.7	0.387

CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; RC, regression coefficient
* University educational level is the reference category. Model 1 is unadjusted; model 2 is adjusted by total energy intake, age, and geographic location.

for dietary plant sterol intake in our cohort are similar to those recently reported for the Spanish population [7] and compare favorably with similar data reported from other European countries [4,5].

Adjusted plant sterol intake in the Spanish EPIC cohort was higher in subjects with a lower educational level, but the differences were minor and reflect a homogeneous food consumption pattern in Spain across different socioeconomic groups. Healthy Mediterranean foods are costly and have limited availability, quality, and palatability in northern European countries compared with southern Europe [14]. This fact could explain in part the observation that in some northern European populations, where food intake is less homogeneous across socioeconomic groups [13], the most disadvantaged groups have a poorer diet and worse health compared with the wealthier ones [15]. Recent evidence, however, points to gradual changes of food supply in Spain toward a Western dietary pattern [16]. Furthermore, characteristic Mediterranean foods have become less affordable in recent years [17]. It appears that a firm nutritional policy action is necessary to preserve the traditional Mediterranean diet and associated high intake of plant sterols across all education groups in Spain.

The study has a limitation that is inherent to the evaluation of food consumption by questionnaires, which is subjective and can be biased. However, a validated dietary history was used and data were adjusted for energy.

Conclusion

Few differences in nutrient and plant sterol intake existed in men and women with different educational level in the Spanish EPIC cohort, indicating the usual diet homogeneity and reflecting healthy food choices at the time of the survey. A current challenge is to preserve the availability of traditional Mediterranean foods for all population groups.

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4. RESUM DELS RESULTATS

4.1. Article 1: “Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet”.

En el present estudi s’analitza l’efecte del consum d’aliments rics en fitoesterols sobre el perfil lipídic d’individus sense història de malaltia cardíaca coronària però amb un elevat risc cardiovascular, al cap d’un any d’intervenció nutricional. En un subgrup de 106 participants de l’estudi PREDIMED es van analitzar factors de risc cardiovascular, perfil lipídic i ingesta d’aliments i nutrients, incloent els fitoesterols, així com les concentracions d’esterols no-colesterol en sang en el moment del reclutament i al cap d’un any d’intervenció nutricional. Els subjectes de l’estudi es van aleatoritzar en tres grups, dues dietes Mediterrànies suplementades amb fruites seques o oli d’oliva verge (que són aliments rics en fitoesterols) i un grup amb recomanacions per seguir una dieta baixa en greix (DBG).

Els participants de l’estudi eren d’edat avançada, obesos i amb diversos factors de risc cardiovascular. En la mesura basal no es van observar diferències significatives entre els tres grups en els factors de risc cardiovascular, els paràmetres sèrics analitzats, o la ingesta d’aliments i nutrients. Al cap d’un any d’intervenció nutricional, els resultats més notables en la ingesta d’aliments i nutrients van ser:

- L’augment del consum de fruites seques en el grup suplementat amb aquest aliment. El grup suplementat amb oli d’oliva bàsicament va substituir l’oli d’oliva refinat de consum habitual per l’oli d’oliva verge aportat.
- L’increment en el consum de fitoesterols en els grups de dieta Mediterrània. Els principals aliments contribuents a la ingesta de fitoesterols van ser l’oli, els cereals, els vegetals i les fruites, i les fruites seques en el grup suplementat amb aquest aliment.

- L’augment de la ingesta d’energia, fibra, greix i AGP en el grup de fruites seques, mentre que en el grup DBG va disminuir el consum de colesterol.

En cap dels grups va haver-hi un canvi significatiu del pes corporal.

Els canvis en el perfil lipídic dels participants al cap d’un any d’intervenció van ser:

- La reducció en els grups suplementats amb oli d’oliva o fruites seques del colesterol-LDL (-4.2% i -6.8%, respectivament) i del quocient LDL/HDL (-6.1% i -9.5%, respectivament), en comparació a les concentracions basals.
- L’augment del colesterol HDL (5.2%) en el grup suplementat amb fruites seques, en comparació a les concentracions basals.
- La reducció del colesterol LDL (8.3%) i del quocient LDL/HDL (11.5%) en el grup suplementat amb fruites seques en comparació al de DBG.
- La correlació inversa del canvi del colesterol total, cLDL i triglicèrids amb les seves concentracions basals.
- La correlació negativa del quocient sèric de sitosterol amb el colesterol total i cLDL.

L’exploració dels factors que influenciaren el canvi del cLDL en aquest estudi va desvetllar l’associació independent de les xifres basals de cLDL, el canvi del sitosterol/colesterol del sèrum i el tractament amb estatines.

4.2. Article 2: “Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort ”.

En aquest treball s’explora la relació entre els fitoesterols de la dieta i del sèrum amb malaltia cardíaca coronària incident en 306 casos i 592 controls aparellats de la cohort espanyola de l’estudi EPIC.

En els individus control es van analitzar els fitoesterols de la dieta i el sèrum en relació a factors de risc i alimentació.

- L’anàlisi del consum dels fitoesterols de la dieta va mostrar que una ingesta elevada es relacionava directament amb el sexe masculí, la ingesta d’energia, la concentració de sitosterol en sèrum, i la ingesta d’aliments i nutrients saludables (fruita, llegums, cereals, fibra, proteïna vegetal i AGP). En canvi, es relacionava inversament amb l’edat i la ingesta de nutrients “perjudicials” (proteïna animal, AGS i colesterol).
- Les concentracions de sitosterol/colesterol es van associar directament amb el contingut en fitoesterols de la dieta i el colesterol HDL i inversament amb l’índex de massa corporal (IMC), el perímetre de cintura i les concentracions de glucosa, triglicèrids i quocients colesterol total/colesterol HDL i lathosterol/colesterol.

L’estudi de casos i controls aparellats no va mostrar diferències importants entre ambdós grups en la ingesta de nutrients o aliments, ni tampoc en la concentració de fitoesterols de la dieta o del plasma. En tot cas, l’anàlisi multivariant del risc de patir malaltia cardiaca coronària segons la concentració de sitosterol ajustat va mostrar un efecte protector significatiu de les concentracions més elevades de sitosterol.

4.3. Article 3: “Plasma phytosterols are inversely associated with the metabolic syndrome and its components”.

En el present estudi vam investigar l’associació entre les concentracions d’esterols no-colesterol del plasma i els components de la síndrome metabòlica en una mostra de 674 subjectes dislipèmics procedents de clíniques de lípids i en 361 participants sans de l’estudi EPIC.

En els dos grups analitzats, els homes eren més obesos, tenien pitjor perfil lipídic i concentracions més elevades de lathosterol i inferiors de sitosterol, en comparació amb les dones.

Quan vam estudiar els dos grups en relació a la síndrome metabòlica vam observar que els individus del grup dislipèmic tenien una major proporció de components del síndrome metabòlica o la pròpia síndrome, excepte per l'obesitat abdominal. Els resultats de l'anàlisi multivariant en relació al risc de patir algun dels components de la síndrome metabòlica o la pròpia síndrome per diferents definicions NCEP-ATPIII (122) i International Diabetes Federation (IDF) (123)] van mostrar que, en els pacients dislipèmics, un increment d'una desviació estàndard en el quocient sitosterol/colesterol s'associava a una reducció del risc de patir qualsevol component de la síndrome metabòlica i aquesta reducció del risc era a la meitat quan es considerava la pròpia síndrome. En canvi, el quocient lathosterol/colesterol va seguir el patró exactament invers, incrementant les possibilitats de patir la síndrome quan les concentracions eren elevades. Aquests resultats es van reproduir a la mostra EPIC, excepte per la pressió arterial segurament perquè en aquest estudi només es disposava de la dada de hipertensió autoreportada, no de xifres de pressió.

4.4. Article 4: "Plant sterol intake and education level in the Spanish EPIC cohort".

Aquest treball va estudiar la dieta i la ingesta de fitoesterols en relació a variables contextuals, el gènere i la posició socioeconòmica (mesurada a partir del nivell assolit d'estudis) en 41.167 subjectes separats per sexe (25.615 dones i 15.552 homes).

En l'anàlisi cru, un nivell més elevat d'estudis es va associar amb menor edat, però quan es va analitzar la ingesta de nutrients no es van trobar diferències rellevants entre els diferents nivells d'estudis ni en homes ni en dones. Mitjançant un model de regressió lineal ajustada de la ingesta de fitoesterols, vam observar que el consum disminuïa en les classes més benestants en ambdós sexes.

Els homes consumien més fitoesterols que les dones, tant en l'anàlisi cru com quan es va analitzar per regressió lineal en tota la població i el sexe es va introduir a l'anàlisi.

5. DISCUSSIÓ

La dieta habitual té una gran influència en la nostra salut. Hipòcrates (s. V a.C.) ja ho va expressar amb la cita “Que el menjar sigui el teu aliment i el teu aliment la teva medicina”. En aquest context, la dieta mediterrània és considerada com un dels models de dieta saludable degut als components que conté i als efectes beneficiosos que produeix sobre diversos factors de risc cardiovascular (119). Per l’elevat consum de productes vegetals en la dieta mediterrània, els fitoesterols en són components importants, i està plenament demostrat que el consum d’aquests composts en dosis farmacològiques té efecte hipocolesteromiant (69). En l’anàlisi realitzat en un centenar de participants de l’estudi PREDIMED, hem observat com l’increment del consum de fitoesterols amb la dieta mediterrània s’associa a l’augment de la ingesta d’altres nutrients beneficiosos, com els àcids grassos insaturats o la fibra. Els participants que van seguir el patró de dieta Mediterrània suplementat amb fruites seques o oli d’oliva verge no van variar gaire la seva dieta habitual ni van augmentar de pes, però van augmentar significativament el consum de fitoesterols i van aconseguir una millora del perfil lipídic al cap d’un any d’intervenció nutricional, suggerint que la quantitat de fitoesterols present en els aliments naturals és suficient per produir un efecte biològic. Aquest efecte hipolipemiant s’ha estudiat en dues poblacions europees on es van detectar xifres de colesterol més baixes en les persones que consumien més fitoesterols amb la dieta habitual en comparació als que seguien una dieta pobre en aquests composts (80;81). Degut a que els aliments rics en fitoesterols, com el mateix oli d’oliva, els cereals, les llegums i les fruites seques (124) són tradicionals en la dieta espanyola (120;125), les recomanacions per augmentar-ne el consum poden contribuir a reduir les concentracions de colesterol LDL que s’obseven al passar d’una dieta occidental a una dieta mediterrània (126). Les fruites seques son els aliments que més fitoesterols van aportar a la dieta en la branca corresponent del nostre **estudi 1**, i és

ben conegut que l'efecte reductor del colesterol de les fruites seques és superior al que es pot atribuir al seu contingut en àcids grassos insaturats (127). Per tant, tal com s'ha proposat (124), és molt probable que els fitoesterols siguin responsables en part de la reducció de colesterol produïda pel consum habitual de fruites seques. A més, els aliments naturals rics en fitoesterols aporten altres nutrients amb efectes beneficiosos per la salut, com fibra (128), vitamines i minerals (124). Per altra banda, l'augment del consum de greix total i d'àcids grassos insaturats en el grup de l'estudi PREDIMED suplementat amb fruites seques, probablement va contribuir en l'increment del colesterol HDL (127), un important factor de prevenció de risc cardiovascular (18;129). En el cas de persones hipercolesterolèmiques, s'ha observat una reducció efectiva de les concentracions elevades colesterol LDL mitjançant la combinació de dosis farmacològiques de fitoesterols amb dietes que contenen diversos components hipolipemiants (dieta portfolio) (130) o amb estatines (aquesta combinació té un efecte sinèrgic i mantingut al llarg del temps) (131).

Un augment en el consum de fitoesterols es tradueix en un increment de les seves xifres sèriques en major o menor grau, depenent de l'eficiència d'absorció d'esterols a l'intestí (82-84). En **l'estudi 2** de casos i controls dut a terme en la cohort EPIC-Espanya, aquest augment no va implicar un risc cardiovascular major, a diferència dels resultats publicats per altres autors (90-95). Al contrari, les xifres elevades de sitosterol en sèrum, el fitoesterol més abundant en la dieta (29), s'associaven a un efecte protector de desenvolupar malaltia cardíaca coronària, coincidint amb els resultats de 3 estudis recents en cohorts poblacionals d'Anglaterra (96), Alemanya (97) i Holanda (98). Per altra banda, en **l'estudi 1** PREDIMED, l'augment de les concentracions circulants de sitosterol es va relacionar amb la reducció de colesterol LDL, un dels principals factors de risc cardiovascular (132). Aquesta aparent contradicció sobre el risc cardiovascular dels fitoesterols sèrics en diversos treballs podria ser deguda a diversos factors. Per una banda, el fet d'estudiar la relació entre les xifres augmentades de fitoesterols i el risc cardiovascular sense controlar per factors

confusors (90;95) fa que sigui difícil la interpretació dels resultats. A més, alguns d'aquests estudis tenen un tamany mostral petit, dificultat afegida per poder fer comparacions (91;92;94). Un altre factor que influenciaria aquests resultats contradictoris seria el fet de mesurar el risc amb les xifres sèriques crues de fitoesterols i no les ajustades per colesterol. Els esterols no-colesterol s'expressen com a quocients de colesterol perquè, com el colesterol, aquestes molècules es transporten exclusivament en les lipoproteïnes transportadores de colesterol i les seves concentracions s'alteren per canvis en les mateixes. L'anàlisi amb xifres crues (90;95) podria dur a errors en la interpretació del metabolisme dels esterols; els individus amb hipercolesterolèmia familiar heterozigota en són un bon exemple, ja que tenen xifres crues d'esterols no-colesterol elevades en comparació a un grup control, però les xifres ajustades són similars en els dos grups (133). Està clar que a l'hipercolesterolèmia familiar les concentracions elevades de colesterol son degudes a la funció defectuosa del receptor LDL i no a una absorció intestinal augmentada (33;133), per tant, el no ajustar les xifres d'esterols no-colesterol pot dur a interpretacions clarament errades sobre la causa de la hipercolesterolèmia. Finalment, l'adipositat és segurament el factor més important en determinar l'eficiència d'absorció intestinal i, per tant, de les xifres de fitoesterols en sang. Bona part dels estudis que relacionen les xifres sèriques elevades de fitoesterols amb risc cardiovascular reporten un IMC semblant entre casos i controls i fins i tot, una mica més elevat en els controls (91-93;95), mentre que en els estudis que afirmen el contrari (96-98), els casos tenen més factors de risc que els controls, incloent un IMC més elevat. Així doncs, l'adipositat sembla jugar un paper clau en determinar les xifres sèriques de fitoesterols, com veurem de seguida al comentar l**'estudi 3.**

Per altra banda, les xifres de fitoesterols sèrics obtingudes en els nostres estudis són comparables a les reportades per altres estudis epidemiològics a Estats Units (90;101), Finlàndia (91;92), Alemanya (93;95;97;109), Anglaterra (96) i els Països

Baixos (98), suggerint que les troballes no són específiques per la població Espanyola i es poden generalitzar a altres poblacions occidentals.

Els quocients dels fitoesterols, campesterol i sitosterol, s'accepten com a marcadors d'eficiència d'absorció de colesterol, mentre que el precursor del colesterol, lathosterol, és un índex fiable de síntesi de colesterol (89). Els estats de resistència a la insulina com són la obesitat, la diabetis tipus 2 o la síndrome metabòlica estan associats a xifres disminuïdes de fitoesterols en sang perquè hi ha una menor taxa d'absorció i una major taxa de síntesi (105-108). Els resultats de **l'estudi 3** en subjectes dislipèmics i participants sans de l'estudi EPIC en relació al risc de patir algun dels components de la síndrome metabòlica o la pròpia síndrome van mostrar que un increment d'una desviació estàndard en el quocient sitosterol/colesterol estava associat amb una reducció significativa del risc de presentar qualsevol component de la síndrome metabòlica. En el cas de la propia síndrome, la reducció del risc amb l'augment de 1 DE del quocient sitosterol/colesterol era d'aproximadament la meitat (44% o 51%, depenent de la definició). En canvi, el quocient lathosterol/colesterol va seguir el patró invers, incrementant les possibilitats de patir el síndrome quan les concentracions eren elevades. Aquests resultats quadren amb la lògica esperada per mantenir l'homeòstasi del colesterol i amplien els resultats de l'estudi d'Assman et al. (109), en el qual no es va analitzar la pressió arterial. Miettinen et al. i altres autors van obtenir resultats similars en petits estudis on es descriu la important influència de l'obesitat, la diabetis tipus 2 i la síndrome metabòlica en l'homeòstasi del colesterol (105-108;110). Miettinen et al. van estudiar 268 pacients hipercolesterolèmics de malaltia coronària i van trobar marcadors de síntesi elevada i baixa absorció de colesterol en aquells que tenien sobrepès, triglicèrids elevats i colesterol HDL baix (134), i aquesta troballa es va confirmar en un grup de 263 subjectes assimptomàtics amb hipercolesterolèmia moderada (135). En l'**estudi 3**, l'efecte més important de les xifres ajustades de sitosterol sèric es va observar en l'obesitat visceral, que sembla ser el factor clau per desenvolupar la síndrome metabòlica i és també determinant de

l'augment de la síntesi de colesterol i reducció concomitant de la seva absorció (122). L'obesitat s'ha associat a estrès oxidatiu i inflamació i l'obesitat abdominal, en particular, es considera un important factor de risc cardiovascular, ja que es relaciona amb trastorns metabòlics com la diabetes mellitus, hipertensió o dislipidèmia (136;137). En un estudi recent, l'obesitat visceral es va associar amb el colesterol LDL oxidat independentment de l'IMC en homes i dones (138). Per altra banda, la síndrome metabòlica es caracteritza per un elevat grau d'estrès oxidatiu (139;140). Malgrat que l'evidència científica és encara limitada, s'ha descrit que els fitoesterols tenen un efecte antiinflamatori (141). Per tant, aquests composts podrien ser no només marcadors d'una absorció intestinal augmentada i d'un perfil cardiometabòlic més saludable sinó que també podrien contribuir a un menor grau d'inflamació en l'organisme, disminuint el risc de patir síndrome metabòlica.

El consum de fitoesterols en els estudis realitzats va ser de 300 mg/d en l'estudi EPIC i 360 mg/d en l'estudi PREDIMED, essent major en homes que en dones. Aquesta ingesta era similar a la reportada per un estudi realitzat en població espanyola (142) i un altre en població anglesa (80). En canvi el consum era menor en estudis realitzats en població sueca (81) i alemanya (97), i major en població japonesa (143). La dieta habitual i el consum de determinats aliments està influenciat per múltiples factors, no només factors biològics, sinó també factors socials, que poden tenir un paper clau en els hàbits alimentaris. En **l'estudi 4** en la cohort completa de 41.446 persones de l'estudi EPIC d'Espanya realitzat amb participants reclutats als anys 90, hem observat que no hi ha diferències importants en el consum de fitoesterols (i els nutrients principals) quan analitzem la població segons el sexe i la classe social (analitzada a través del nivell d'escolarització). En altres països europeus però, es veuen clares diferències en el consum d'aliments saludables en relació a la classe social (112;113). Aquesta sembla ser la tendència a seguir a Espanya, ja que s'ha descrit un augment en el preu dels aliments característics de la dieta Mediterrània com les verdures, les fruites o l'oli d'oliva (144), així com un canvi en l'alimentació

dirigit cap a una dieta més occidental i de menjar ràpid, seguint el patró que han adoptat altres països europeus (145;146). Aliments com les llegums o els cereals (que són rics en fitoesterols) eren àmpliament consumits per les classes més populars degut al seu preu assequible. Actualment però, el consum d'aquests aliments s'està reduint i ha augmentat el consum d'aliments refinats, productes làctics i carn (146).

6. CONCLUSIONS

- L'augment de la ingesta de fitoesterols amb aliments naturals és responsable en part de la reducció de colesterol LDL que s'observa al implementar la dieta Mediterrània en comparació amb una dieta baixa en greix.
- La ingesta de fitoesterols amb la dieta habitual augmenta de forma moderada les xifres de fitoesterols circulants.
- Les xifres augmentades de fitoesterols circulants (dins dels rangs fisiològics) es relacionen amb un elevat consum d'aliments saludables amb la dieta habitual.
- Les xifres elevades de fitoesterols plasmàtics no s'associen a un augment del risc de malaltia cardíaca coronària en la població espanyola, caracteritzada per consumir una dieta amb abundància de fitoesterols.
- Les concentracions sèriques elevades de sitosterol, el fitoesterol més abundant en els aliments, s'associen a un risc cardiometabòlic menor i assenyalen individus amb una prevalença menor de síndrome metabòlica i els seus components.
- La ingesta de fitoesterols als anys 90 era similar en homes i dones, i entre les diferents classes socioeconòmiques, reflexant uns hàbits alimentaris saludables generalitzables a tota la població espanyola en aquell moment. Degut al canvi cap a una dieta més occidental, el preservar l'accessibilitat d'aliments tradicionals de la dieta Mediterrània, que són rics en fitoesterols, és un objectiu rellevant de salut pública.

7. CONCLUSIÓ FINAL

Els fitoesterols del sèrum són marcadors d'una dieta saludable com la que es consumia preferentment a Espanya als anys 90 en les classes socials més populars. Aquests components es relacionen amb la reducció del colesterol observada després del consum d'aliments naturals rics en fitoesterols i s'associen inversament a les característiques del síndrome metabòlic. Els resultats dels estudis presentats en aquesta tesi suggereixen una reinterpretació del significat de les concentracions de fitoesterols circulants. En persones que segueixen una dieta mediterrània sense suplements de fitoesterols, els fitoesterols circulants son marcadors de l'abundància de productes vegetals en la dieta i s'associen a un perfil cardiometabòlic saludable i a un menor risc de desenvolupar malaltia cardíaca coronària, en lloc de ser biomarcadords d'un risc cardiovascular augmentat.

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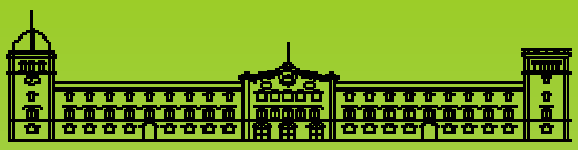

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