UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA:
CIÊNCIAS MÉDICAS

ENSAIO CLÍNICO SOBRE O IMPACTO DA INIBIÇÃO DE UM TRANSPORTADOR DE MEMBRANA DO COLESTEROL NA ABSORÇÃO DA VITAMINA D

MARIANA COSTA SILVA

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DEDICATÓRIA

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LISTA DE SIGLAS E ABREVIATURAS

1,25OH$_2$D: 1,25-dihidroxivitamina D ou calcitriol

7DHC: 7-Deidrocolesterol

25OHD ou 25(OH)D: 25-Hidroxivitamina D

ABCA1: ATP-binding cassete A1

CBP: Cirrose biliar primária

CD 36: Cluster dominant 36

DP: Desvio padrão

GC: Proteína ligadora da vitamina D

IMC: Índice de massa corporal

NPC1L1: Transportador de membrana Niemann–Pick C1-Like 1

PTH: Hormônio paratireoidiano

SR-BI: Receptor scavenger classe B do Tipo 1

TCLE: Termo de Consentimento Livre e Esclarecido

UVB: Ultravioleta tipo B

VDR: Receptor de vitamina D

RESUMO

Base teórica: Suplementos dietéticos são comumente empregados para prevenir e tratar a deficiência de vitamina D, apesar disso, o mecanismo de absorção intestinal da vitamina D é pouco compreendido até o momento.

Objetivo: Avaliar o impacto do ezetimibe na absorção da vitamina D em indivíduos saudáveis e, com isso, determinar se o transportador de colesterol *Niemann–Pick C1-Like* 1 participa deste processo.

Métodos: Ensaio clínico randomizado, controlado e duplo-cego (ClinicalTrials.gov: NCT02234544), realizado no Hospital de Clínicas de Porto Alegre, onde 51 médicos residentes foram randomizados para ezetimibe 10 mg ou placebo por cinco dias. No quinto e no décimo nono dias, foram coletadas, em jejum, amostras de sangue para dosagem de 25-hidroxicolecalciferol (25OHD), hormônio paratireoidiano (PTH), cálcio e albumina. Após a primeira coleta de sangue, todos os participantes receberam uma dose única oral de 50.000 UI de colecalciferol com um lanche contendo 15 gramas de gordura. Níveis plasmáticos de 25OHD foram dosados pelo imunoensaio Diasorin Liaison®. Medidas foram comparadas por regressão linear múltipla e corrigidas pelo teste de Bonferroni.

Resultados: Níveis séricos basais de 25OHD foram <30 ng/mL e <20 ng/mL em, respectivamente, todos e 82,3% dos participantes. Quatorze dias após dose única de colecalciferol 50.000 UI, a média (DP) das variações de 25OHD foi similar entre o grupo que recebeu ezetimibe e o grupo placebo, respectivamente, 8,7 (3,7) ng/mL *versus* 10,0 (3,8) ng/mL, p = 0,26, após ajuste para IMC e 25OHD basal. A média dos níveis séricos de 25OHD, PTH, cálcio e albumina permaneceram semelhantes em ambos os grupos.

Conclusão: Conclui-se que o ezetimibe não influenciou a variação nas médias de 25OHD após consumo de dose única de colecalciferol 50.000UI, nestes jovens saudáveis.

Palavras chave: Vitamina D, 25-Hidroxicolecalciferol, Absorção, Proteínas Transportadoras de Membrana, Ezetimibe.

ABSTRACT

Background: *Oral supplements are important to prevent and treat vitamin D deficiency. Despite the growing number of prescriptions, vitamin D's absorptive mechanisms are not clearly elucidated.*

Objective: *By evaluating the effect of ezetimibe on vitamin D absorption, we aimed to determine if the cholesterol transporter Niemann–Pick C1-Like 1 transporter contributes to it.*

Methods: *This randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov NCT02234544) was developed in a South Brazilian University Hospital. Fifty-one medical students were randomized to ezetimibe 10 mg/day for 5 days or placebo. On the fifth and 19th days, blood samples for 25-hydroxycholecalciferol (25OHD), parathyroid hormone (PTH), calcium, and albumin were collected. After the first blood sample collection, all participants received a single oral 50,000 IU cholecalciferol dose during a 15 g-fat meal. Serum 25OHD levels were measured by the immunoassay Diasorin Liaison®. Measurements were compared in a general linear model adjusted for multiple comparisons by the Bonferroni test.*

Results: *Before cholecalciferol administration, 25OHD was <30 ng/mL and <20 ng/mL, respectively, in all and in 82.3% of the participants. Fourteen days after a single 50,000 IU oral dose of cholecalciferol, mean (SD) changes in serum 25OHD were similar in both groups, after adjustment to BMI and 25OHD levels, before cholecalciferol administration ($p = 0.26$): 8.7 (3.7) ng/mL in the ezetimibe group, versus 10.0 (3.8) ng/mL in the placebo group. Mean serum 25OHD, PTH, calcium and albumin levels remained similar in both groups.*

Conclusion: *We concluded that ezetimibe had no effect on the mean change in serum 25OHD after a single oral dose of cholecalciferol, in healthy and young adults.*

Key words: *Vitamin D, 25-Hydroxycholecalciferol, Absorption, Membrane transport proteins, Ezetimibe.*

LISTA DE FIGURAS

REVISÃO DA LITERATURA

ARTIGO 1

ARTIGO 2

ARTIGO 3

LISTA DE TABELAS

ARTIGO 1

ARTIGO 2

ARTIGO 3

SUMÁRIO

1- INTRODUÇÃO

A elevada prevalência de deficiência de vitamina D é uma preocupação comum a diversos países (1,2). Esta vitamina difere das demais, pois pode ser sintetizada em quantidades suficientes pelo corpo humano e atua como pró-hormônio esteroide no metabolismo ósseo e homeostase do cálcio (3). A suplementação de vitamina D é uma estratégia cada vez mais empregada nas últimas décadas, contudo estudos desenhados com o propósito de avaliar sua absorção, biodisponibilidade e desfechos clínicos ainda são esperados.

A identificação de nutrientes dietéticos essenciais principiou no século XVIII e possibilitou o tratamento de doenças comuns à época, como escorbuto, beribéri e raquitismo. A propriedade de cura do raquitismo foi identificada no óleo de fígado de bacalhau e foi atribuída à vitamina A inicialmente. Ao se constatar que esta propriedade terapêutica se mantinha mesmo após degradação da vitamina A, conclui-se haver outro nutriente no óleo de fígado de bacalhau. Paralelamente, observou-se que crianças raquíticas eram curadas com a exposição à luz solar ou luz artificial ultravioleta (UVB). Estes experimentos levaram ao reconhecimento de uma quarta vitamina, a vitamina D (4).

Vitaminas ou nutrientes dietéticos essenciais são, por definição, aqueles que os seres humanos não podem sintetizar e necessitam obter através da alimentação. Entretanto, quantidades suficientes de vitamina D podem ser obtidas quando a pele é devidamente exposta à radiação UVB e seu aporte em alimentos costuma ser baixo (5). Hábitos da vida moderna, com maior permanência em ambientes fechados e menor exposição solar desde a infância, parecem constar entre os principais responsáveis pela elevada prevalência de hipovitaminose D na atualidade.

Níveis adequados de vitamina D estimulam a absorção intestinal de cálcio e fósforo, necessários para a mineralização óssea. Baixos níveis resultam em hiperparatireoidismo, com aumento da reabsorção óssea, além de fraqueza muscular e maior risco de quedas (6). Um aumento expressivo no número de fraturas de quadril é estimado para os próximos anos, com projeções superiores a 310% em homens e 240% em mulheres para o ano de 2050 quando comparado a 1990 (7). A suplementação dietética da vitamina D é apontada como importante arma para combater o aumento no número de fraturas ósseas (8-10).

Além disso, observa-se que a suplementação de vitamina D se popularizou nos últimos anos não apenas em decorrência da necessidade de prevenção e tratamento de doenças osteomusculares. Também contribuíram para isso estudos epidemiológicos e metanálises que demonstraram associação entre baixos níveis de vitamina D e doenças cardiovasculares, metabólicas, respiratórias, autoimunes e neoplásicas (11-13). Contudo, a causalidade entre suplementação e possíveis efeitos pleiotrópicos da vitamina D não foi estabelecida e são necessárias maiores evidências para a prática de sua suplementação com estes fins (14). Grandes ensaios clínicos com o propósito de avaliar desfechos relacionados aos níveis de vitamina D estão em andamento (15,16).

Apesar de amplamente prescrita, pouco se sabe a respeito da absorção intestinal da vitamina D. Estabeleceu-se que esta vitamina seria absorvida por difusão passiva, entretanto, estudos recentes questionam este pressuposto e sugerem que seu processo de absorção envolve mecanismos mais complexos, uma vez que a vitamina D é absorvida mesmo na ausência de gordura ou veículos oleosos (17).

Por se tratar de uma molécula lipossolúvel e estruturalmente semelhante ao colesterol, é possível que seus mecanismos de absorção sejam semelhantes. O ezetimibe é um inibidor farmacológico da absorção do colesterol dietético e biliar, que age através da inibição do transportador de membrana *Niemann-Pick C1 tipo 1* (NPC1L1) no enterócito e no fígado (18). Pesquisas *in vivo* e *in vitro* demostraram uma diminuição da absorção da vitamina D na presença de ezetimibe (19).

A vitamina D é sintetizada pelo corpo humano e participa de eixos metabólicos - complexos, portanto vai além de sua qualificação inicial e tem amplo potencial biológico e terapêutico a ser explorado. Estudos científicos que transponham resultados de pesquisa básica para a prática clínica são almejados. O presente trabalho se propõe a avaliar o efeito do ezetimibe na absorção intestinal da vitamina D em seres humanos saudáveis e poderá contribuir para o tratamento e prevenção de doenças relacionadas à hipovitaminose D.

2- REVISÃO DA LITERATURA

2.1 ESTRATÉGIAS PARA LOCALIZAR E SELECIONAR INFORMAÇÕES

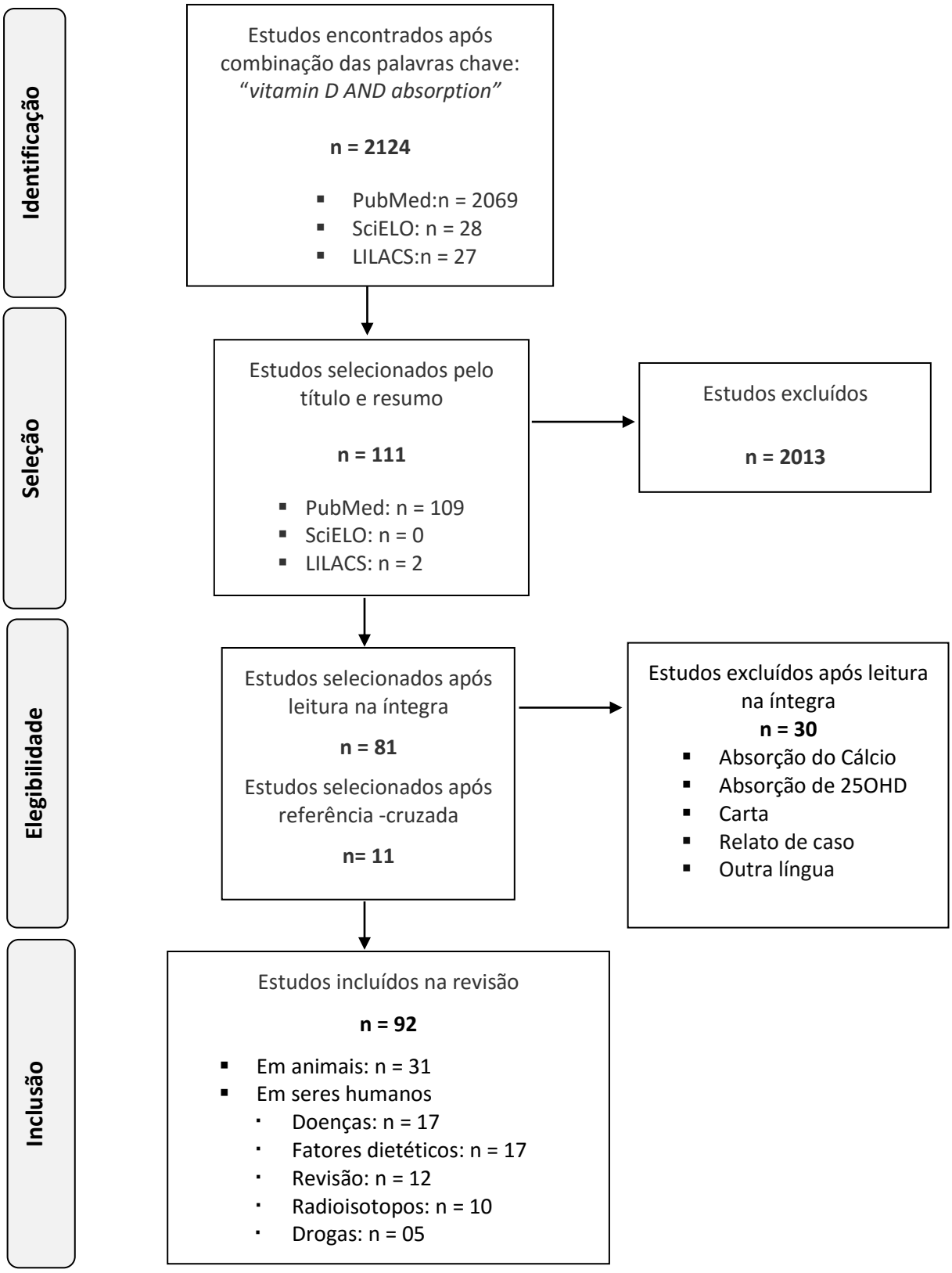
Realizou-se revisão de estudos que avaliaram a fisiologia e o metabolismo da vitamina D, com ênfase em seu processo absortivo e biodisponibilidade. A estratégia de busca envolveu as seguintes bases de dados: LILACS, SciELO e PubMed, até janeiro de 2016. Foram realizadas buscas utilizando-se os termos vitamina D e absorção, em língua inglesa ou portuguesa. Os detalhes da busca foram os seguintes:

- LILACS e SciELO: *vitamina D AND absorção* ou *vitamin D AND absorption*;
- PubMed e Embase: *("Vitamin D/administration and dosage"[Mesh] OR "Vitamin D/pharmacokinetics"[Mesh] OR "Vitamin D/physiology"[Mesh]) AND ("absorption"[MeSH Terms] OR "absorption"[All Fields]).*

Localizaram-se 2124 títulos, destes 111 foram selecionados para leitura na íntegra. Os artigos selecionados também serviram de base para localização de estudos que não entraram no critério de busca. Os critérios de inclusão foram estudos *in vitro* ou *in vivo* sobre o processo absortivo da vitamina D, testes absortivos que utilizaram vitamina D radiomarcada, ensaios clínicos que avaliaram a absorção de uma dose única oral de vitamina D, além de revisões sistemáticas sobre o assunto. A estratégia utilizada para seleção de estudos encontra-se exposta na **Figura 1**.

O **Artigo 1** foi elaborado a partir desta revisão sistemática conforme protocolo de revista internacional e com ênfase no processo absortivo da vitamina D através da membrana apical dos enterócitos.

Figura 1. Revisão sistemática da literatura.



2.2 FISIOLOGIA E METABOLISMO DA VITAMINA D

A síntese cutânea é a principal fonte de vitamina D em humanos e pode contribuir com mais de 90% das concentrações séricas desta vitamina. O 7-desidrocolesterol, ou pró-vitamina D, está presente na pele e é convertido em colecalciferol (vitamina D3), a partir da radiação UVB , seguida por reação calor dependente (5). Os alimentos contribuem com uma pequena parcela de suas necessidades diárias e, quando a síntese cutânea está limitada, muitas vezes a suplementação dietética se faz necessária (9). Esta vitamina pode ser adquirida na forma de ergocalciferol (vitamina D2), quando obtida a partir de leveduras e plantas, ou de colecalciferol quando de origem animal, encontrada especialmente peixes gordurosos de água fria e profunda.

Tanto a vitamina D provinda da síntese cutânea quanto da dieta, são biologicamente inertes e necessitam ser ativadas (5). Para isso, sofrem duas etapas de hidroxilação:

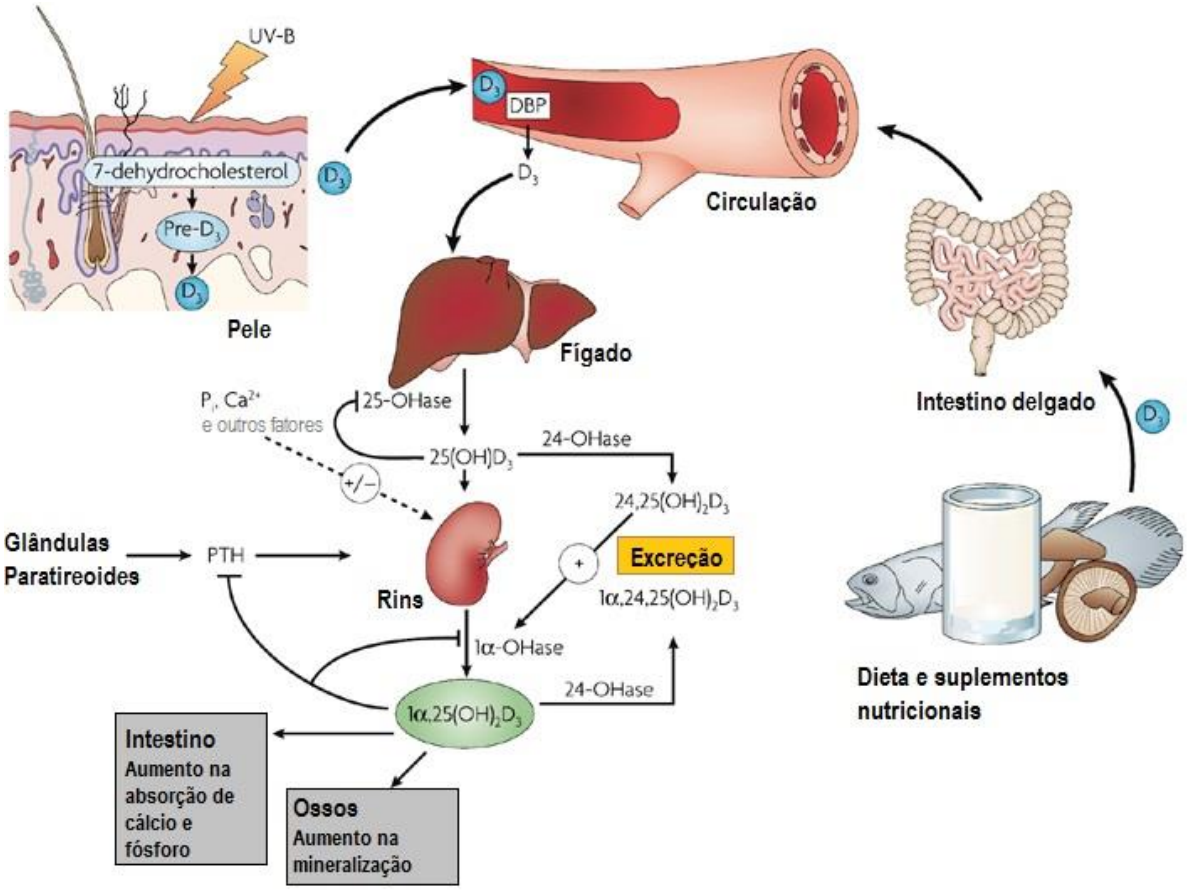
1) Hidroxilação hepática em 25-hidroxivitamina D ou calcidiol (25OHD), pela ação da enzima 25-hidroxilase;

2) Hidroxilação renal, onde a 25OHD é transformada em 1,25-dihidroxivitamina D ou calcitriol (1,25OH_2D), a forma biologicamente ativa da vitamina D, pela ação 1α-hidroxilase (**Figura 2**).

A 1,25OH_2D age através de ligação a receptor nuclear (VDR) e regula a homeostase cálcio-fósforo e o metabolismo ósseo, principalmente (5). A descoberta da expressão de VDR em células envolvidas na resposta imune, proliferação e inflamação, além da expressão extra-renal da enzima 1α-hidroxilase são o racional para investigações de ações não osteometabólicas, da vitamina D (13,20).

Apenas uma pequena fração dos metabólitos da vitamina D circulam na sua forma livre (<0,1%), e cerca de 85 a 90% estão ligados à proteína ligadora da vitamina D (GC) e 10 a 15% à albumina (21). Os metabolitos da vitamina D são catabolizados pela enzima 24-hidroxilase (22). A GC afeta o *clearence* dos metabólitos da vitamina D (23).

Figura 2. Via metabólica da vitamina D.



Fonte: Figura modificada de Deeb, KK. Nature Reviews Cancer 7, 684-700, September 2007.

2.3 DEFICIÊNCIA DE VITAMINA D

Medidas séricas de 25OHD são estabelecidas atualmente como o método de escolha para o diagnóstico da deficiência de vitamina D, pois apesar de não representarem a fração ativa da vitamina, seus níveis são mais estáveis e refletem melhor os estoques obtidos por via dietética ou síntese cutânea (12).

As Sociedades Americana e Brasileira de Endocrinologia e Metabologia determinam que níveis de 25OHD menores que 30 ng/mL caracterizam deficiência e menores que 20 ng/mL insuficiência de vitamina D (9,12). O hormônio paratireoidiano (PTH) é um marcador biológico indireto dos níveis de vitamina D, e o ponto de corte para deficiência desta vitamina foi estimado a partir do nível sérico de 25OHD em que os níveis de PTH começam a aumentar (24).

A resposta à suplementação de vitamina D varia muito entre os indivíduos e isso dificulta o cálculo da dose necessária para atingir os níveis séricos de 25OHD (25). São reconhecidos inúmeros fatores que interferem nos níveis séricos de 25OHD, como índice de massa corporal (IMC) (26), medicações que interferem na hidroxilação hepática (anticonvulsivantes, antirretrovirais), doenças granulomatosas (27), condições que afetam os níveis de circulantes de GC, como síndrome nefrótica, insuficiência hepática e estados inflamatórios agudos (28,29), além de polimorfismos em genes relacionados ao metabolismo da vitamina D (30-33).

Fatores relacionados à absorção da vitamina D também são responsáveis pela variabilidade na resposta à suplementação, sendo necessário maior conhecimento dos mecanismos envolvidos na absorção da vitamina D.

2.4 ABSORÇÃO INTESTINAL DA VITAMINA D

A vitamina D faz parte da classe das vitaminas lipossolúveis e pode ser ingerida na forma de ergocalciferol e colecalciferol. Apesar de pouco detalhado até o momento, entende-se que seu padrão absortivo é semelhante ao das gorduras e compreende primeira etapa intraluminal de emulsificação e solubilização micelar, seguida de incorporação através da borda em escova (membrana apical) dos enterócitos (1,34,35).

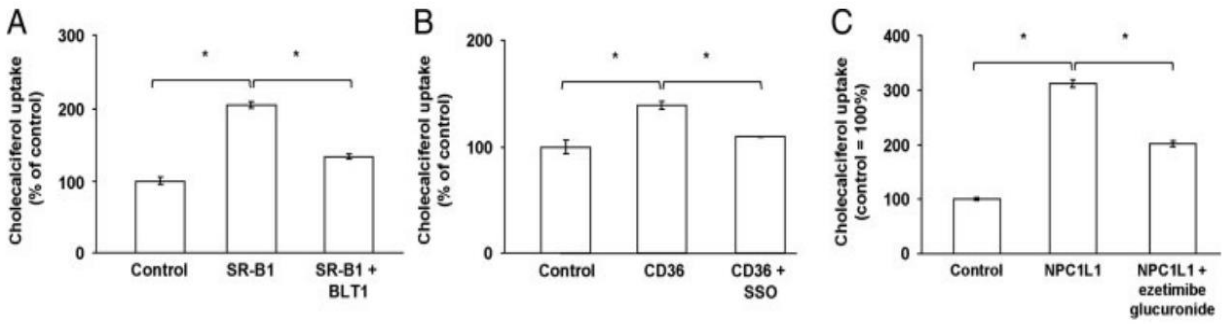
Conhecimentos sobre o transporte da vitamina D através da membrana apical dos enterócitos partiram de estudos da década de 70. Hollander D *et al* demonstraram, em experimentos com ratos vivos e sacos intestinais, uma maior taxa de absorção da vitamina D no jejuno e no íleo, com aumentos lineares conforme se incrementava sua concentração ou, ao se administrar íons de hidrogênio, os quais diminuem a resistência da membrana celular à difusão de micelas (36,37). Tais achados os fizeram sugerir que a vitamina D seria absorvida exclusivamente por difusão passiva.

Estudos experimentais realizados na última década trazem, contudo, evidências fortes de que os mecanismos de incorporação da vitamina D pelos enterócitos são mais complexos do que os sugeridos anteriormente. Demonstrou-se, por exemplo, que o excesso de colesterol limita a absorção da vitamina D e que fatores que interferem no funcionamento de transportadores de membrana do colesterol modificam a absorção da vitamina D (19,38,39).

Sabe-se que o principal transportador de membrana do colesterol é o *Niemann–Pick C1-like 1* (NPC1L1) (18), seguido pelos transportadores *Receptor Scavenger classe B do Tipo 1* (SR-BI) (40), *Cluster dominant 36* (CD 36) (41) e *ATP-binding cassete A1* (ABCA1) (42). Reboul, E. *et al* demonstraram que modificações na expressão ou na função destes receptores de colesterol podem interferir na absorção da vitamina D (19).

No estudo de Reboul E *et al* foram realizados experimentos com camundongos, células humanas Caco-2, que são modelos de epitélio intestinal humano frequentemente empregados na avaliação do transporte intestinal de lipídios, e células HEK 293-T, que são modelos já validados de super-expressão de SR-BI, CD-36 e NPC1L1.A absorção da vitamina D foi maior nas células que super-expressavam SR-BI, CD-36 e NPC1L1 e menor após administração de inibidores específicos de cada transportador (**Figura 3**).

Figura 3. Efeitos da inibição de transportadores do colesterol na absorção do colecalciferol. **(A)** Efeito do inibidor do transportador SR-BI (BLT1) na captação de colecalciferol por células 93-T HEK que super-expressam SR-BI **(B)** Efeito do inibidor do transportador CD36 (SSO) na captação de colecalciferol por células 93-T HEK que super-expressam CD36. **(C)** Efeito do inibidor do transportador NPC1L1 (ezetimibe) na captação de colecalciferol por células 93-T HEK que super-expressam NPC1L1.



Fonte: Reboul, E. Mol Nutr Food Res. 2011 May;55(5):691-702.

O ezetimibe é um inibidor farmacológico do NPC1L1 nos enterócitos e no fígado (43), que reduziu a absorção da vitamina D nos modelos de epitélio intestinal humano estudados por Reboul E *et al* (19). Entretanto, a diminuição na absorção de vitamina D não foi estatisticamente significativa em camundongos vivos tratados com ezetimibe. Resultados semelhantes foram encontrados previamente por equipe que avaliou o efeito do ezetimibe na absorção de vitamina D em roedores (44).

Fitoesteróis, comumente empregados para a redução da absorção do colesterol dietético, também diminuíram a absorção de vitamina D em modelos experimentais. Alteração na estrutura micelar e concorrência pelo transportador NPC1L1 foram aventadas como possíveis causas (39). Além disso, ácidos graxos livres, que modulam a absorção do colesterol, também parecem interferir na absorção da vitamina D (38). Observou-se que eles provocam modificação na carga elétrica micelar e modulação do efluxo de colecalciferol pela membrana basolateral do enterócitos. Aventaram-se as hipóteses de competição por transportadores de membrana e modulação genética da expressão destes transportadores (38).

Diversos métodos foram empregados para avaliar a absorção da vitamina D em seres humanos. A administração de formas radiomarcadas de vitamina D e sua monitorização no sangue, tecidos, urina e fezes pode ser considerado o método mais direto para avaliar a absorção da vitamina D em seres humanos. Ensaios clínicos de absorção da vitamina D que seguiram os princípios de dose única oral, tanto de ergocalciferol como de colecalciferol apresentaram resultados semelhantes aos que avaliaram suas formas marcadas.

2.4.1 Testes absortivos com vitamina D marcada

A equipe de Thompson GR foi a primeira a aplicar e descrever um protocolo para avaliação da absorção da vitamina D (45). Tal protocolo consistia na administração de um isótopo de vitamina D3 (^{3}H-D3) em dose única por via oral para seres humanos saudáveis ou com doenças gastrointestinais diversas, seguida de monitorização da radioatividade plasmática total por um período de quatro dias. Eles constataram maiores níveis plasmáticos de radioatividade após seis a 12 horas da administração, com níveis menores em indivíduos com esteatorreia ou doença celíaca.

De maneira semelhante, outros seis estudos avaliaram a absorção de formas radiomarcadas de vitamina D em indivíduos com doenças disabsortivas e grupos controle (46-51). Estas doenças não pareceram influenciar o tempo médio em que foram atingidos os picos plasmáticos, contudo, os indivíduos doentes apresentaram menores níveis séricos em resposta à dose oral de vitamina D marcada. Comparativamente a portadores de doenças disabsortivas, idosos hospitalizados também apresentaram menores níveis plasmáticos da vitamina D marcada que jovens saudáveis (52).

Além disso, foram realizadas comparações entre a absorção das formas radiomarcadas de colecalciferol (^{3}H-D3 ou ^{14}C-D3) e de calcidiol (^{3}H-25OHD3) administradas oralmente. Observou-se que ^{3}H-25OHD3 atingia concentrações plasmáticas mais elevadas e mais rapidamente que ^{3}H-D3 ou ^{14}C-D3 (46,48-50). Aparentemente a absorção de ^{3}H-25OHD3 independe de ácidos biliares (46), sendo melhor absorvido inclusive por indivíduos com doenças colestáticas e inflamatórias intestinais (48-50).

Em uma diferente abordagem, Danielson A *et al* avaliaram a resposta nos níveis plasmáticos de ^{3}H-25OHD3 após administração de ^{3}H-D3 em mulheres com cirrose biliar primária (CBP) e grupo controle. Com isso, objetivou-se avaliar se a hidroxilação hepática também se encontra alterada na CBP (51). Evidenciou-se resposta semelhante em ambos os

grupos, com aumento gradual nos níveis plasmáticos de ^{3}H-25OHD3, à medida que os níveis de ^{3}H-D3 reduziam.

2.4.2 Ensaios Clínicos: Impacto de doenças na absorção da vitamina D

Lo CW *et al* avaliaram a resposta plasmática à 50.000 UI de ergocalciferol não marcado após sua administração em dose única oral a sete participantes com disabsorção de gorduras e sete saudáveis (53). Nos participantes saudáveis, os níveis plasmáticos de ergocalciferol começaram a aumentar dentro de quatro horas, com detecção de níveis máximos em 12 horas e queda gradual a níveis basais em três dias. Resultados semelhantes foram observados em dois dos sete participantes com disabsorção, contudo não se detectou aumento nos níveis plasmáticos nos cinco demais.

De maneira semelhante, Lark RK *et al* e Farraye FA *et al* observaram uma menor absorção de ergocalciferol em, respectivamente, indivíduos com fibrose cística em suplementação de enzimas pancreáticas e em indivíduos com doença de Crohn, quando comparados a grupo controle. Identificou-se, entretanto, grande variabilidade nas respostas plasmáticas ao ergocalciferol em indivíduos doentes de ambos os estudos.

Lark RK *et al* também avaliou os níveis plasmáticos de 25OHD em resposta a administração de ergocalciferol 100.000 UI em indivíduos com fibrose cística e grupo controle (35). Em ambos os grupos, os níveis plasmáticos máximos de ergocalciferol foram atingidos após 24 horas de sua administração. No grupo controle, os níveis plasmáticos de 25OHD aumentaram conforme os níveis de ergocalciferol gradualmente reduziram. Contudo, não houve aumento significativo dos níveis de 25OHD nos indivíduos com fibrose cística.

O by-pass gástrico em Y de Roux também modificou a absorção de colecalciferol em um estudo conduzido por Aarts E *et al* (54). A diferença entre os níveis basais e os níveis máximos após dose única de colecalciferol 50.000 UI diminuiu de 92 ± 6,5 para 63,5 ± 10,3 nmol/L após a cirurgia, $P< 0,02$, com grande variação entre os pacientes. Por outro lado, fatores como obesidade, idade avançada e uso de anticonvulsivantes pareceram não interferir na absorção da vitamina D.

A interpretação dos resultados de estudos que avaliaram a absorção da vitamina D em indivíduos doentes demanda cuidado, uma vez que fatores diversos à absorção intestinal podem interferir na resposta plasmática da vitamina D e seus metabólitos (28). Por exemplo,

baixos níveis plasmáticos de 25OHD são comumente encontrados em estados inflamatórios, mesmo quando o trato gastrointestinal se encontra intacto. Razões possíveis para isso são modificações na atividade da enzima 25-hidroxilase, nas concentrações das globulinas de ligação, ou no *clearence* da 25OHD. O **Artigo 2** trata de revisão sistemática com o objetivo de avaliar o impacto de um insulto inflamatório agudo nos níveis plasmáticos da 25OHD (28).

2.4.3 Ensaios Clínicos: Absorção da vitamina D em indivíduos saudáveis

Ellis G *et al* avaliaram o aumento nos níveis plasmáticos de 25OHD após dose única oral de colecalciferol 40.000 UI em indivíduos saudáveis (55). Eles compararam a resposta em ingleses *versus* imigrantes indianos e encontraram um aumento imediato na concentração de 25OHD em ambos os grupos. Entretanto, ao longo de cinco dias a média do aumento nas concentrações de 25OHD foi maior nos indianos que nos ingleses, possivelmente porque os níveis basais de 25OHD eram menores nos indianos.

O ergocalciferol pode ser considerado vantajoso para testes absortivos pois não é sintetizado na pele e apresenta concentrações muito baixas na dieta, constatou-se, contudo, que este composto é menos potente que o colecalciferol (56). Armas LA *et al* compararam a farmacologia do ergocalciferol e do colecalciferol após administração de 50.000 UI de um dos calciferóis a homens saudáveis (56). Ambos provocaram aumentos semelhantes nas suas respectivas concentrações plasmáticas, indicando absorção semelhante, e ambos resultaram em aumentos semelhantes nas concentrações de 25OHD durante os primeiros três dias. Contudo, os níveis de 25OHD continuaram a aumentar nos indivíduos tratados com colecalciferol, com níveis máximos em 14 dias, enquanto os níveis de 25OHD reduziram rapidamente nos indivíduos tratados com ergocalciferol, retornando aos seus níveis basais menos 14 dias.

Sequencialmente, Ilahi M *et al* avaliaram a farmacologia de uma dose única alta de colecalciferol (100.000 UI) em indivíduos saudáveis (57). Níveis plasmáticos máximos de colecalciferol foram atingidos oito a 24 horas após sua administração. Já os níveis plasmáticos de 25OHD aumentaram gradualmente conforme os níveis de ergocalciferol reduziram, com níveis máximos atingidos por volta de sete dias após a intervenção e se mantiveram diferentes dos níveis basais por 84 dias. Os indivíduos não apresentaram efeitos colaterais significativos.

Em uma diferente abordagem, Holemberg I *et al* observaram que, em situações de jejum, o colecalciferol é melhor absorvido quando administrado em óleo de amendoim, um

ácido graxo de cadeia longa, que com triglicerídeo de cadeia média. Entretanto sua absorção independe do veículo quando administrado junto a um lanche (58).

Johnson JL *et al* realizaram um estudo para determinar se a biodisponibilidade do ergocalciferol é maior em queijo fortificado ou dissolvido em água, e se a absorção difere entre jovens e idosos. Observou-se que o ergocalciferol é melhor absorvido quando administrado em queijo do que em água e que o padrão absortivo é semelhante em jovens e idosos (59).

Grande parte dos estudos mais recentes realizados em indivíduos saudáveis tiveram como objetivo comparar a influência de diferentes teores de gordura na absorção do colecalciferol (55,57,58,60,61). Alimentos gordurosos favorecerem a absorção da vitamina D, possivelmente porque aumentam a secreção biliar e pancreática. Observa-se, contudo, que a presença de gordura não é fundamental para que haja absorção (60,62). Indivíduos saudáveis que receberam lanche contendo gordura, apresentaram maiores aumentos nos níveis de colecalciferol e 25OHD, respectivamente, 12 horas (60) e 14 dias (62) após administração de colecalciferol 50.000 UI em dose única. Entretanto, indivíduos que receberam lanche pobre em gorduras também apresentaram aumento significativo nos níveis de 25OHD quando comparados ao placebo, o que sugere mecanismos mais complexos de incorporação da vitamina D pela borda de escova dos enterócitos.

Apesar dos avanços recentes em estudos experimentais sobre o transporte da vitamina D através da membrana apical dos enterócitos, poucos foram realizados em seres humanos. O ezetimibe é o único inibidor farmacológico de um transportador de colesterol disponível para uso clínico na atualidade. Demonstrou-se que a associação de 10mg de ezetimibe a 10 mg de sinvastatina resultava em metade do aumento nos níveis de 25OHD quando comparado ao aumento resultante da administração de 40 mg de sinvastatina isolada, em pacientes dislipidêmicos (63). Motivos pelos quais as estatinas elevam os níveis de 25OHD são desconhecidos até o momento e a dose de sinvastatina administrada em monoterapia foi maior que aquela administrada junto ao ezetimibe. Não se pode dizer, portanto, se os resultados foram relacionados a dose de sinvastatina ou ao ezetimibe.

Pacientes com dislipidemia primária não apresentaram maior incidência de deficiência de vitamina D após 12 semanas do uso de ezetimibe em estudo de avaliação de segurança da droga (64). Não se sabe, porém, se esses indivíduos usavam suplementação oral de vitamina

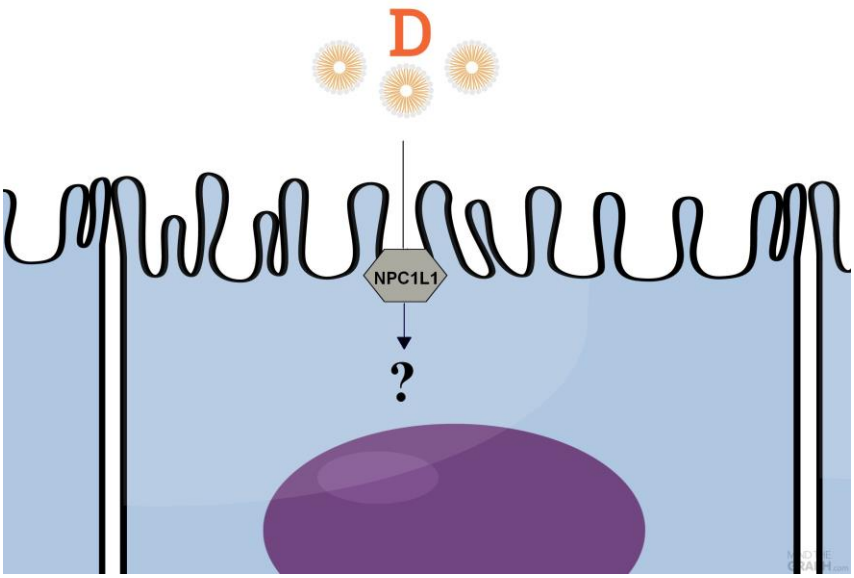
D ou se tinham exposição à luz ultravioleta suficiente para manter níveis adequados dessa vitamina.

O **Artigo 3** foi o primeiro ensaio clínico randomizado e controlado para avaliar o impacto de um transportador de membrana do colesterol na absorção da vitamina D (65).

3- MARCO TEÓRICO

Espera-se determinar o impacto do transportador de membrana do colesterol NPC1L1 na absorção da vitamina D em seres humanos através da inibição deste transportador pelo ezetimibe.

Figura 4. Marco teórico. O transportador de membrana do colesterol NPC1L1 está envolvido na absorção intestinal da vitamina D em seres humanos?



Fonte: Criado pela autora com auxílio da ferramenta mindthegraph.com.

4- JUSTIFICATIVA

O efeito da inibição do transportador de membrana do colesterol NPC1L1 na absorção da vitamina D em seres humanos nunca foi avaliado e poderá contribuir para a melhor compreensão do processo absortivo da vitamina D.

5- OBJETIVOS

5.1 OBJETIVO PRIMÁRIO

Avaliar os níveis séricos de 25OHD em resposta à suplementação oral de colecalciferol associada a ezetimibe ou a placebo em indivíduos saudáveis.

5.2 OBJETIVO SECUNDÁRIO

Avaliar os níveis séricos de PTH em resposta à suplementação oral de colecalciferol associada a ezetimibe ou a placebo em indivíduos saudáveis.

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7- ARTIGO 1

Título do manuscrito: Vitamin D Intestinal Uptake: A Systematic Review
Autores: Mariana Costa Silva, MD, MsSc e Tania Weber Furlanetto, MD, PhD
Situação: Submetido

ABSTRACT

Context: Vitamin D supplementation is frequently prescribed to prevent and treat its deficiency. Nevertheless, vitamin D absorption remains poorly understood. **Objective:** The aim of this systematic review is to evaluate data concerning mechanisms involved on vitamin D absorption, focusing on its uptake through the brush border membrane of enterocytes. **Data Sources:** PubMed, Embase and Cochrane Library databases were searched based on the combination of medical subject headings (MeSH) "vitamin D" and "absorption" until January 2016. **Data Extraction:** From 2069 articles identified, 42 studies met the inclusion criteria. Studies were categorized in: experimental research concerning vitamin D intestinal uptake, absorption tests using radiolabeled vitamin D, and clinical trials of a single vitamin D dose. **Data Synthesis:** Recent laboratory and clinical research suggest that vitamin D absorption is not an exclusive simple diffusion process, as previously assumed. Factors which interfered on cholesterol absorption also modified vitamin D absorption. **Conclusion:** In healthy human subjects, vitamin D is probably absorbed through two different mechanisms: a passive diffusion, which could be optimized by fat intake, and an active process involving membrane carriers, especially cholesterol transporters, which could explain vitamin D absorption at fasting, although data concerning this topic remain scarce, especially from clinical research.
Key words: vitamin D, absorption, membrane transport, enterocyte, bioavailability

INTRODUCTION

Vitamin D deficiency is a global concern today, particularly due to its special role in bone health and other possible systemic benefits (1-4). It is a steroid pro-hormone which can be supplied by skin synthesis in humans when skin is exposed to UVB radiation (5). Nevertheless, many people need dietary supplements, mainly due to low sun exposure. Therefore, recently, researchers have shown an increased interest in vitamin D physiology and metabolism, especially concerning its absorption when taken by the oral route.

Until recently, it was accepted that vitamin D absorption was just a simple passive diffusion process. However, recent findings suggest that complex mechanisms of vitamin D

incorporation through the enterocyte membrane might be present. For instance, absorption of vitamin D supplements occurs even with non-fat meals and non-oily vehicles (6,7). In these situations, absorption would be difficult to explain by simple diffusion.

This systematic review will focus on studies that evaluated the intestinal uptake of vitamin D. An electronic search of the literature was conducted up to January 2016 using Ovid MEDLINE, Embase, and the Cochrane Library, by combining the MESH terms "vitamin D" and "absorption". Knowing how vitamin D absorption occurs and the factors that might interfere with this process is important to better treat and prevent vitamin D deficiency.

METHODS

This review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (8). The Population, Intervention, Comparison, Outcomes, and Study Design criteria (PICOS) were used to formulate and narrow the focus of the following research question: What are the mechanisms involved on the vitamin D uptake through the brush border (apical) membrane of enterocytes? (**Table 1**)

Search methods for studies identification

Original research studies were identified from the databases PubMed, Embase and Cochrane Library, based on the combination of medical subject headings (MeSH) "vitamin D" and "absorption". Search details were: ("Vitamin D/administration and dosage"[Mesh] OR "Vitamin D/pharmacokinetics"[Mesh] OR "Vitamin D/physiology"[Mesh]) AND ("absorption"[MeSH Terms] OR "absorption"[All Fields]).

Article eligibility, inclusion and exclusion criteria

All original studies in English, Portuguese, and Spanish, up to January 2016, were considered for this review. Studies were included if they were 1) Experimental laboratory studies concerning vitamin D absorption through the enterocyte brush-border membrane, 2) Absorption tests using radiolabeled vitamin D or, 3) Clinical trials of a single cholecalciferol or ergocalciferol dose, which reported at least two measurements of serum cholecalciferol, ergocalciferol or 25OHD. Studies were excluded if they were performed in a pediatric sample, systematic reviews, books chapters, conference proceedings, correspondences and authors' comments.

Data extraction analysis

Both authors screened titles and abstracts identified from the electronic search. All abstracts with disagreement between the reviewers were re-visited and agreement was found by discussion and consensus. Full texts were obtained and once more, they were evaluated for

eligibility by the authors. Other sources for obtaining the papers were screened by cross-referencing texts. Studies quality was not rated due to differences in their design. No meta-analyses were performed.

RESULTS

From 2069 titles and abstracts, 109 articles were selected for full-text review. From these, 79 were excluded due to study design and 30 studies were included, as well as 12 others obtained by cross-referencing articles, as shown in **Figure 1**. Those 42 studies fulfilled one of the three inclusion criteria and, subsequently, they were classified into the following groups: 16 laboratory experiments concerning vitamin D absorption through the enterocyte apical membrane (**Table 2**); nine absorption tests using radiolabeled vitamin D (**Table 3**); 17 clinical trials of a single vitamin D dose, which were distributed in seven studies performed in patients with some kind of health concern (**Table 4**) and 10 studies performed in healthy people (**Table 5**).

DISCUSSION

Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are the vitamin D presentations commonly prescribed to prevent and treat its deficiency (9). The first is a plant-derived form of vitamin D, and the second is the animal form of vitamin D, which can be synthetized by irradiation of 7-dehydrocholesterol in the skin by UVB light. Both structures are very similar and both need to be hydroxylated in liver to 25-hydroxyvitamin D (25OHD) and in kidneys to its active form, the 1,25-dihydroxyvitamin D [1,25(OH)2D] (10).

Different vitamin D forms and methods were employed to evaluate vitamin D absorption. Altogether, the selected studies may provide good insights about mechanisms involved on vitamin D intestinal uptake and will be discussed in the next topics.

BASIC SCIENCE RESEARCH

An overview of experimental *in vivo* and *in vitro* studies concerning vitamin D uptake through the apical membrane of the enterocytes is exposed on **Table 2**.

Knowledge of vitamin D uptake through the brush border (apical) membrane of the enterocytes comes from experimental studies in the mid-seventies, when Hollander *et al* demonstrated a linear relationship between vitamin D absorption rate and its intraluminal concentration in two studies using evert gut sacs and live rats (11,12). Moreover, they demonstrated that vitamin's rate of absorption was raised by an increase in either the perfusate's flow rate or the hydrogen ion concentration, which decreases the resistance of the

cell membrane to the diffusion of micelles. These results indicated lack of saturation kinetics and supported the idea of a passive diffusion vitamin D transport.

For many years, the mechanistic process of vitamin D intestinal uptake was no longer the emphasis of investigations and laboratory experiments focused only on factors that could interfere on vitamin D uptake such as uremia (13), gastrectomy (14), aging (15-17) and vitamin D status (18). For example, the effect of aging upon intestinal absorption of vitamin D was evaluated in three experimental studies; two of them indicated that age did not impact significantly the vitamin D absorption in rats (15,17).

The influence of vitamin D status on the intestinal absorption and body retention of vitamin D was evaluated by Lorentzon R & Danielson A (18). Radiolabeled cholecalciferol (^{3}H-D3) was administered intragastrically to rats previously fed with different amounts of vitamin D. Animals with vitamin D deficiency accumulated high levels of serum radioactivity, which were to a great extent confined to polar fractions of 25OHD and 1,25(OH)2D, and there was significantly less radioactivity in the 3-day faecal collection from these animals. These results indicate that a higher vitamin D intestinal absorption may occur in states of its deficiency, but its serum concentrations reflects several different processes as absorption, distribution between body tissues, metabolism and excretion.

In a wider perspective, Bikhazi AB & Hasbi AR (19) investigated the brush border mechanistic passage of vitamin D and its metabolite 1,25(OH)2D, and evaluated their intracellular binding protein translocation and their subsequent release into the pre-hepatic systemic circulation. Radiolabeled cholecalciferol (^{14}C-D3) and 1,25(OH)2D (^{3}H-1,25D3) were measured in intestinal perfusates and portal blood samples of rats injected with cycloheximide, which is an inhibitor of protein biosynthesis, and compared with a control group. The ^{14}C-D3 amount lost from the perfusate remained the same in both groups. However, the treated rats presented a drastic increase of ^{14}C-D3 retention in intestinal segments and a reduction in ^{14}C-D3 portal plasma. The authors concluded that cholecalciferol might be transferred through the cytosol by carrier-binding proteins.

Moreover, the same authors observed that apical membrane absorption was different for cholecalciferol and 1,25(OH)2D, since ^{3}H-1,25D3 uptake from the perfusates was significantly reduced in cycloheximide-treated rats (19). Another polar metabolite of vitamin D, 25OHD, also presented a different absorption pattern from cholecalciferol in a study by Sitrin MD *et al* (20), although the mechanistic processes remained misunderstood.

More than 30 years after the first experiments about vitamin D intestinal uptake, researchers have brought evidences about similarities between cholesterol and vitamin D

absorption mechanisms (21-24). These new studies demonstrated that cholesterol and factors that are known to interfere with cholesterol uptake, like phytosterols and free fatty acids, also reduced the absorption of vitamin D in experimental models (21,23).

Knowledge about cholesterol absorption has made great advances in the last decades and it is well recognised that it involves the participation of protein membrane transporters (25). The major cholesterol membrane carrier is the Niemann-Pick C1-like 1 carrier (NPC1L1) (25), followed by scavenger receptor class B type 1 (SR-BI) (26), cluster determinant 36 (CD36) and ATP-binding cassette A1(ABCA1) (27).

Reboul *et al* evaluated vitamin D uptake in human Caco-2 cells, which are models of human intestinal epithelium often employed in the assessment of lipids intestinal transport, and in HEK 293-T cells, which are validated models of SR-BI, CD-36 and NPC1L1 overexpression in mice (22). Vitamin D uptake was higher in HEK 293-T cells and decreased after administration of carrier-specific inhibitors. Ezetimibe, a NPC1L1 pharmacological inhibitor, and another inhibitor of SR-BI similarly reduced the vitamin D uptake in Caco-2 cells. Vitamin D absorption decreased in live mice treated with ezetimibe, but it was not statistically significant, like a prior study conducted by Van Heek *et al*(28).

Free fatty acids modulate cholesterol absorption and they seemed to interfere with vitamin D absorption in the study conducted by Gonçalves A *et al* (23). As potential causes for the reduction in vitamin D absorption, the authors evented a possible modification in the micellar electrical charge or a modulation of cholecalciferol outflow through the basolateral membrane of enterocytes.Therefore, other possible explanation given by the authors was the competition between free fatty acids and cholecalciferol for the same transporter, especially CD36 or SR-BI, since these two proteins are known to be involved in FAs uptake.

Phytosterols are commonly employed to reduce dietetic cholesterol absorption and the same research group evaluated *in vitro* and *ex vivo* the effect of different sterols on cholecalciferol micellar incorporation, cholecalciferol apical uptake and basolateral efflux (21). In mice, cholecalciferol bioavailability was 15-fold lower in the presence of b-sitosterol ($p<0.05$). The significantly impaired cholecalciferol incorporation into mixed micelles (from -16 to -36% depending on sterol micellar composition) and the significantly lowered cholecalciferol apical uptake (from -13 to -39%) were cited by the authors as possible causes for the decreased vitamin D bioavailability. They also observed vitamin D and phytosterol competition for a common membrane transporter, supposedly NPC1L1.

CLINICAL RESEARCH

Radiolabelled absorption tests

Administering radiolabeled forms of vitamin D and monitoring it in blood, tissues, urine, and feces are the most direct ways to evaluate vitamin D absorption. Studies concerning intestinal absorption of radiolabeled vitamin D forms in humans are exposed on **Table 3** and will be in this section.

Thompson GR e*t al* were the first group to define and to implement a protocol for vitamin D absorption assessment in humans (29). Such protocol consisted in administering a single oral dose of ^{3}H-D3 to healthy people or patients with different gastrointestinal diseases, followed by monitoring total plasma radioactivity during four days. They found higher plasma radioactivity levels six to 12 hours after ^{3}H-D3 administration, with lower plasmatic radioactivity concentrations in patients with steatorrhea or coeliac disease.

Similarly, six subsequent studies evaluated different malabsorptive conditions, such as severe cholestasis (30-32), short bowel disease (33,34), and inflammatory bowel disease (34), and control groups. These conditions seemed not to interfere with the time of vitamin D plasmatic peak and its highest concentrations occurred about six to 24 hours after its oral administration (30-36). However, they seemed to modify absorption, with lower plasmatic levels occurring in patients with malabsorptive conditions than in control groups.

Paralleling to patients with malabsorptive disease, hospitalized elderly also had lower plasmatic radioactivity concentrations compared to healthy adults (35). On the other hand, plasma radioactivity and shape of the radioactive peak and decline were similar in patients taking anticonvulsant and healthy individuals (37).

In addition, comparisons between the intestinal absorption of labeled cholecalciferol and labeled 25OHD (^{3}H-25OHD3) were performed in three different studies involving patients with multiple gastrointestinal diseases and control groups (31,33,34). Administration of ^{3}H-25OHD3 resulted in higher serum radioactivity levels, and it was also reached faster, than administration of labeled cholecalciferol. Therefore, apparently 25OHD is best absorbed by individuals with inflammatory bowel disease or cholestasis. Additionally, Compston JE *et al* observed, in healthy men, that 25OHD absorption may be independent of bile acids, withsome absorption occurring directly into the portal vein (36).

In another prospective, Danielson A *et al* investigated whether hepatic hydroxylation is also changed in primary biliary cirrhosis (PBC) (32). They assessed serum radiolabeled 25OHD (^{3}H-25OHD3) response after ^{3}H-D3 administration in women with PBC and in a control group. Similar response was found in both groups, with gradual increase in ^{3}H-25OHD3 levels as ^{3}H-D3 levels reduced, showing that hepatic hydroxylation was not impaired in that sample.

Clinical trials

Clinical trials that used a single non-labeled dose of ergocalciferol or cholecalciferol showed similar results to those studies using its radioactive forms. Because ergocalciferol is rarely found in human diet and it is not synthetized by skin, it may be considered an advantageous supplement for vitamin D absorption tests when compared to cholecalciferol. The aims of these studies were, in general, to assess the impact of diseases (**Table 4**) or the influence of dietary compounds (**Table 5**) on vitamin D absorption.

Impact of diseases on vitamin D absorption

Lo CW *et al* were the first group to evaluate plasmatic ergocalciferol response after a high non-labeled ergocalciferol dose, calling it a "challenge test" (38). A capsule of ergocalciferol 50,000 IU was offered to seven patients with clinical fat malabsorption and seven healthy volunteers. Plasmatic ergocalciferol levels began to rise within four hours, peak concentrations were reached by 12 hours, and it gradually declined to baseline levels within three days. This result was similar in two patients with malabsorption, but in marked contrast in the other five patients with malabsorption, who did not present increasing ergocalciferol levels.

In a similar way, Lark RK *et al* and Farraye FA *et al* conducted ergocalciferol challenge tests, respectively, in volunteers with cystic fibrosis and Crohn disease (39,40). Comparable to Lo CW *et al* study, these two studies also found lower ergocalciferol response when the unhealthy groups were compared to their respective control groups. It is important to note that both authors found great variability between unhealthy subjects' responses.

In addition to analyzing ergocalciferol concentrations, Lark RK *et al* examined the 25OHD response to a single oral dose of ergocalciferol 100,000 IU in cystic fibrosis and control groups over 36 hours (40). In both groups, maximum levels of ergocalciferol were detected in plasma around 24 hours after its administration. As ergocalciferol serum levels gradually reduced, serum 25OHD levels gradually increased in the control group. In the cystic fibrosis group, 25OHD concentrations did not increase significantly at any time point.

The Y in Roux bariatric surgery also modified cholecalciferol absorption in a study conducted by Aarts E *et al*(41). The difference between baseline and the highest post-absorptive cholecalciferol level, after a single dose of cholecalciferol 50,000 IU, decreased from 92 ± 6.5 to 63.5 ± 10.3 nmol/L after bariatric surgery, $P< 0.02$. But these results also varied markedly between patients.

On the other hand, obesity and aging did not seem to interfere in vitamin D absorption after a single dose of ergocalciferol 50,000 IU (42,43). Peak ergocalciferol concentrations,

difference between peak and basal concentrations and mean (± SEM) serum 25OHD were not statistically different in obese and normal body mass index (BMI) groups in a study performed byWortsman J *et al* (42). Likewise, Clemens TL *et al* found similar plasmatic ergocalciferol peak response in both young volunteers and in institutionalized elderly with normal kidney function (43).

Moreover, von Restorff C *et al* analyzed serum 25OHD response to a single oral dose of cholecalciferol 30,0000 IU in elderly with severe vitamin D deficiency. It was evaluated on admission to acute care over a course of 4 months (44). This intervention increased serum 25OHD concentration in most patients to at least 50 nmol/L and 48% of patients reached the desirable range of at least 75 nmol/L at 3 months. Despite a decline at 6 months, mean 25OHD were still more than 4 times higher when compared to baseline.

Overall, these results highlight the need of caution when interpreting data about vitamin D absorption in health disorders, since several other factors not directly related to vitamin D intestinal uptake may interfere on plasma vitamin D response. For instance, lower serum 25OHD levels are commonly found in inflammatory conditions, even when gastrointestinal tract is not affected (45). Possible reasons for lower serum 25OHD levels could be an altered concentration or activity of the 25-hydroxylase enzyme, a changed vitamin D binding protein concentrations, or high rates of metabolic clearance of 25OHD.

Vitamin D absorption in healthy people

Ellis G *et al* were the first group to evaluate serum 25OHD response in healthy individuals before and at intervals after taking a single oral dose of cholecalciferol 40,000 UI (46). They compared the 25OHD response between two groups comprehending seven Indian immigrants and eight Europeans and they found an immediate increase in serum 25OHD concentrations after the oral dose in both groups. Over the first five days the mean increase was greater in the Indians than in the Europeans, possibly due to lower 25OHD baseline levels in the Indian group.

Subsequently, Armas LA *et al* evaluated the pharmacology of cholecalciferol and ergocalciferol by administrating single doses of 50,000 IU of the respective calciferols to 20 healthy male volunteers (47). Doses were administered weakly for a total of 12 doses. The two calciferols produced similar rises in serum concentration of the administered vitamin, indicating equivalent absorption. Both produced similar initial rises in serum 25OHD over the first three days, but 25OHD continued to rise in the cholecalciferol-treated subjects, peaking after 14 days, whereas serum 25OHD fell rapidly in the ergocalciferol-treated subjects and

was not different from baseline after 14 days of ergocalciferol administration. They also indicated that ergocalciferol potency is less than one third that of cholecalciferol.

Moreover, Ilahi M *et al* evaluated the pharmacology of a single large dose of cholecalciferol (100,000 IU) in healthy people (48).Plasmatic peaks of plasma cholecalciferol occurred eight to 24 hours after its administration, while highest plasma 25OHD concentrations occurred seven days after cholecalciferol administration. During 84 days, mean 25OHD concentrations were not significantly different from baseline. It is interesting to note that the 25OHD plasmatic peak concentrations were reached faster in this study of a single 100,000 IU cholecalciferol dose than in the earlier study of a single 50,000 IU cholecalciferol dose (47).

In a different approach, Holemberg *et al* have shown that cholecalciferol was more efficiently absorbed after administration in peanut oil, a long chain fatty acid, than in a medium chain triglyceride in the fasting state. But they found no difference between the two formulations when vitamin D was administered together with food (49).

Additionally, Johnson JL *et al* conducted a study to determine whether the bioavailability of ergocalciferol delivered in cheese is similar to the one dissolved in water and whether absorption differs between younger and older adults (50). Peak serum vitamin D and area under the curve were similar between younger and older adults, and ergocalciferol was absorbed more efficiently from cheese than from water.

Recent studies conducted in healthy people aimed to evaluate the influence of meal fat-compounds on cholecalciferol absorption (6,7,51,52). Raimundo *et al* conducted two double-blind randomized trials about the influence of dietary fat on the absorption of a single cholecalciferol 50,000 IU oral dose (7,52). Young medical doctors were divided in three groups according to meals fat-content (0, 15 or 30 grams of fat) given with the cholecalciferol dose or placebo in the newest study conducted by those authors (7). Mean serum 25OHD concentrations were higher in those who received lunch containing at least 15 grams of fat, but the group that received fat-free meal also presented an increased 25OHD levels compared to placebo, suggesting that vitamin D intestinal absorption probably does not occur by a simple passive diffusion process only.

Based on laboratory experimental results, in which polyunsaturated fatty acids (PUFAs) were demonstrated to decrease vitamin D absorption,Dawson-Hughes B *et al* also tested if monounsaturated fatty acid to polyunsaturated fatty acid ratio (MUFA:PUFA) were related to vitamin D absorption in men and postmenopausal women both older than 50 years (6). MUFA:PUFA had no significant influence in vitamin D absorption in this population.

Clinical trials addressing factors that may interfere on vitamin D apical membrane uptake are scarce. Silva, MC *et al* conducted a randomized, double-blind, placebo-controlled trial intended to determine if there is a participation of NPC1L1 cholesterol transporter in vitamin D absorption (53). They examined the effect of ezetimibe on serum 25OHD levels 14 days after a single cholecalciferol 50,000 IU oral dose was evaluated in medical residents. Ezetimibe produced no differences, comparing to placebo, on the mean change of serum 25OHD 14 days after cholecalciferol, in those young adults. As previously discussed in this review, similar results were demonstrated in live mice, corroborating that NPC1L1 may not be an important vitamin D transporter (22).

Ezetimibe is the only pharmacological inhibitor of a cholesterol transport available for clinical use at the moment. This is probably the reason why no clinical studies evaluating the effect of inhibition of other cholesterol transporters, as SR-BI and CD-36, on vitamin D absorption have been performed yet.

CONCLUSION

This systematic review was designed to analyze data concerning mechanisms involved on vitamin D absorption, with emphasis on the uptake through the apical membrane of enterocytes. Recent experimental *in vivo* and *in vitro* studies demonstrated that vitamin D absorption is not an exclusive simple diffusion process as previously assumed, but membrane carriers participate on this process. Cholesterol transporters may also be responsible for vitamin D uptake, since factors that interfere on cholesterol absorption also interfered on vitamin D absorption in laboratory and clinical studies. However, despite NPC1L1 being the major cholesterol transporter, it does not seem to perform a fundamental part for vitamin D absorption in studies using live rodents and in a clinical trial administrating ezetimibe to healthy adults. A limitation regarding the present systematic review was the heterogeneity of the included studies, with different methods employed to evaluate vitamin D absorption. Even though the attention for mechanisms involved on vitamin D absorption has been risen lately, data concerning this topic remains scarce, particularly from clinical research. The present review provides significant insights for future research, specially revealing the need for both identifying vitamin D membrane transporters and translating basic research findings into clinical research.

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Figure 1. Study selection flow diagram.

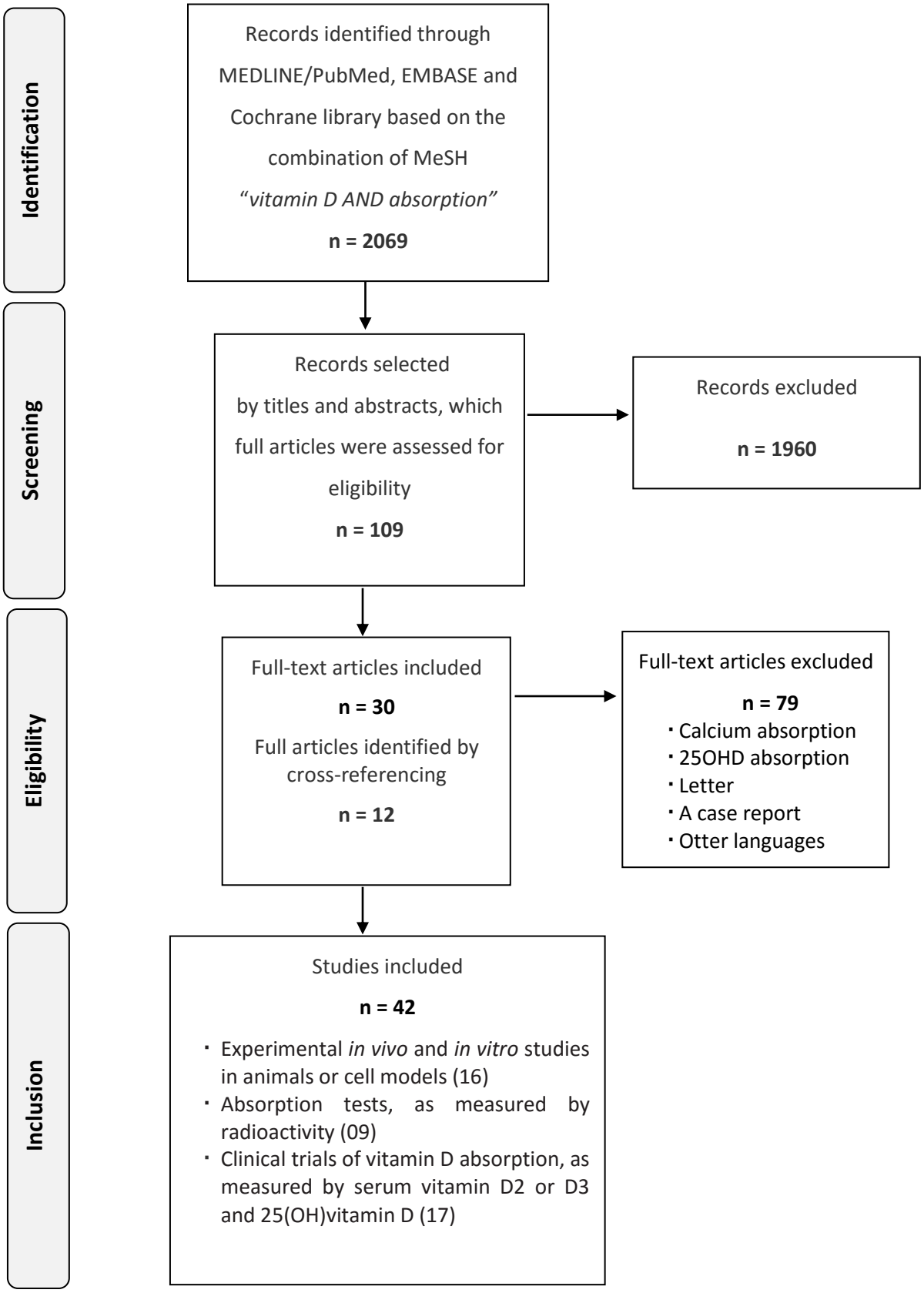

Table 1. PICOS criteria used to define the research question for the systematic review.

Criteria	**Description**
Population	Experimental models of mammalian physiology or human volunteers
Intervention	Administration of cholecalciferol, ergocalciferol, or its radiolabeled forms
Comparison	Plasmatic concentrations before and after vitamin D administration
Outcomes	Plasmatic concentrations of cholecalciferol, ergocalciferol, 25-hydroxyvitamin D, or its radiolabeled forms
Study design	Experimental laboratory studies, absorption tests using radiolabeled vitamin D, or clinical trials of a single cholecalciferol or ergocalciferol dose

Table 2. Experimental *in vivo* and *in vitro* studies concerning vitamin D uptake through the brush border (apical) membrane of the enterocytes.

Author, year	Addressed points	Methods	Outcomes
Gonçalves A, 2015	Fat-soluble vitamins absorption profile along the duodenal–colonic axis and interactions between FSVs during their uptake	· Mouse intestine *in vivo* · Caco-2 cells	**Fat-soluble vitamins** · Intestinal absorption location in mouse intestine: vitamins A = proximal, D = median, E and K = distal · In Caco-2 cells: Competitive interactions for uptake of D, E, and K vitamins. Vitamin A also decreased the uptake of the other FSVs. Vitamin A uptake was not impaired by vitamins D and K and even promoted by vitamin E.
Gonçalves A, 2013	Fatty acids effects on vitamin D3 intestinal absorption: Could FAs modulate vitamin D3 absorption in the same way as they impact cholesterol metabolism?	· Physicochemical properties of micelles formed with different FAs were analyzed · Micelles were administered to human Caco-2 cells · Cholecalciferol uptake and basolateral efflux · Regulation of genes coding proteins involved in lipid absorption process were analyzed	**Long-chain FAs in micelles** · ↓Vitamin D3 uptake in Caco-2 cells · Mixed with other FAs: annihilated the decrease · Oleic acid improved D3 basal efflux · Genes coding for lipid transport proteins such as NPC1L1, SR-BI were modulated, and could partly explain these results.
Reboul E, 2011	Is vitamin D intestinal absorption a protein-mediated process? Is there a possible involvement of cholesterol transporters in vitamin D absorption?	Vitamin D3 apical transport was examined in: ·Human Caco-2 and transfected HEK cells ·Wild-type mice, mice overexpressing SR-BI at the intestinal level ·Mice treated or not with ezetimibe, a NPC1L1 inhibitor	**Vitamin D3 uptake** · In caco-2 cells: was concentration, temperature and direction-dependent and ↓co-incubation with cholesterol or tocopherol, ↓SR-BI inhibitor or ezetimibe · ↑SR-BI, CD-36 and NPC1L1 transfection of HEK cells · ↓CD-36 inhibitor or ezetimibe on CD-36 and NPC1L1 transfected HEK cells, respectively · ↑ Mice overexpressing SR-BI · Ezetimibe effect was not significant in mice · Similar results were obtained in mouse intestinal explants
Gonçalves A, 2011	Phytosterols and cholecalciferol absorption	· β-Sitosterol effect on vitamin D3 postprandial response was assessed in mice · The effect of different sterols on vitamin D3 micellar incorporation, apical uptake and basolateral efflux were evaluated *in vitro* and *ex vivo*	**β-sitosterol** · ↓ Vitamin D3 bioavailability in mice - 15x, $p<0.05$ **Phytosterols** · Impaired cholecalciferol incorporation into mixed micelles · ↓ Vitamin D3 apical uptake in Caco-2 cells and mouse intestinal explants · No effect on vitamin D3 secretion at the basolateral side of Caco-2 cells
Van Heek M, 2001	Ezetimibe and the absorption of cholesteryl ester, TGC, ethinylestradiol, progesterone, vitamin	· Experiments were conducted in hamster or rats · A biliary anastomosis model was established in the rats	**Ezetimibe** · No effect on absorption of cholesteryl ester, TGC, ethinylestradiol, progesterone, vitamins A and D,

	A and D, taurocholic acid		taurocholic acid · Eliminating pancreatic function did not affect the ability of ezetimibe to block absorption of free cholesterol
Bikhazi, 1989	Brush border mechanistic passage of vitamin D3 and 1,25-vitamin D3, their intracellular binding protein translocation and their subsequent release into the pre-hepatic systemic circulation	· Intestinal perfusion technique for the study of ^{14}C-D3 or ^{3}H-1,25D3 absorption through intact jejunal segments of rats · Samples of intestinal perfusates, homogenates and portal blood were assayed for ^{14}C-D3 or ^{3}H-1,25D3 at specified time intervals in control rats and in rats injected with cycloheximide, an inhibitor of protein biosynthesis	**Cycloheximide-treated groups** · ^{14}C-D3 uptake from the perfusates = controls · ↑^{14}C-D3 retention in the perfused intestinal segments · ↓^{3}H-1,25D3 uptake from the perfusates · ↑^{3}H-1,25D3 intestinal retention · Intracellular binding proteins may be involved in the transport of ^{14}C-D3 or ^{3}H- 1,25D3 through rat enterocytes
Lorentzon R, 1985	Influence of vitamin D status on the intestinal absorption and body retention of vitamin D	· Rats were kept on a diet deficient in vitamin D for 2 months · Randomly assigned to one of three groups with different supplies of vitamin D for nine days · ^{3}H-D3 was administered intragastrically · Serum radioactivity was recorded after various periods of time · The animals were kept in metabolic cages and urine and faeces were collected	**Vitamin D deficient rats** · ↑ Serum radioactivity, mostly confined to the 25OHD e 1,25D fractions · ↓ Radioactivity in the 3-day faecal collection
Meyer MS, 1984	Intestinal absorption of cholecalciferol in gastrectomized rats	· Rats were gastrectomized and intestinal absorption and fecal excretion of cholecalciferol were studied following the administration of ^{3}H-D3 by subcutaneous injection or with the aid of a gastric tube · Measurements of radioactivity in feces and serum were performed	**Gastrectomized rats** · ↓ ^{3}H-D3 intestinal absorption · ↑^{3}H-D3fecal excretion
Hollander D, 1984	Aging and intestinal absorption of vitamin D3	· Vitamin D absorption and mucosal accumulation were measured using single-pass technique in male rats 9 to 101 weeks of age	· Vitamin D3 absorption was higher at 41 weeks of age, compared to 9 weeks of age, and remained relatively constant thereafter
Vaziri ND, 1983	Uremia and vitamin D3 intestinal absorption	· An *in vivo* perfusion technique was used to determine the rate of intestinal absorption of vitamin D3 in uremic and normal rats	**Uremic rats** · Rate of vitamin D3 absorption < control animals ($p<0,001$)
Sitrin MD, 1982	Compare vitamin D and 25OHD absorption	· Physiological amounts of vitamin D and 25OHD · *In vivo* from jejunal sacs in rats with thoracic and bile duct cannulas	**Absorption of 25OHD > vitamin D** · The majority of absorbed vitamin D and 25OHD was transported from the intestine in portal blood rather than lymph · When the luminal fluid contained 2.5 mM oleic acid and monoolein, the presence of taurocholate did not affect total intestinal absorption of vitamin D or 25OHD but

			increased recovery of vitamin in lymph
Fleming BB, 1982	Aging and intestinal absorption of vitamins A and D	· Rats were fasted for 12 hours and were then anesthetized and treated by stomach tube with 1.6 uCi ^{3}H-vitamin A and 1.0 uCi ^{4}C-D3	**Aged rats** · Did not have a significant decrease on absorption of either vitamin A or vitamin D · The pattern of distribution of the dose of A and D also did not differ significantly as a function of age
Holt PR, 1981	Aging and intestinal absorption of vitamin D3, fatty acids and monoglycerides	· ^{3}H-Trioleyl glycerol and ^{14}C-D3 were perfused intraduodenally for 5 h in aged and young adult rats	**Aged rats** · ↑ in the intestinal content of vitamin D3 at all perfusion rates when compared to young adult controls · ↑trioleylglycerol at the higher perfusion rates · Intestinal reesterification of absorbed ^{3}H-labeled fatty acid and partial glycerides was unchanged, so impaired intestinal triglyceride formation did not cause the increased content of tissue ^{3}H-labeled lipids · No accumulation of ^{14}C-D3 metabolites was detected
Hollander, 1978	Absorption of physiological concentrations of vitamin D3	· Rat was anaesthetised and its abdomen was opened, catheters were used to isolate proximal jejunal and distal ileal segments · Intestinal loops were replaced into peritoneal cavity and abdomen was closed · The animal was allowed to awaken and was placed in a restraint cage	**^{3}H-D3 uptake** · ↑ Concentration↑ uptake rate: linear relationship in jejunum and ileum · ↑ by increases in H^{+} concentration or perfusate's flow rate · ↓by the addition of 2-5 mM FAs of varying chain length and degrees of saturation · Increasing sodium taurocholate concentration in the perfusate did not change ileal absorption rate but did decrease jejunal absorption rate
Hollander, 1976	Mechanism and site of vitamin D3 intestinal uptake in rat bowel sacs	· ^{3}H-D3 in pharmacological concentrations · Everted rat small bowel sacs, from three different regions of the small bowel, incubated in a micellar medium	**^{3}H-D3 uptake** · ↑ Concentration ↑ uptake rate: linear relationship · Proximal and medial small bowel > distal small bowel ($p < 0.01$)
Thompson WG, 1969	Cholestyramine and the absorption of radioactive vitamin D and calcium in rats	· Absorption of 10 ug ^{3}H-D3 and 1 mg ^{47}calcium after the addition of cholestyramine to diet in an amount sufficient to cause steatorrhoea in rats, and control	**^{3}H-D3 uptake** · ↓ by the addition of cholestyramine to diet · ^{47}Calcium absorption was similar in both control and cholestyramine-fed rats

FSVs: fat soluble vitamins , NPC1L1: Niemann-pick C1-like 1, SR-BI scavenger receptor class B type I, HEK: Transfected human embryonic kidney, CD 36: Cluster Determinant 36, [^{3}H]Trioleyl glycerol (TO), ^{14}C-D3, ^{3}H-25OHD3: 25-[^{3}H]hydroxyvitamin D3, ^{3}H-vit A:[1-^{3}H (N)] vitamin A; 25OHD: 25-hydroxyvitamin D, ^{4}C -D3: [4-^{4}C] vitamin D3, 1,25-vitamin D3: 1,25-hydroxyvitamin D3, TGC: triglyceride

Table 3. Studies concerning intestinal absorption of radiolabeled vitamin D in humans.

Author, year	Participant (n)	Objective	Intervention	Plasma radioactivity: post intervention time	Plasma peaks	Results concerning vitamin D absorption
Leichtmann GA, 1991	· Crohn's disease + bowel resection (12) · CG (04)	Compare absorption of ^{3}H-D^{3} X ^{3}H-25OHD3 administered separately	^{3}H-D3 or ^{3}H-25OHD3 5.9-8.9 10^{-9} uCi + 100 ug VitD + dietary formula	^{3} H total: 0, 2, 4, 8, 12, 24 h	^{3} H total: 12h after ^{3}H-D3 8h after ^{3}H-25OHD3	· ^{3}H-D3<^{3}H-25OHD3 · CD + BR< CG (both presentations) · ↑bowel resection ↓ absorption
Sitrin MD, 1987	· Chronic cholestasis, female, 36-63 y.o. (08) · Chronic cholestasis, male, 44 y.o. (01) · CG (05)	Compare absorption of ^{3}H-D^{3} X ^{3}H-25OHD3 administered simultaneously	^{3}H-D3 + ^{3}H-25OHD3 8-10 uCi + 100 ug VitD + dietary formula	^{3}H-D3, ^{3}H-25OHD3: 0, 4, 8, 12, 24h	^{3}H-D3:12h ^{3}H-25OHD3:8h	· ^{3}H-D3<^{3}H-25OHD3 · ^{3}H-D3: severe cholestasis (04) < mild cholestasis (05) = CG · ^{3}H-25OHD3 ≈ in all groups · not correlated with basal serum 25OHD
Danielsson, 1982	· Primary biliary cirrhosis, female, 41-71 y.o. (08) · CG (08)	Evaluate the absorption, metabolism and excretion of ^{3}H-D3	^{3}H-D3 12.5 uCi + 2000IU D3 by the oral route after 48 h: 12.5 uCi ^{3}H-D3 + 2000IU D3 IV	^{3}H-D3, ^{3}H-25OHD3: 1, 2, 3, 4, 5, 6 days	^{3}H-D3:6h ^{3}H-25OHD3: 6 days	· PBC < CG · ↑ steatorrhea↓absorption · metabolism of D3 to 25HD3 = PBC x CG
Compston, JE, 1981	· Healthy young men (12)	Compare the appearance of ^{3}H-D^{3} X ^{3}H-25OHD3 in chylomicrons fraction of plasma and plasma	^{3}H-D3 6.25 uCi and ^{3}H-25OHD3 4-10 uCi administered concurrently	^{3}H: 2, 3, 4, 6h	Not evaluated	· Chylomicrons fraction: · ^{3}H-D3 >^{3}H-25OHD3 · ^{3}H-25OHD3: first in plasma
Davies M, 1980	· Gastrectomy (05) · Short bowel (05) · Coeliac disease (03) · CG (05)	Compare the absorption of the ^{14}C-D^{3} X ^{3}H-25OHD3 administered concurrently	^{14}C-D3 2 uCi + ^{3}H-25OHD3 8 uCi + meal and milk	^{14}C-D3, ^{3}H-25OHD3: 2, 4, 8, 12h and 13 days	^{14}C-D3: 6-24h ^{3}H-25OHD3: 4-12h	· ↑ steatorrhea↓absorption · ^{14}C-D3<^{3} H-25OHD3 · ^{14}C-D3: Gastrectomy and Celiac Disease ≈GC>BR · ^{3}H-25OHD3: Celiac disease≈CG · partial gastrectomy < CG at 8 and 24h, but earlier peak

Barragry JM, 1978	· Healthy adults (22), 30-58 y.o. · Hospitalized elderly, 68-94 y.o. (20) · Adults with malabsorption, 31-66 y.o. (05)	Experiment 1 Pilot evaluation of ^{3}H-D3 absorption Experiment 2 Compare ^{3}H-D3 absorption, and compare the metabolism of D3 to 25OHD3 in healthy young X hospitalized elderly	Experiment 1 ^{3}H-D3 1-3.5 uCi + meal: 08g fat, after 3h (02) 50g fat, at 0h (02) 50g fat, after 8h (01) Experiment 2 ^{3}H-D3 6 uCi + meal 30g fat, at 0h (47)	Experiment 1 ^{3}H-D3: 1, 2, 3, 4, 5, 6, 7, 8, 9h Experiment 2 ^{3}H-D3,^{3}H-25OHD3: 1, 2, 3, 4, 5, 6h	Experiment 1 8g after 3h: 6h 50g at 0h: 9h 50g after 8h: 9h Experiment 2 Last measurement was at 6th h, so peak was not determined	Experiment 2 · hospitalized elderly and adults with malabsorption< healthy subjects · ^{3}H-25OHD3: the response was < in the hospitalized elderly
Krawitt EL, 1977	· PBC (06) · CG (04)	Evaluate ^{3}H-D3 absorption	^{3}H-D3 8 uCi + meal with milk + ^{14}C-D3 2 uCi EV	^{3}H total,^{14}C-D3, ^{14}C-25OHD: from 10 min until 13 days	Not reported	· PBC with steatorrhea (04)< PBC without steatorrhea (02) and CG
Schaefer K, 1972	· Patients taking anticonvulsant drugs (08) · CG (05)	Compare ^{14}C-D3 absorption in patients taking anticonvulsant X CG	^{14}C-D3 10 uC + meal with milk	^{14}C total: 4, 8, 24, 72, 144 h	^{14}C-D3: 8-24h	· Plasma radioactivity and shape of the radioactive peak and decline were similar in both groups
Thompson GR, 1966	· Gastrointestinal diseases (10) · CG (09)	Aapply a protocol for the evaluation of ^{3}H-D3 absorption in humans	^{3}H-D3 1.5-55 uCi + milk	^{3}H total: 2, 4, 6, 8, 10, 12h and 1, 2 3, 4 days	^{3}H total: 6-12h	· Coeliac disease and patients with steatorrhea< CG · radioactivity after 3h: 45-100% in chylomicrons fraction

GC: control group; y.o.: years old; PBC: primary biliary cirrhosis; CD: Crohn's disease, BR: bowel resection; IV: intravenous;^{3}H-D3: [^{3}H]cholecalciferol; ^{3}H-25OHD3: [^{3}H] 25-hydroxycholecalciferol, ^{14}C-D3:[^{14}C]cholecalciferol; uCI: microcurie; Vitd: non-radiolabeled vitamin D; PO: orally; <:lesser; >: greater; ≈: similar

Table 4. Studies about absorption of a single vitamin D oral dose in adults with some kind of health concern.

Author, year	**Participant (n)**	**Objective**	**Intervention**	**Serum measurement: post intervention time**	**Conclusion concerning vitamin D absorption**
Farraye FA, 2011	· Quiescent Crohn's disease (37) · Healthy (10)	Compare vitamin D absorption in healthy people x Crohn´s disease, and evaluate if Crohn´s location and previous surgeries interfere on vitamin D absorption	D2 50,000 IU	D2: 0, 12h	**Crohn's disease:** · ↓ D2 peak 30%, $P < 0.01$ · great variability among participants · location of disease, past of surgery or surgery type did not interfere
Aarts E, 2011	· Obese premenopausal women, 20-50 y.o. pre and post Y in Roux bariatric surgery (14)	Create a method to quantify changes in D3 absorption after RYGB	· D3 50,000 IU pre RYGB · D3 50,000 IU post RYGB	· D3 pre RYGB: 0, 1, 2, 3, 14 days · D3 post RYGB: 0, 1, 2, 3, 14 days	**Post RYGB** · ↓ 26.6 ± 3.7% the maximal change in D3, $P = 0.02$ · D3 peak: 01 day (= pre) · great variability among participants
von Restorff C, 2009	· Vitamin D deficient elderly during admission for: musculoskeletal pain, bone disease or gait changes (33)	Evaluate 25OHD levels after a high vitamin D dose	D3 300,000 IU + calcium 500-1000mg	25OHD: 0, 3, 6 months	**Elderly patients** mean 25OHD nmol/l (SD) baseline =15 (±5.5) 3rd month = 81.4 (±29.7) 6rd month = 69 (±17.9)
Lark RK, 2001	· Cystic fibrosis (10) · Healthy (10)	Assess absorption of vitamin D, 25OHD response, and compare healthy x cystic fibrosis subjects	D2 100,000 IU + a meal + pancreatic enzymes	D2, 25OHD: 0, 5, 10, 24, 30 and 36 h	**Cystic fibrosis** · great variability · ↓ D2 in 50% $P < 0.001$ · ↓ 25OHD $P = 0.0012$
Wortsman J, 2000	· Obese (19) · Normal BMI (19)	Determine if obesity alters the absorption of D2	· D2 50,000 IU	· D2, 25OHD: 0, 5, 10, 15, 20, 25 hours after D2	**Obesity** · Did not differ significantly the D2 peak, the difference between D2 peak and basal concentrations, neither the mean (± SEM) serum 25OHD
Clemens TL, 1986	· Institutionalized elderly, 57-88 y.o., normal kidney function (07) · Young, 22-28 y.o. (08)	Study the impact of age on vitamin D absorption	D2 50,000 IU	D2: 0, 4, 8, 16, 24, 48, 72 h	**Institutionalized elderly and young: similar** · plasmatic peak: 8-16 h · return to basal levels in 3 days

Lo CW, 1985	• Malabsorptive syndromes (07) • Controls (07)	Develop a test for clinical evaluation of vitamin D absorption	D2 50,000 IU	D2, 25OHD:0, 4, 8, 12 h and 1, 2, 3, 14 days	**Malabsorptive patients** • D2 did not raise in 05 of the 07 participants • same result as controls in 02 of the 07: • D2 peak at 12 h • return to baseline in 3 days

y.o.: years old, D2: ergocalciferol or vitamin D2; D3: cholecalciferol or vitamin D3; 25ohd: serum 25-hydroxyvitamin D; MUFA PUFA: diet/between monounsaturated fatty acids/polyunsaturates; BMI: body mass index; UVB: ultraviolet

Table 5. Studies about absorption of a single vitamin D oral dose in healthy people.

Author, year	**Participant (n)**	**Objective**	**Intervention and groups (n)**	**Serum measurement: post intervention time**	**Results concerning vitamin D absorption**
Silva MC, 2015	· Young men and women (51)	Evaluate if NPC1L1 cholesterol transporter is involved in D3 absorption	D3 50,000 IU + 15g-fat meal + · ezetimibe (24) · placebo (27)	25OHD: 0 and 14 days	↑ **D3** ≈ in ezetimibe and placebo group, $P = 0.26$
Dawson-Hughes B, 2015	· Men and postmenopausal women, >50 y.o. (50)	Evaluate if D3 is best absorbed: · With dietary fat · With ↑MUFA/ PUFA	D3 50,000 IU + · fat meal (19) · non-fat meal (31) · MUFA/PUFA high (20) x low (11)	D3:0, 10, 12, 14 h	↑**D3:** · fat meal > non- fat · 32%, $P = 0.003$ · MUFA/PUFA: high = low · plasma peak: 12h
Raimundo FV, 2015	· Young men and women (64)	Compare 25OHD levels after D3 took with ≠ fat content meals	D3 50,000 IU /placebo + meal containing: · 30g fat (15/05) · 15g fat (17/05) · 0g fat (15/07)	25OHD: 0 and 14 days	↑ **25OHD:** · D3 >placebo, $P< 0.001$ · 15g and 30g >0 g fat, $P< 0.05$ · 15g ≈ 30g
Dawson - Hughes B, 2013	· Men and postmenopausal women, 50-59 y.o. (62)	Evaluate if the presence of meal and if its fat content influence on D3 absorption and 25OHD levels	D3 50,000 IU + · no meal (21) · meal containing: 11.1g fat (20) 35.2g fat (21)	D3: 0 and 12 h 25OHD: 0, 30, 90 days	↑**D3:** · 11.1g > 32.2g fat and 0g fat meal ↑**25OHD:** ≈ in all groups
Raimundo FV, 2011	· Young men and women (30)	Compare 25OHD levels after D3 took with meals containing high x low fat	D3 50,000 IU + meal: · 25.6g fat (15) · 1.7g fat (15)	25OHD: 0, 7, 14 days	↑**25OHD:** · after 14 days · 25.6g >1.7g, $P< 0.01$
Ilahi M, 2008	· Elderly, 61-84 y.o. (20)	Evaluate the response of 25OHD levels after D3	· D3 100,000 (20 elderly + 10 adults)	25OHD: 0, 1, 3, 5, 7, 14, 21, 28, 42, 56, 70, 84, 96,	↑**25OHD:** · plasma peak: 7 days

	· Adults, 27-47 y.o. (10) · CG, 63-91 y.o. (10)		· placebo (10)	112 days	· non-toxic levels · not correlated with basal levels · progressive decline with no statistics ≤ 84 days
Johnson JL, 2005	· Adults,23-50 y.o. (04) · Elderly,72-84 y.o. (04)	Determine the bioavailability of vitamin D2 in cheese X in water, and whether absorption differs between younger and older adults	Single acute feedings of: · D2 10,000 IU in cheese · D2 10,000 IU in water Procedures repeated with the other delivery vehicle on the 14th day	D2: 0, 6, 12, 24 h	↑**D2** · cheese: 15 ± 1 ng/mL per 10,000 IU > Water: 2 ± 0.4 ng/mL per 10,000 IU, *P*<0.001 · from cheese and water: younger~older groups
Armas LA, 2004	· Men, 20-61 y.o. (30)	Test the hypothesis of superiority of D3 over D2	· D2 50,000 IU (10) · D3 50,000 IU (10) · no supplement (10)	D2 or D3: 0, 1, 3 days 25OHD: 0, 1, 3, 5-7, 14, 28 days	↑**25OHD:** · D3>D2 treated groups · began to fall earlier in the D2-trated group ↑**D2 and D3:** D3~D2 treated groups
Holmberg I, 1990	· Adults, 22-46 y.o. (24)	Compare the absorption of D3 dissolved with ≠ vehicles: peanut oil, which is a long chain TGC X medium-chain TGC	D3 1.3 umol in ≠ vehicles (TGC long/medium chain) · with meal (06/06) · fasting (06/05)	D3 and 25OHD: 0, 2, 4, 6, 8, 10, 12 h 1, 2, 7, 14, 28 days	↑**D3** · plasma peak: 8-24 h · fasting:TCG medium >long chain vehicle · with lunch: TCG medium ≈ long chain ↑**25OHD** · plasma peak: 7 days · ≈ in all groups
Ellis G, 1978	· Indian immigrants, with low 25OHD levels (07) · Europeans, 19-33 y.o. (08)	Assess D3 absorption in Indians and Europeans descendants	D3 40,000 IU + milk	25OHD: 0, 1, 5, 9, 20, 46, 97, 191 days	↑**25OHD:** · plasma peak · Europeans: 6 days · Indians: 10 days

y.o.: years old; D3: cholecalciferol or vitamin D3; 25OHD: serum 25-hydroxyvitamin D; MUFA/PUFA: monosaturated/polysaturateds fatty acids; TCG: triglycerides; ≈: similar; ↑: increase; ↓: fall; >: greater; <: lesser; AUC: area under the curve

8- ARTIGO 2

Título do manuscrito: Does serum 25-hydroxyvitamin D decrease during acute-phase response?

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Situação: Publicado

CLINICAL SIGNIFICANCE

- Serum 25(OH)D levels decreased during the acute-phase response in most of the reviewed studies.
- The metabolic meaning and the functional importance of 25(OH)D changes in the context of an inflammatory state are not yet well understood.
- 25(OH)D measurements to evaluate vitamin D deficiency, during the acute-phase response, should be interpreted with care.

ABSTRACT

Low levels of 25-hydroxyvitamin D [25(OH)D] are commonly associated to inflammatory diseases. These associations could be due to an increased prevalence of inflammatory diseases in hypovitaminosis D, although reverse causality cannot be excluded. We aimed to systematically review the longitudinal studies which reported serum 25(OH)D during an acute inflammatory response in humans. An electronic search of the literature was conducted up to January 2014 using Ovid MEDLINE, EMBASE, and the Cochrane Library, by combining the MESH terms: vitamin D and acute phase reactants. Other sources for obtaining papers were used as cross-referencing texts. From 670 titles and abstracts, 40 articles were selected for full-text review and eight of these studies met the final inclusion criteria. In six of the reviewed studies, 25(OH)D dropped after the inflammatory insult; this decrease was abrupt in the studies which measured 25(OH)D early after the insult. In two studies, there was no change of 25(OH)D during the course of the disease, but baseline levels were measured in both after days of symptoms onset. One study suggested that hemodilution decreased 25(OH)D, with no effect of inflammation. Serum C-reactive protein concentrations were used as inflammatory markers in almost all studies. The metabolic meaning and

the functional importance of these changes are unknown. In light of the current evidence, 25(OH)D measured during acute-phase response should be interpreted with care. Future researches, including other markers of vitamin D adequacy, would help to clarify if hypovitaminosis D might be the cause or the consequence of inflammatory diseases.

Key words: 25-hydroxyvitamin D, vitamin D, acute-phase reactants, inflammation

INTRODUCTION

Hypovitaminosis D, defined by low 25-hydroxyvitamin D [25(OH)D] levels, has been widely associated with chronic and acute diseases, illness severity, poor outcome, and increased mortality (1-9). These associations have been credited to the vitamin D anti-inflammatory and anti-proliferative properties (10-13). Acute-phase response has been associated with significant metabolic changes (14), so variations on blood concentrations of vitamin D would not be unexpected. Several mechanisms could be responsible for a decrease in serum 25(OH)D levels in these situations, such as decrease in vitamin D carrier proteins (15), increased conversion of 25(OH)D to 1,25-dihydroxyvitamin D, and hemodilution (16). The aim of this study was to review the longitudinal studies, which evaluated the association of acute phase response with serum 25(OH)D levels during an inflammatory state. An electronic search of the literature was conducted up to January 2014 using Ovid MEDLINE, EMBASE, and the Cochrane Library; by combining the MESH terms: vitamin D and acute phase reactants. Original studies that were written in Portuguese, English or Spanish were included.

METHODS FOR SELECTION OF LITERATURE REVIEWED

Search strategy

We searched Ovid MEDLINE, EMBASE and the Cochrane Library from database inception, combining the MESH terms: vitamin D and acute phase reactants. The search details were: ("vitamin d"[MeSH Terms] OR "vitamin d"[All Fields] OR

"ergocalciferols"[MeSH Terms] OR "ergocalciferols"[All Fields]) AND ("acute-phase proteins"[MeSH Terms] OR ("acute-phase"[All Fields] AND "proteins"[All Fields]) OR "acute-phase proteins"[All Fields] OR ("acute"[All Fields] AND "phase"[All Fields] AND "reactants"[All Fields]) OR "acute phase reactants"[All Fields]). Original studies in English, Portuguese or Spanish, up to January 2014, were included.

Article eligibility, inclusion and exclusion criteria

Eligibility criteria: All studies obtained by combining the combining the MESH terms: vitamin D and acute phase reactants, in English, Portuguese or Spanish, up to January 2014, were eligible. Inclusion criteria: Longitudinal studies, which reported 25(OH)D outcomes during an acute inflammatory response in humans, caused by a medical procedure or an acute illness, with at least two serum 25(OH)D measurements: the first prior to the inflammatory insult or at its beginning, and the second during follow-up, were included. Exclusion criteria: Chapters of books, conference proceedings, correspondences, and author comments, were not included.

Data extraction analysis

The two authors reviewed the citations and abstracts identified from electronic searches using the eligibility criteria. Full-text articles of potentially relevant references were independently evaluated for eligibility by the authors. Other sources for obtaining the papers were used as cross-referencing texts. The quality standard considered for selection of studies was the longitudinal design. No meta-analysis was performed; the studies were clinically heterogeneous: serum 25(OH)D was measured at different moments, the acute inflammatory response was due to several causes, and in one study, patients were submitted to hemodilution due to cardiopulmonary bypass.

FINDINGS OF SERUM 25(OH)D DURING ACUTE-PHASE RESPONSE

From 670 titles and abstracts, 40 articles were selected for full-text review. Of these, 32 were excluded for the following reasons: study design (n=27), no 25(OH)D outcome (n=5). Eight studies were included, as shown in Figure 1. Four studies evaluated 25(OH)D levels before and after elective surgery (16-19), another, before and after the acute-phase response originated from the first dose of intravenous amino-

bisphosphonate administration (20). In the remaining three, 25(OH)D levels were measured soon after the diagnosis of an acute illness and during its course (21-23). These studies are described below, and summarized in Table 1.

Louw et al were the first authors to conduct a study testing the hypothesis that blood 25(OH)D concentrations might change after the onset of an acute phase-response (17). In their study, healthy adult volunteers, submitted to uncomplicated orthopedic surgery, had significant decreases in 25(OH)D concentrations during the acute-phase response, which mirrored the changes in serum C-reactive protein concentrations (CRP). Serum 25 (OH)D levels differed between males and females; for men, levels were significantly lower than the baseline at the 24th hour; for women, at the 2nd, 3rd and 7th days. Data about 25(OH)D levels are shown in Table 2.

In line with these findings, most subsequent studies found a rapid decrease in serum 25(OH)D levels, after an inflammatory insult. Reid et al. showed that plasma 25(OH)D concentrations began to fall within the first 6-12 hours after elective knee surgery, and reached a statistically significant nadir of 42.5% within 24–48 hours. Levels were still significantly lower, a 25% decrease, after 3 months, time by which the systemic inflammatory response (SIR) was already resolved (18). Similarly, Waldron et al showed a significant decrease of serum 25(OH)D concentrations 48 hours after elective knee or hip arthroplasty (19). Bang et al also observed a decline in serum 25(OH)D levels 48 h after admission for severe acute pancreatitis (21).

Only two of the reviewed studies have found no changes in 25(OH)D levels, during the course of disease. In one, the insult was an acute myocardial infarction, in the other, the insult was a severe malarial infection, and that was the sole retrospective study of this review. In both, baseline serum 25(OH)D levels were measured days after symptom onset and possibly after the early fall in serum 25(OH)D, shown by Reid et al (18), and Louw et al (17). It was observed that 25(OH)D levels after myocardial infarction follow-up were collected concomitant with the recovery of CRP levels (Table 2).

In two of the reviewed studies, which evaluated 25(OH)D levels in the first 12 hours after the insult, mean serum 25(OH)D levels at this time were lower than baseline. Louw et al (17), Reid et al (18), and Waldron et al (19) intended to avoid

disease state as a potential confounder by evaluating patients submitted to elective surgery. Mean serum 25(OH)D concentrations decreased after surgery, and this endorses the hypothesis that the acute inflammatory response itself may reduce 25(OH)D levels.

Only one of the five studies, which evaluated patients before the pro-inflammatory condition, had a long follow-up (18) and observed low 25(OH)D concentrations after 3 months, when SIR had already resolved. Likely, factors not taken into consideration might have contributed to those differences, as sun exposure, and vitamin D, calcium and sodium intake.

Hemodilution

Krishnan et al aimed to demonstrate the impact of fluid loading on serum vitamin D, after cardiopulmonary bypass (16). There was a 35% reduction in serum 25(OH)D concentration immediately after mixture with the pump, although it returned to baseline levels when the excess of fluid improved. Serum CRP measurements were high 24 h after the procedure; at that time, 25(OH)D levels were already close to the baseline. Therefore, this study suggested that hemodilution influenced 25(OH)D concentrations, with no effect of inflammation. The study conducted by Bertoldo et al avoided hemodilution as a potential confounder, since bisphosphonate infusions did not require great amounts of intravenous fluids (20). They reported a decrease of 30.1 ± 8.1% on the third post-dose day of IV amino-bisphosphonate in subjects with normal baseline 25(OH)D levels, which had an acute phase reaction after the administration of the drug.

Serum C-reactive protein (CRP)

Serum CRP concentrations were used as an inflammatory marker in almost all studies. In four of the five studies, which evaluated patients before medical procedures (17-20), CRP levels increased, and 25(OH)D levels decreased. In one study (16), which included patients submitted to extracorporeal circulation, there was an initial decrease in both CRP and 25(OH)D levels, attributed to hemodilution subsequently, there was an increase in CRP levels without a change in 25(OH)D levels. In another study (22), which evaluated CRP and 25(OH)D levels initially two days after acute myocardial

infarction, there was a decrease in CRP levels without change in 25(OH)levels during the follow-up. These data are shown in Table 2.

Binding proteins

Serum VDBP concentrations, as measured by its actin-free fraction, dropped after surgical interventions in patients evaluated by Reid et al (18) and Waldron et al (19). It is important to point out that inflammation has not the same effect on the two different forms of VDBP: it decreases the actin-free VDBP fraction, while increasing the actin-bound VDBP (24). In addition, plasma albumin concentrations decreased significantly during SIR in patients in all five studies (16-19,21), which evaluated it. As more than 90 percent of circulating 25(OH)D in human serum is protein-bound, changes in the binding proteins and albumin can alter measured total 25(OH)D concentrations without influencing its free concentrations (25).

Vitamin D status and illness

Measurements of serum total 25(OH)D concentration are the best way to assess vitamin D status in the general population; however, it is possible that they do not accurately indicate the vitamin D status during illness. Furthermore, free 25(OH)D is the physiologically important fraction in regard to target tissue. As free 25(OH)D is technically difficult to measure (25), none of the reviewed studies have done it. Reid et al have estimated it through an equation (18); however, that method has not been validated for acutely ill patients.

It is remarkable that, despite the significant drop in total and in calculated free 25(OH)D, there was no change in parathyroid hormone levels, an indirect indicator of vitamin D status, in one of the studies which evaluated it (18). Moreover, in a group of medical inpatients with low 25(OH)D levels, secondary hyperparathyroidism was less intense in hypoalbuminemic patients, suggesting that free serum 25(OH)D levels were less altered (26). Similarly, another steroid hormone, cortisol, has been shown to be decreased in patients with hypoproteinemia, when total levels were measured, although adrenal function was normal, as measured by free cortisol levels (27). These findings suggest that, like other hormones that circulate bound to proteins, fluctuations in binding proteins promote changes on total 25(OH)D concentrations, without changing its free levels.

SUMMARY

This systematic review summarizes results of longitudinal studies that assessed 25(OH)D levels during acute inflammatory processes and offers useful information for clinical practice. A large majority of the reviewed studies was prospective and designed in an orderly manner to assess the 25 (OH)D performance during an acute inflammatory reaction, and most of them demonstrated a rapid decrease in serum 25(OH)D levels after an inflammatory insult. Nevertheless, the studies have some clinical heterogeneity that must be considered: serum 25(OH)D was measured at different moments, the acute inflammatory response was due to several causes, and in one study patients were submitted to hemodilution due to cardiopulmonary bypass. Selection bias concerning the fact that the authors assessed only studies written in English, Spanish and Portuguese may have affected this review.

MISSING KNOWLEDGE AND FUTURE RESEARCH

Serum 25(OH)D levels decreased during the acute-phase response in most of the reviewed studies. The fundamental question about the metabolic meaning and the functional importance of 25(OH)D changes in the context of an inflammatory state remains open. In light of the current evidence, biochemical 25(OH)D measurements performed during the acute-phase response should be interpreted with care. Future researches, including other markers of vitamin D adequacy, would help to clarify if hypovitaminosis D might be the cause or the consequence of inflammatory diseases.

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Figure 1. Process of study selection for the systematic review. 25(OH)D: 25-hydroxyvitamin D

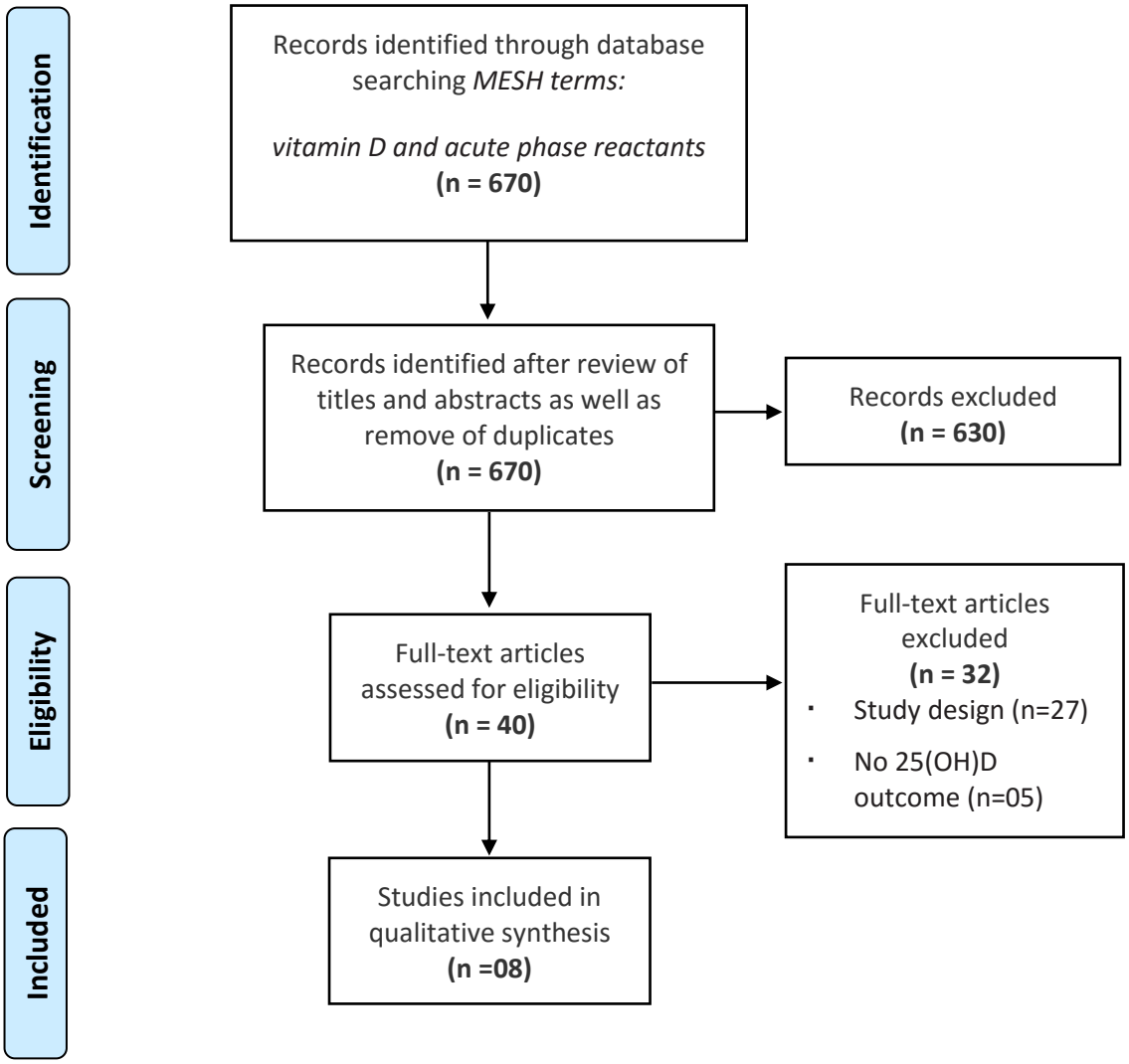

Table 1: Cohort Studies (n=8) which evaluated 25-hydroxyvitamin D levels and acute inflammation.

Author, year	Source	n	Insult	Significant changes	25(OH)D assay
Waldron, 2013 [21]*	J Clin Pathol	30	Knee / hip arthroplasty	↓25(OH)D ↑CRP ↓VDBP ↓albumin	LC-MS/MS
Barth, 2012 [24]**	Ann Clin Biochem	32	AMI treated successfully with PPCI	25(OH)D = ↑CRP	LC-MS/MS
Bang, 2011 [23]***	Endocr Res	73	Acute pancreatitis	↓25(OH)D ↓albumin	HPLC
Reid, 2011 [20]*	Am J Clin Nutr	33	Knee arthroplasty	↓25(OH)D ↑CRP ↓VDBP ↓albumin	LC-MS/MS
Bertoldo, 2010 [22]*§	J Bone Miner Res	90	1º dose IV bisphosphonate	↓25(OH)D ↑CRP	DiaSorin Liaison
Krishnan, 2010 [18]*	Crit Care	19	Cardiopulmonary bypass	↑ volume ↓25(OH)D ↓CRP ↓albumin	LC-MS/MS
Newens, 2006 [25]‖	Trans Soc Trop Med Hyg	14	Malarial infection	25(OH)D =	DiaSorin RIA
Louw, 1992 [19]*	Crit Care Med	12♂ 14♀	Orthopaedic surgery	↓25(OH)D ↑CRP ↓albumin	HPLC

25(OH)D: 25-hydroxyvitamin D (ng/mL); CRP: C-reactive protein (mg/L). Baseline levels were measured: *before the procedure; **2 days after symptoms onset; ***43% <24 hours, 14% 24-48 hours, and 43% >48 hours after symptoms onset; 16 patients were excluded due to 25(OH)D < 4 ng/mL, and 8 were excluded for other reasons. §Data are shown for the 19 patients with 25(OH)D levels ≥30ng/mL at baseline, who had acute inflammatory reaction after zoledronic acid infusion. ‖Few days after symptoms onset14 of the original 49 patients had follow-up data collected. ♂, ♀: male and female subjects.

Table 2. 25-hydroxyvitamin D levels and acute inflammatory insult.

Author, year		**Baseline**	**5min**	**6-12h**	**24h**	**2d**	**3d**	**5-14d**	**90d**
Waldron, 2013*	25(OH)D CRP	22.5 ±12.1 5 ±5.5				18.4 ±11¶ 116 ±81.2¶			
Barth, 2012**	25(OH)D CRP					18.4(10.6/25.4) 22		≈18 ≈6	≈18 ≈ 0
Bang, 2011***	25(OH)D	15.6 ±7.2			14.9 ±6	14.5 ±6.4¶		15.5±7.6	
Reid, 2011*	25(OH)D CRP	16 (4.8-49.7) 2.8 (0.6-9.3)		10.4(3.2-37.2)¶	9.2 (3.2-38)¶ 56 (5-105)¶	9.2 (3.2-31.6)¶ 164 (74-298)¶	10 (3-28)¶ 189(61-296)¶	11.6(3.2-30.5)¶ 113(25-234) ¶	12 (3.2-31.6)¶ 3.2 (0.4-10)
Bertoldo, 2010*§	25(OH)D	42.8 ±8.1					27.2 ±1.7¶		
Krishnan, 2010*	25(OH)D CRP	23.6 ±6.4 6 ±9	15.2 ±5.6¶ 4 ±5		≈22 82 ±40¶			≈24.8 134 ±58¶	
Newens, 2006‖	25(OH)D	25.6 (23/40.2)				25.2 (19.2/32.1)			
Louw, 1992*	25(OH)D ♂ ♀ CRP ♂ ♀	 13.6 ±1.1 14.7 ±1.4 ≈0 ≈0		 ≈12.4 ≈11.6¶ ≈35¶ ≈13	 ≈10.9¶ ≈12.4 ≈125¶ ≈70¶	 12.3±1.3 12.1±1.1¶ ≈170¶ peak ≈110¶ peak	 ≈12.0 ≈12.9¶ ≈85¶ ≈80¶	 13.9 ±1.6 12.6 ±1.0¶ ≈65¶ ≈0	

25(OH)D: 25-hydroxyvitamin D (ng/mL); CRP: C-reactive protein (mg/L). Data are shown as mean ± standard deviation, median (range), or median (P25/P75).
≈ Approximately data, estimated from graphs. ¶ Significantly different from baseline levels: $p<0.05$ or lower. Baseline levels obtained: *Before the procedure; ** 48 hours after symptoms onset; *** 43% >48 hours, 14% 24-48 hours, and 43%<24 hours of symptoms onset; 16 patients were excluded due to 25(OH)D <4ng/mL and 8 were excluded for other reasons. §Data are shown for the 19 patients with 25(OH)levels ≥30ng/mL at baseline, who had acute inflammatory reaction after zoledronic acid infusion.‖Few days after symptoms onset. ♂,♀: male and female subjects.SI conversion factors: To convert 25(OH)D to mmol/L, multiply values by 2.496.

9- ARTIGO 3

Título do manuscrito: Impact of a cholesterol membrane transporter´s inhibition on vitamin D absorption: A double-blind randomized placebo-controlled study

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Situação: Publicado

ABSTRACT

Oral supplements are important to prevent and treat vitamin D deficiency. Despite the growing number of prescriptions, vitamin D`s absorptive mechanisms are not clearly elucidated. By evaluating the effect of ezetimibe on vitamin D absorption, we aim to determine if the cholesterol transporter Niemann-Pick C1-Like 1 transporter contributes to it. This randomized, double-blind, placebo-controlled trial (*ClinicalTrials.gov NCT02234544)* was developed in a South Brazilian University Hospital. Fifty-one medical students were randomized to ezetimibe 10 mg/day or placebo for 5 days. On the fifth and 19th days, blood samples for 25-hydroxycholecalciferol (25OHD), parathyroid hormone (PTH), calcium, and albumin were collected. After the first blood sample collection, all participants received a single oral 50,000 IU cholecalciferol dose during a 15g-fat meal. Serum 25OHD levels were measured by the immunoassay Diasorin Liaison®. Measurements were compared in a general linear model adjusted for multiple comparisons by the Bonferroni test. Before cholecalciferol administration, 25OHD was < 30 ng/mL and < 20 ng/mL, respectively, in all and in 82.3% of the participants. Fourteen days after a single 50,000 IU oral dose of cholecalciferol, mean (SD) changes in serum 25OHD were similar in both groups, after adjustment to BMI and 25OHD levels before cholecalciferol administration (p=0.26): 8.7 (3.7) ng/mL in the ezetimibe group, *versus* 10.0 (3.8) ng/mL in the placebo group. Mean serum 25OHD, PTH, calcium and albumin levels remained similar in both groups. We conclude that ezetimibe had no effect on the mean change in serum 25OHD after a single oral dose of cholecalciferol, in these healthy and young adults.

Key words: vitamin D, 25-hydroxycholecalciferol, absorption, membrane transport proteins, ezetimibe

INTRODUCTION

Vitamin D is essential for human health (1,2), so the high prevalence of its deficiency has become a big concern in the modern world (3-5). Despite oral vitamin D supplements are frequently employed to treat it (6-9), mechanisms of vitamin D absorption remain poorly understood (10). It has being attributed to a passive process (11), but as vitamin D is also absorbed with fat-free food, membrane transporters could be involved (12-15). Moreover, it is possible that vitamin D and cholesterol share the same transporters, since they are structurally similar.

Ezetimibe is a pharmacological inhibitor of the uptake of dietary and biliary cholesterol, which acts through inhibition of Niemann-Pick C1-Like 1 transporter (NPC1L1) in the enterocyte and liver (16). Researchers have shown in rodents and *in vitro* that the absorption of vitamin D decreased by ezetimibe and increased with the overexpression of NPC1L1, scavenger receptor class B type I (SR-BI) and cluster of differentiation 36 (CD36) cholesterol transporters (15).

Identifying vitamin D membrane transporters and inter-individual differences in their expression is a great challenge. So, the aim of this study was to evaluate the effect of ezetimibe on vitamin D absorption in young healthy people.

MATERIALS AND METHODS

Study design

This randomized, double-blind, placebo-controlled clinical trial was developed during the spring (October 2014) in a South Brazilian University Hospital (30°S latitude).

Participants

All medical students and residents working in the hospital were able to participate. The exclusion criteria were body mass index (BMI), calculated by weight in kilograms (Kg) divided by height in meters squared, >25.0 or <18.5 kg/m^2; age <18 years old or >40 years old; known malabsorptive, liver, kidney or endocrine disease; current use of orlistat, barbiturates, carboxamides, GABA analogs, fatty acids anticonvulsants, glucocorticoids, calcium or vitamin D supplements; travel outside the Brazilian south region during the previous 120 days. Skin phototype was evaluated according to Fitzpatrick (17).

Study intervention

Participants were randomized to receive 10 mg ezetimibe or placebo daily for five days. On the fifth day, an oral 50,000 IU cholecalciferol capsule was administrated to each

subject concurrently with a 15g fat-containing meal (cream-crackers, cheese, chocolate and orange juice: 392 Kcal). The first and last capsules of ezetimibe or placebo and the cholecalciferol capsule were taken under supervision. All participants received the interventions on the same days and were instructed to not consume any food or drink in the two hours after cholecalciferol administration, and to avoid changes in their usual sun exposure and eating pattern for the next two weeks.

Drugs

Tablets containing 10 mg of ezetimibe were fabricated by Schering-Plough Products (Las Piedras, Puerto Rico, USA). Ezetimibe tablets were coated with two-pieces gel capsules by a pharmacist. Placebo capsules, containing lactose and microcrystalline cellulose, were similar to ezetimibe capsules in taste, color and size, and were prepared by the same pharmacist. Cholecalciferol was fabricated by DSM Nutritional Products Ltd (Kaiseraugst, Switzerland) and commercialized in Brazil by Mantecorp Farmasa (Taquara, RJ, Brazil). Pills counts were performed for the remaining ezetimibe or placebo capsules.

Laboratory testing

Blood samples for 25-hydroxycholecalciferol (25OHD), parathyroid hormone (PTH), total calcium and albumin were collected after overnight fast in two periods of the study: immediately before the cholecalciferol administration and 14 days after that. Serum was kept at – 80 °C until the assays. Serum total 25OHD and PTH levels were measured, respectively, by chemiluminescence (LIAISON, DiaSorin Inc., Stillwater, MN, USA, Dynamic range: 4.0 – 150 ng/mL, intra-assay and inter-assay coefficient of variation of 1.62% and 5.61%, respectively) and electrochemioluminescence (Roche Diagnostics, Indianapolis, IN, USA, intra-assay and inter-assay coefficient of variation of 1.01% and 6.45%, respectively), all other biochemical measurements were made by routine methods. All parameters were measured in two runs, with the samples from each participant in the same run. To convert 25OHD test results in ng/mL to nmol/L, multiply by 2.5.

Outcomes

Primary outcome was the mean change in serum 25OHD levels 14 days after oral cholecalciferol in participants taking ezetimibe or placebo. Secondary outcomes were serum PTH and total calcium levels.

Sample size

The number of subjects was estimated to be 23 for each group, to detect a difference of 5 ng/mL in the mean change of serum 25OHD levels between ezetimibe and placebo

groups after intervention, with standard deviation of 6 ng/mL, power of 80%, p <0.05, two-tailed test (18). Five additional subjects were included in each group to allow for losses.

Randomization, blinding, and formation of study groups

Fifty-six subjects were paired by sex, BMI and age, in two groups, which were assigned to placebo or ezetimibe using the sides of a coin (heads - control, tails - treatment). The content of the capsules was disclosed to the research team after the statistical analyses; assignment groups were unknown to the researchers who collected the samples, as well as to the technicians responsible for biochemical measurements. Subjects of both groups received 01 capsule containing 50,000 IU of cholecalciferol.

Statistical methods

Normality of variables distribution was determined by One-Sample Kolmogorov–Smirnov test. Continuous variables were compared using the Student's t test. Correlations between numerical variables were evaluated by the Pearson correlation coefficient. Categorical variables were compared by the chi-square test. Mean serum 25OHD levels and mean change in serum 25OHD levels were compared by ANCOVA, in a general linear model, with BMI and serum 25OHD levels before cholecalciferol administration as covariates. Pairwise comparisons were adjusted for multiple comparisons by Bonferroni test and p value of less than 0.05 was considered statistically significant. The SPSS software, version 18.0, was used to perform all tests (SPSS, Chicago, IL, USA).

Ethical aspects

The study was approved by the Ethics Committee of HCPA (GPPG 14-0378), and participants were included after written informed consent. This study conformed to “Principles for Protecting Integrity in the Conduct and Reporting of Clinical Trials”, published by the American Association of Medical College, and the World Medical Association Declaration of Helsinki. It was registered at ClinicalTrials.gov with the identifier NCT02234544.

RESULTS

Baseline characteristics of participants and trial structure

Sixty residents and medical students were invited to participate, and fifty-nine agreed. Three were excluded due to travel outside the southern Brazilian region in the last 120 days. Four did not come for the first appointment, so were excluded before any intervention (Figure 1). Baseline characteristics are shown in Table 1. Before cholecalciferol administration, serum

25OHD levels were below 30 ng/mL in all 55 participants, of which 36 had serum 25OHD levels between 10 and 20 ng/mL, and in 6 it was below 10 ng/mL. PTH levels ranged from 10.2 to 61.5 pg/mL, and one participant had low serum total calcium and high normal range serum PTH levels, respectively, 8.3 mg/dL (8.8-10.2 mg/dL), and 61.5 pg/mL (10.0-65.0 pg/mL). Serum albumin levels were in the normal range in all. No participant smoked. Six female participants were taking oral contraceptives in the ezetimibe group and seven in the placebo group.

Follow-up

Only one participant in the placebo group forgot to take a capsule. One participant in the placebo group threw up one hour after taking the cholecalciferol capsule and was excluded from the analyses. Two participants in the ezetimibe group reported headache. The incidence of diarrhea was equal in both groups. No other adverse drug reactions were reported.

Outcomes

After 14 days, a single oral 50,000 IU dose of cholecalciferol increased mean serum 25OHD levels in both groups. When associated with ezetimibe, the mean change in serum 25OHD levels was 8.7 ± 3.7 ng/mL (*SEM* 0.76; 95% CI 7.2-10.3), *versus* 10.0 ± 3.8 ng/mL in the placebo group (*SEM* 0.72; 95% CI 8.5-11.4); no statistical significance was found after adjustment to BMI and serum 25OHD levels before cholecalciferol administration, p =0.26. At day 14, mean serum 25OHD and PTH levels remained similar in both groups, p =0.39 and 0.73, respectively. No participant developed hypercalcemia. These results are shown in Table 2.

DISCUSSION

In these healthy and young volunteers, ezetimibe consumption for 5 days did not significantly affect cholecalciferol absorption, when compared with placebo. Absorption was estimated by serum 25OHD 14 days after a single oral 50,000IU cholecalciferol dose taken with a 15g fat meal. Results were adjusted for BMI and serum 25OHD levels before cholecalciferol administration, which have been demonstrated to predict the supplementation response (19).

The study was conducted during spring in the southern hemisphere, when 25OHD levels are likely to have the lowest values in this area (20). All participants had insufficient serum levels of vitamin D and among them, 82.3% had vitamin D deficiency (8). Despite that,

in all serum PTH levels were within the normal range, with wide ranging from 10.2 to 61.5 pg/mL for 25OHD levels lower than 20 ng/mL.

The mean serum 25OHD levels increase, observed 14 days after a single oral dose of cholecalciferol taken with a 15g fat meal, is consistent with other studies of our group (14,18). Although serum 25OHD measurements could be less sensitive to estimate variations on vitamin D absorption than serum cholecalciferol measurements (21), this metabolite of vitamin D has been shown to increase rapidly after vitamin D supplementation, and it has been used to evaluate vitamin D status as well as the effect of vitamin D supplementation (22-24).

Membrane transporters could be involved in fat-soluble vitamins intestinal absorption since cholesterol and other fat-soluble molecules share a number of transport mechanisms (25). Ezetimibe is a potent and selective inhibitor of NPC1L1, the main intestinal cholesterol transporter (16). Following oral administration, ezetimibe is rapidly absorbed and extensively metabolized (>80%) to the pharmacologically active ezetimibe-glucuronide, total concentrations reach a maximum 1-2 hours post-administration, followed by enterohepatic recycling and slow elimination (26).

Absorption of vitamin D at dietary amounts decreased after ezetimibe use in experiments with rodents and *in vitro* (15). In this randomized, double-blind, placebo-controlled trial, despite a good drug adherence during more than five half-lives of ezetimibe, the same effect was not replicated with a single 50,000 IU cholecalciferol dose.

We cannot know whether similar results would have been observed had we used smaller daily doses of vitamin D3. Nevertheless, a prior study also demonstrated no difference in the serum concentrations of lipid-soluble vitamins in patients with primary hypercholesterolemia after 12 weeks of ezetimibe 10 mg/day (27), which suggests that ezetimibe does not impair dietary vitamin D absorption.

Although the interest in the effects of ezetimibe on serum 25OHD levels in humans has been rising lately, data concerning this topic remains scarce. A recent study with hypercholesterolemic patients showed that the administration of simvastatin/ezetimibe 10/10 mg was associated with less than one half of the increase in 25OHD levels seen with the simvastatin 40 mg, for similar low-density lipoprotein cholesterol lowering (28). Since the mechanisms which mediate statins raise in 25OHD levels are not clearly elucidated, the amount of simvastatin administered was lower in the association of simvastatin/ezetimibe and there was no group receiving monotherapy with ezetimibe, it is not possible to determine if

those results could be attributed to a dose dependent effect of simvastatin or to a decline of vitamin D intestinal absorption by ezetimibe.

The present clinical trial was carefully designed to avoid main factors known to influence 25OHD levels (body weight, calcium and vitamin D supplementation, seasonality, comorbidities, drugs) (29,30), even though less recognized factors could have interfered. Genetic polymorphisms could also be associated with differences in vitamin D supplemental response (29).

Ezetimibe is the only cholesterol transporter inhibitor currently available for clinical use (16), so we could not evaluate other cholesterol transporters, particularly SR-BI and CD 36.

Finally, it is important to point out the lower mean change in serum 25OHD levels in the ezetimibe group, so a significant decrease in vitamin D absorption could have not been identified in this group due to sample size.

In conclusion, ezetimibe for 5 days did not significantly affect cholecalciferol absorption, as measured by serum 25OHD, when compared with placebo, in young and healthy volunteers. Thus NPC1L1 seems not to be a major vitamin D transporter in this group of individuals. The high prevalence of vitamin D deficiency demands improvements in supplementation strategies, so understanding the mechanisms involved in vitamin D absorption remains a challenge.

Acknowledgments

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Figure 1. Study flow diagram

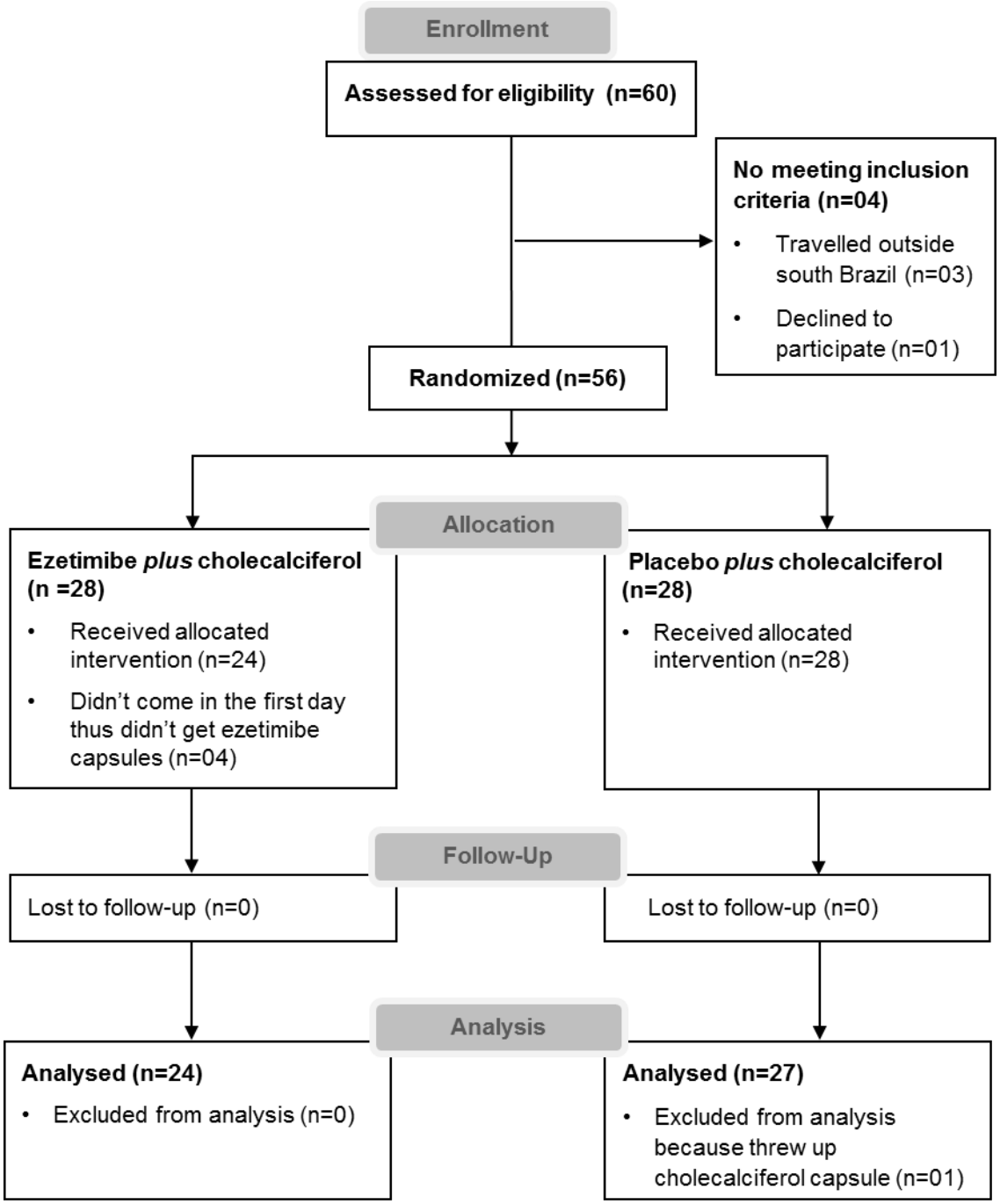

Table 1. Baseline Characteristics of the 51 Participants

Characteristics	Ezetimibe	Placebo
Participants, *n*	**24**	**27**
Sex, *n*		
Male	**10**	**11**
Female	**14**	**16**
Fitzpatrick, *n*		
1 and 2	**16**	**12**
3 and 4	**8**	**15**
Age, years	**27.8 ± 3.4**	**27.2 ± 2.7**
Weight, kg	**65.4 ± 9.3**	**66.1 ± 10.7**
BMI, kg/m^2	**22.3 ± 1.7**	**22.5 ± 1.9**
25OHD, ng/mL	**15.9 ± 5.6**	**14.5 ± 4.3**
Calcium, mg/dL	**9.4 ± 0.4**	**9.4 ± 0.5**
Albumin, g/dL	**4.5 ± 0.3**	**4.6 ± 0.3**
PTH, pg/mL	**31.3 ± 14.7**	**33.1 ± 12.8**

Values are shown as mean ± SD or *(n)*.
BMI: body mass index; 25OHD: 25-hydroxyvitamin D; PTH: parathyroid hormone.

Table 2. Biochemical responses 14 days after 50,000 IU oral dose of vitamin D3

Measure	**Ezetimibe**	**Placebo**	***p***
25OHD, ng/mL	24.7 ± 5.2	24.5 ± 6.2	0.39
Δ25OHD, ng/mL	8.7 ± 3.7	10.0 ± 3.8	0.26
Calcium, mg/dL	9.3 ± 0.4	9.4 ± 0.4	0.47
Albumin, g/dL	4.5 ± 0.3	4.6 ± 0.3	0.36
PTH, pg/mL	33.5 ± 14.4	34.9 ± 12.8	0.73

25OHD: 25-hydroxyvitamin D; **Δ:** delta/variation; PTH: parathyroid hormone.

10- CONSIDERAÇÕES FINAIS

Com base nos resultados obtidos em revisão sistemática da literatura, entende-se que a vitamina D é absorvida através de dois diferentes mecanismos prováveis:

1) Difusão passiva, otimizada pela ingestão de gorduras;

2) Transporte ativo, que envolve proteínas de membrana e explicam a absorção da vitamina D mesmo em jejum.

É possível que proteínas transportadoras do colesterol estejam envolvidas neste processo, visto que fatores que interferem na absorção do colesterol também interferem na absorção da vitamina D.

O ensaio clínico apresentado foi o primeiro estudo delineado com o propósito de avaliar o impacto de um transportador de membrana do colesterol na absorção da vitamina D. Dessa forma, fatores que poderiam interferir nos resultados foram potencialmente controlados (IMC, estação do ano, doenças, medicamentos). O NPC1L1, apesar de ser o principal transportador do colesterol, não parece, portanto, desempenhar um importante papel na absorção da vitamina D em seres humanos jovens e saudáveis.

Fatores pouco conhecidos podem, contudo, ter interferido nos resultados, por exemplo, não se sabe se o status vitamínico basal dos participantes, a dose de vitamina D oferecida ou se polimorfismos genéticos relacionados ao metabolismo da vitamina D possam ter influenciado os resultados. Além disso, o ezetimibe é o único transportador do colesterol disponível para uso clínico no momento, e outros transportadores do colesterol não foram avaliados. Espera-se, portanto, que mais estudos sobre a absorção e a biodisponibilidade da vitamina D sejam desenvolvidos.

11- PERSPECTIVAS FUTURAS

O interesse sobre a absorção intestinal da vitamina D cresceu nos últimos anos. A participação de proteínas de membrana na absorção da vitamina D foi demonstrada em estudos recentes, contudo pesquisas permanecem escassas. É possível que variabilidade interindividual na expressão e no desempenho de proteínas transportadoras determinem maior vulnerabilidade à deficiência de vitamina D e influenciem a resposta à suplementação. A avaliação de polimorfismos genéticos de transportadores de membrana do colesterol pode ser uma forma promissora de avaliar a participação de outros transportadores na absorção da vitamina D. Espera-se, portanto, que mais estudos sejam realizados com o propósito de avaliar os mecanismos celulares envolvidos na absorção da vitamina D e de transpor estes conhecimentos para a prática clínica. A identificação de proteínas de membrana envolvidas no seu transporte poderá contribuir para a prevenção e tratamento da deficiência de vitamina D.

ANEXOS

ANEXO 1. Instrumento de coleta de dados.

ESTUDO CLÍNICO RANDOMIZADO, CONTROLADO E DUPLO-CEGO DO IMPACTO DE UM TRANSPORTADOR DE MEMBRANA DO COLESTEROL NA ABSORÇÃO DE UMA VITAMINA

Dados de identificação e contato	
Nome:	nome
Número do prontuário:	num_pront
Data da avaliação: ____/_____/_____	data_aval
Data de nascimento: _____/_____/_____	data_nasc
Telefone residencial: (____)	tel_resid
Telefone celular: (____)	tel_celular
Outro telefone de contato: (____)	tel_outro
E-mail de contato:	email

Características sócias demográficas	
Idade: _____ anos	idade
Sexo: Feminino Masculino	sexo
Fototipos – Classificação de Fitzpatrick: Branca – Sempre queima – Nunca bronzeia Branca – Sempre queima – Bronzeia muito pouco Morena clara – Queima (moderadamente)– Bronzeia (moderadamente) Morena moderada – Queima (pouco) – Sempre bronzeia Morena escura – Queima (raramente) – Sempre bronzeia Negra – Nunca queima – Totalmente pigmentada	fototipo

Comorbidades ***Já teve diagnóstico de uma das condições abaixo ou fez cirurgia do aparelho digestivo:***				
Doença hepática crônica	(0) Não	(1) Sim	(9) IGN	hepat
Insuficiência renal	(0) Não	(1) Sim	(9) IGN	insuf_renal
Doença Endocrinológica	(0) Não	(1) Sim	(9) IGN	endocr
Tuberculose	(0) Não	(1) Sim	(9) IGN	tuberc
Cirurgia do aparelho digestivo	Não	Sim	(9) IGN	cirurg

Medicações				
O senhor faz uso regular de alguma das medicações abaixo?				
Diuréticos	(0) Não	(1) Sim	(9) IGN	diuret
Cálcio	(0) Não	(1) Sim	(9) IGN	calcio
Vitamina D	(0) Não	(1) Sim	(9) IGN	vitamd
Polivitamínicos	(0) Não	(1) Sim	(9) IGN	polivit
Glicocorticóides sistêmicos	(0) Não	(1) Sim	(9) IGN	cortic
Anticonvulsivantes	(0) Não	(1) Sim	(9) IGN	anticonv
Orlistat	(0) Não	(1) Sim	(9) IGN	orlistat
Laxantes	(0) Não	(1) Sim	(9) IGN	laxantes
Remédio para colesterol alto	(0) Não	(1) Sim	(9) IGN	hipolip
Quais outras medicações o senhor fez uso regular nos últimos 30 dias (anotar os nomes):				
				med1
				med2
				med3

Viagem - O Sr (a) viajou para fora da região sul do Brasil nos últimos 120 dias?

Não Sim	viagem

Dados de antropometria	
Peso (kg):	peso
Altura (cm):	altura
IMC:	IMC

Critérios de inclusão			
Incluído no estudo?	(0) Não	(1) Sim	incluido
Motivo da exclusão: IMC <18,5 Kg/m2 ou >25 Kg/m2 Doença hepática, renal ou endocrinológica previamente diagnosticada Suplementos com cálcio e/ou vitamina D Uso de anticonvulsivantes, barbitúricos ou glicocorticoides Viagem para fora da região sul do Brasil nos últimos 120 dias Recusa			motivo_excl

Randomização	
Grupo A Grupo B	grupo

Quantidade de comprimidos perdidos, esquecidos ou não tomados	
nenhum um dois três quatro todos	compr
Se não tomou, o motivo foi:	
Esquecimento ou perda Efeito adverso. Qual_____	

Exames Laboratoriais 5º Dia		
Data da coleta:_____/_____/_____		data_coleta
Anotar ao menos duas casas decimais quando for o caso		
Creatinina		ex_crea
Uréia		ex_ureia
PTH		ex_pth
25(OH)D		ex_vitad
Cálcio total		ex_calciot
Albumina		ex_albumina
Exames Laboratoriais 19º Dia		
Data da coleta:_____/_____/_____		data_coleta
Anotar ao menos duas casas decimais quando for o caso		
Creatinina		ex_crea
Uréia		ex_ureia
PTH		ex_pth
25(OH)D		ex_vitad
Cálcio total		ex_calciot
Albumina		ex_albumina

ANEXO 2. Termo de Consentimento Livre e Esclarecido**.**

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Você está sendo convidado(a) a participar de um projeto de pesquisa: **Estudo clínico randomizado, controlado e duplo-cego do impacto de um transportador de membrana do colesterol na absorção de uma vitamina***. Este projeto tem o objetivo de identificar dados da avaliação médica, nutricional ou de exames laboratoriais que possam nos ajudar a identificar os mecanismos de absorção intestinal da vitamina em estudo. Os resultados desta pesquisa poderão aprimorar a prevenção e o tratamento de hipovitaminose.

Caso concorde em participar deste estudo, você deverá comparecer ao Centro de Pesquisa Clínica no HCPA no início da manhã em 03 dias de semana previamente agendados (1º, 6º e 19º dias da pesquisa). No primeiro dia você será entrevistado, seu peso e sua altura serão medidos. Após randomização (sorteio), você receberá 05 cápsulas contendo a medicação em estudo ou placebo e será orientado a tomar por via oral uma cápsula ao dia, pela manhã, durante 5 dias. No quinto dia, você deverá comparecer à unidade de pesquisa pela manhã, em jejum de 8 horas, para coleta de sangue por punção de veia periférica; com posterior ingestão de 01 cápsula de suplemento vitamínico e 01 cápsula da medicação em estudo ou placebo, acompanhados de lanche. Você será orientado a não alterar hábitos cotidianos por duas semanas (alimentação, exposição solar, exercício) e a retornar no 19º dia para nova coleta de sangue de veia periférica pela manhã após jejum de 8 horas. Deverá trazer o frasco de medicação ou placebo, para contagem das cápsulas remanescentes.

Você receberá uma ligação telefônica e/ou SMS durante os primeiros 5 dias da pesquisa para confirmar a ingestão da medicação ou placebo.

O material coletado será destinado aos exames da pesquisa (como vitaminas, eletrólitos e albumina) que serão realizados no laboratório do HCPA. As informações obtidas serão analisadas em conjunto com outros participantes, não sendo divulgada a identificação de nenhum deles.

Durante a pesquisa, nenhum dos envolvidos, inclusive participantes, pesquisadores e funcionários do laboratório, terão conhecimento de qual grupo (medicação ou placebo) cada indivíduo irá participar; da mesma forma que ninguém poderá escolher a qual grupo pertencer.

Os riscos são pequenos e associados às coletas de sangue venoso (dor no local da punção e/ou formação de hematoma local) e ao uso da medicação em estudo (raramente podem ocorrer sintomas gripais, fadiga, diarréia, dores de cabeça, articulares ou musculares, infecção respiratória ou alergia a algum componente da fórmula).

A participação no estudo é voluntária e é garantida a liberdade da não participação ou da desistência em qualquer momento após ingressar no estudo; sem prejuízo da avaliação profissional, da avaliação curricular ou do vínculo institucional.

Não há despesas pessoais para o participante em qualquer fase do estudo, incluindo exames e consultas. Também não há benefício direto para o participante, nem compensação financeira relacionada à sua participação. Se existir qualquer despesa adicional, ela será absorvida pelo orçamento da pesquisa.

Você tem o direito de ser mantido atualizado sobre os resultados parciais das pesquisas, assim que os mesmos forem de conhecimento dos pesquisadores.

Em qualquer etapa do estudo, você terá acesso aos profissionais responsáveis pela pesquisa para esclarecimento de eventuais dúvidas. A pesquisadora responsável é a Dra. Tania Weber Furlanetto (Serviço de Medicina Interna do HCPA – ramal: 8152; e-mail: taniafurlanetto@gmail.com). Outros investigadores envolvidos são a Dra. Mariana Costa Silva, a e o Dr. Gustavo Adolfo Moreira Faulhaber (mariana.endocrino@gmail.com e gamf@globo.com). Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, o Comitê de Ética em Pesquisa poderá ser contatado das 8 às 17h através do telefone (51)33597640.Este documento é elaborado em duas vias, sendo uma delas entregue ao participante e outra mantida pelo grupo de pesquisadores.

*CAAE**:** 24813913.1.0000.5327 (identifica o projeto na Plataforma Brasil)

Nome do participante: _____ Assinatura:_____

Nome do pesquisador:_____ Assinatura:_____

Local e Data:_____

ANEXO 3. Orçamento do estudo.

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

GPPG - GRUPO DE PESQUISA E PÓS GRADUAÇÃO

SERVIÇO DE GESTÃO EM PESQUISA

SAIR | IMPRIMIR | MODELOS DE ORÇAMENTO | LIMPAR

ORÇAMENTO WEB V.2

Data	sábado, 25 de junho de 2016

Estudo clínico randomizado, controlado e duplo-cego do impacto da inibição de um transportador de membrana do colesterol na absorção da vitamina D.

Financiado por:	**FIPE///PESQUISADOR/**	
N (Número de Sujeitos de Pesquisa)	**48**	

Base de Dados

Item	Código	Financiador	Quantidade	V.U	Valor Total
Cópias Xerográficas no HCPA		FIPE	200	R$ 0,15	R$ 30,00
Papel A4 - pacote com 500 folhas	191663	FIPE	1	R$ 10,00	R$ 10,00
SUBTOTAL (Base de Dados)					R$ 40,00

Exames Clínicos - Sangue

Exames		Financiador	Quantidade	V.U	Valor Total
Albumina		FIPE	96	R$ 1,65	R$ 158,40
Cálcio Total		FIPE	96	R$ 1,85	R$ 177,60
Paratormônio		FIPE	96	R$ 43,13	R$ 4.140,48
Coleta de Sangue (Exames Séricos)		FIPE	96	R$ 5,00	R$ 480,00
SUBTOTAL (Exames)					R$ 4.956,48

Outros Materiais

Ítem		Financiador	Quantidade	V.U	Valor Total
25-hidroxivitamina D		FIPE	96	R$ 22,62	R$ 2.171,52
Ezetimibe 10 mg- 30cps		FIPE	4	R$ 35,00	R$ 140,00
Placebo		PESQUISADOR	120	R$ 1,00	R$ 120,00
Lanche		PESQUISADOR	48	R$ 6,00	R$ 288,00
SUBTOTAL (Outros materiais)					R$ 2.719,52

TOTAL DO PROJETO	R$ 7.716,00
FIPE	R$ 7.308,00
PESQUISADOR	R$ 408,00

ANEXO 4. *Guideline* CONSORT para ensaios clínicos e estudos experimentais preenchido com páginas correspondentes ao **Artigo 3.**

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	338
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	338
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	338
	2b	Specific objectives or hypotheses	338
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	339
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	339
	4b	Settings and locations where the data were collected	339
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	339
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	340

	6b	Any changes to trial outcomes after the trial commenced, with reasons	340
Sample size	7a	How sample size was determined	340
	7b	When applicable, explanation of any interim analyses and stopping guidelines	340
Randomisation:			
uence generation	8a	Method used to generate the random allocation sequence	339
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	339
cation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	339
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	339
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	339
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	339
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	339
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	340
	13b	For each group, losses and exclusions after randomisation, together with reasons	340
Recruitment	14a	Dates defining the periods of recruitment and follow-up	340

	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	340
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	340
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	340
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	340
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	340
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	341
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	341
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	341
Other information			
Registration	23	Registration number and name of trial registry	339
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	341

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.