**Universidade de São Paulo
Faculdade de Saúde Pública**

**Concentrações séricas de 25(OH)D e perfil metabólico
mediados pela adiposidade**

Wyslenny Nascimento de Souza

Tese apresentada ao Programa de Pós-
Graduação em Nutrição em Saúde
Pública para a obtenção do título de
Doutor em Ciências

Área de Concentração: Nutrição em
Saúde Pública

Orientadora: Prof^a. Assoc. Ligia Araujo
Martini

Co-orientadora: Prof^a. Dr^a. Marcela González-
Gross

**São Paulo
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(versão revisada)

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*"Sonhos determinam o que você quer.
Ação determina o que você conquista"*

Edwaldo Novak

RESUMO

Souza, WN. **Concentrações séricas de 25(OH)D e perfil metabólico mediados pela adiposidade** [tese de doutorado]. São Paulo: Faculdade de Saúde Pública da Universidade de São Paulo; 2016

Introdução: Baixas concentrações séricas de hidroxivitamina D (25[OH]D) e o excesso de peso atingiram níveis epidêmicos em todo o mundo. Estudos relatam que concentrações séricas de vitamina D estão associadas às alterações lipídicas, glicolíticas e inflamatórias; e estas alterações são conhecidamente mediadas pela adiposidade. Dessa forma, a vitamina D pode atuar de forma benéfica sobre o perfil metabólico em adolescentes, adultos e idosos.

Objetivo: Investigar e descrever as associações entre as concentrações séricas de 25(OH)D e o perfil metabólico, mediadas pela adiposidade em adolescentes, adultos e idosos.

Metodologia: Indivíduos de três estudos foram incluídos nesta tese. Uma subamostra do Inquérito de Saúde de São Paulo (ISA-Capital), estudo transversal, de base populacional (n=281), foi utilizada para investigar a associação entre as concentrações séricas de vitamina D e marcadores inflamatórios em adultos brasileiros. Também foram utilizados dados do estudo *Healthy Lifestyle in Europe by Nutrition in Adolescents*-(HELENA), estudo multicêntrico transversal da população de adolescentes europeia (n= 1006), com o intuito de avaliar as alterações nos marcadores lipídicos e de homeostase da glicose mediados pela deficiência de vitamina D e obesidade. Finalmente, foi analisada a amostra do estudo PHYSMED, estudo transversal com idosos não institucionalizados (n= 383), para verificar associações entre concentrações séricas de vitamina D, perfil lipídico e composição corporal em idosos espanhóis aparentemente saudáveis. **Resultados:** Nos adultos (> 19 e < 60 anos), observou-se uma associação negativa entre as concentrações de TNF-alfa e de IL-6 e as concentrações séricas de 25(OH)D em indivíduos com peso normal.

Nos adolescentes, as concentrações de 25(OH)D foram associadas de forma independente e positiva com o *Quantitative Insulin Sensitivity Check Index*-QUICKI ($p < 0.001$) e negativamente associada com o IMC ($p < 0.05$). Também foi observado que o aumento do IMC esteve associado com um aumento de 1.93 vezes maior chance de deficiência de vitamina D (IC de 95% = 1.03 - 3.62; $p = 0.04$). Em idosos, verificou-se que as concentrações séricas de 25(OH)D foram associadas negativamente com o IMC ($p = 0.04$), com a circunferência da cintura ($p = 0.004$), com o CT/HDL-c ($p = 0.026$) e com o HDL-c ($p = 0.001$). Adicionalmente, foi observado que idosos com concentrações de HDL-c < 40 mg/dl possuíam 1.7 vezes maior chance de apresentarem deficiência de vitamina D em comparação com aqueles que possuíam concentrações de HDL-c > 40 mg/dl (95% IC = 1.10 a 2.85; $p = 0.017$) e o aumento na circunferência da cintura também foi associado com um maior risco de deficiência de vitamina D (95% IC = 0.96-1.00; $p = 0.04$). **Conclusão:** A composição corporal interage com as concentrações de 25(OH)D modulando a resposta inflamatória, à homeostase da glicose e também o perfil lipídico. Indivíduos sem deficiência de vitamina D apresentam melhor perfil metabólico e também melhor composição corporal, sugerindo que a suficiência de vitamina D pode ter um papel importante nas condições metabólicas mediadas pela adiposidade.

Descritores: vitamina D, excesso de peso, resposta inflamatória, perfil metabólico.

Abstract

Souza, WN. **Serum 25 (OH) D and metabolic profile mediated by adiposity** [thesis]. Sao Paulo: Public Health School the University of São Paulo; 2016

Introduction: Low serum of hydroxyvitamin D (25 [OH] D) and excess weight reached epidemic levels in worldwide. Studies have reported that vitamin D serum concentrations are associated with lipid, glycolytic and inflammatory alterations; and these alterations are known to be mediated by adiposity. Thus, vitamin D may have a benefic action on the metabolic profile in adolescents, adults and elderly. **Objective:** To investigate and describe the associations between 25(OH)D concentrations and the metabolic profile mediated by adiposity in adolescents, adults and elderly. **Methods:** Subjects from three studies were included in this thesis. A subsample from the Health Survey of São Paulo (HS-SP), cross-sectional, population-based study (n = 281), to investigate the association between vitamin D concentrations and inflammatory biomarkers in Brazilian adults. Data from Healthy Lifestyle in Europe by Nutrition in Adolescents study - (HELENA), cross-sectional and multicenter study of European adolescents (n= 1006), in order to evaluate the alterations in lipid markers and glucose homeostasis mediated by vitamin D deficiency and obesity. Finally, the sample from the PHYSMED study, a cross-sectional study with non-institutionalized elderly (n= 393), was analysed to examine associations between vitamin D concentration, lipid profile and body composition in apparently healthy elderly Spanish. **Results:** In adults, a negative association was observed between the concentrations of TNF-alpha and IL-6 and serum 25(OH) D in normal weight subjects. In adolescents, the 25(OH) D concentration was associated positive and independently with QUICKI (p <0.001) and negatively associated with BMI (p <0.05).

It was also observed that increasing BMI was associated with an increase of 1.93 times odds of vitamin D deficiency (95% CI = 1.3 - 3.62; $p = 0.04$). In the elderly, it was found that serum of 25(OH) D was negatively associated with BMI ($p = 0.04$), waist circumference ($p = 0.004$), TC/HDL-c ratio ($p = 0.026$) and HDL -c ($p = 0.001$). Additionally, it was observed that elderly patients with HDL-c <40mg/dl had 1.7 times odds to develop vitamin D deficiency compared to those who had concentrations of HDL-c > 40 mg / dl (95% CI = 1.10 to 2.85 ; $p = 0.017$) and increases in waist circumference were also associated with an increased risk of vitamin D deficiency (95% CI = 0.96-1.00; $P = 0.04$). **Conclusion:** Body composition interacts with 25(OH) D concentrations modulating the inflammatory response, glucose homeostasis and also the lipid profile. Individuals without vitamin D deficiency have better metabolic profile and better body composition, suggesting that vitamin D sufficiency may have an important role in the metabolic conditions mediated by adiposity.

Descriptors: Vitamin D, overweight, inflammatory response, metabolic profile

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LISTA DE ABREVIATURAS

25(OH)D – 25-Hidrovitamina D / 25-Hydrovitamin D

ApoA-I – Apolipoproteína AI / Apolipoprotein AI

ApoB – Apolipoproteína B / Apolipoprotein B

CC/WC – Circunferência da Cintura / Waist Circumference

CETP – Proteína de Transferência do Estér de Colesterol

CMO/ BMC- Conteúdo mineral ósseo / bone mineral content

CT/ TC – Colesterol Total / Total Cholesterol

DM2 – Diabetes Mellitus do Tipo 2

HDL – Lipoproteína de Alta densidade / High Density Lipoprotein

HELENA – Healthy Lifestyle In Europe By Nutrition In Adolescents

HOMA-IR – Modelo de Avaliação da Homeostase da Resistência à Insulina / Homeostatic Model Assessment Insulin Resistance

IL – Interleucina / Interleukin

IMC/BMI – Índice de Massa Corporal / Body Mass Index

ISA – Inquérito de Saúde de São Paulo

LDL – Lipoproteína de Baixa densidade / Low Density Lipoprotein

LPL – Lipase Lipoprotéica

LPS – Lipopolissacarídeo

MAPK – Proteína Quinase Ativada Por Mitógenos / Mitogen-Activated Protein Kinases

MCP-1 – Proteína Quimiotática de Monócitos-1 / Monocyte Chemoattractant Protein- 1

MLTPAQ – Minnesota Leisure Time Physical Activity Questionnaire

MVPA – Atividade Física Moderada à Intensa / Moderate-to-vigorous Physical Activity

NF-kB – Fator Nuclear-kB / Nuclear Factor- kB

OMS/WHO – Organização Mundial da Saúde / World Health Organization

PCR/CRP – Proteína C-Reativa / C-Reactive Protein

PREDIMED – Effects of The Mediterranean Diet on The Primary Prevention of Cardiovascular Diseases

PTH – Paratormônio / Parathyroid Hormone

QUICKI – Índice de Verificação Quantitativa a Sensibilidade à Insulina / Quantitative Insulin Sensitivity Check Index

sICAM-1 – Molécula Solúvel de Adesão Intercelular 1 / Soluble Intercellular Adhesion Molecule-1

sVCAM-1 – Molécula Solúvel de Adesão Vascular-1/ Soluble Vascular Cell Adhesion Molecule-1

TG – Triglicerídeos / Triglycerides

TH – T auxiliares / T-Helper

TNF- α – Fator de Necrose Tumoral-alpha

UVB – Radiação Ultravioleta B

VDR – Receptor de Vitamina D / Vitamin D Receptor

VLDL – Lipoproteína de Muito Baixa Densidade / Very Low Density Lipoprotein

APRESENTAÇÃO

Esta tese é composta por um capítulo introdutório onde é apresentada a epidemiologia da obesidade e da deficiência de vitamina D, assim como suas conseqüências no perfil metabólico. Também é composta por justificativa, objetivos e uma breve seção de métodos. A amostragem, critérios específicos de inclusão e exclusão e a análise dos dados foram descritos em cada um dos três artigos que compõem a seção de resultados e discussão. Finalmente, são apresentadas as considerações finais, as referências bibliográficas (com exceção daquelas apenas citadas nos artigos) e os anexos.

Os artigos que compõem esta tese foram redigidos no idioma e formato exigidos pelo periódico científico ao qual foram ou serão submetidos. Este formato de apresentação de tese corresponde e atende às recomendações do Guia de Apresentação de Teses da Faculdade de Saúde Pública da Universidade de São Paulo.

1 . INTRODUÇÃO

1. 1 OBESIDADE E INSUFICIÊNCIA DE VITAMINA D: RELEVÂNCIA PARA SAÚDE PÚBLICA

O excesso de peso é um crescente problema de Saúde Pública ao redor do mundo. Segundo a Organização Mundial de Saúde (OMS), em 2014, mais de 1,9 bilhões de adultos (≥ 18 anos) estavam acima do peso, correspondendo a 39% da população adulta mundial; desses, mais de 600 milhões (13%) eram obesos. A prevalência do excesso de peso está em ascensão em países de média e baixa renda, especialmente em zonas urbanas. O aumento da prevalência do excesso de peso também é verificado na infância, e nos países subdesenvolvidos essa frequência tem sido cerca de 30% maior que nos países desenvolvidos (OMS, 2015). No Brasil, na última década, a prevalência do excesso de peso apresentou um crescimento de 23% e, em 2014, 52,2% da população adulta brasileira encontrava-se com sobrepeso (VIGITEL, 2014).

O tecido adiposo é notadamente reconhecido como um órgão endócrino ativo, cuja principal função é armazenar energia corporal sob a forma de triglicerídeos (KERSHAW e FLIER, 2004). Esse tecido contém células como adipócitos, pré-adipócitos, células do sistema imunológico e células endoteliais. Ele também é caracterizado por sua função endócrina (leptina, estrogênios, adiponectina), autócrina/parácrina como as citocinas (fator de necrose tumoral- α [TNF- α] e interleucina-6 [IL-6]) e proteínas envolvidas no metabolismo lipídico (lipase lipoprotéica-LPL, proteína de transferência do estér de colesterol-CETP).

Essas características conferem a este tecido importante função metabólica e inflamatória (HALBERG et al., 2008; JUNG e CHOI, 2014).

Estudos confirmaram que a gordura visceral/abdominal tem maior atividade lipolítica e lipogênica com reduzida resposta à sinalização da insulina em comparação a gordura subcutânea (PATEL e ABATE, 2013; ADIELS et al., 2008). Os ácidos graxos livres, produto da lipólise, podem entrar diretamente no fígado via circulação portal, ocasionando aumento na síntese de lipídeos, na gliconeogênese e na resistência à insulina. Estes fatores resultam em hiperlipidemia e intolerância à glicose, pois o excesso de ácidos graxos livres pode induzir resistência periférica à insulina através da inibição da captação pelo músculo esquelético (MATSUZAWA, 1995). Além disso, ácidos graxos livres estimulam a produção de citocinas, (SUGANAMI et al., 2005) modulando a inflamação no tecido adiposo, contribuindo também para complicações metabólicas associadas ao excesso de peso. No excesso de peso, o tecido adiposo sofre hipertrofia, resultando no desequilíbrio do fluxo sanguíneo, levando à hipóxia, inflamação e infiltração de macrófagos. (TRAYHURN, 2013). O aumento na secreção de interleucinas 6 e 8 (IL-6, IL-8), resistina, TNF- α é uma das características notáveis em adipócitos hipertrofiados, bem como a secreção reduzida de adiponectina (WELLEN e HOTAMISLIGIL, 2003), **Figura 1**.

Concomitantemente ao aumento da prevalência mundial do excesso de peso e da obesidade está o aumento da deficiência de vitamina D, definida como concentrações séricas de 25-hidroxivitamina D [25(OH)D], <20 ng/mL (50 nmol/L). Estima-se que mais de um bilhão de pessoas ao redor do mundo apresentem deficiência ou insuficiência (20-29 ng/mL; 50-74.9 nmol/L) de vitamina D (HOLICK, 2010; BISCHOFF-FERRARI et al., 2006).

Estudos relataram que 50 a 100% dos idosos (homens e mulheres) nos Estados Unidos e na Europa apresentam concentrações séricas deficientes de 25(OH)D (HOLICK, 2007; HOSSEIN-NEZHAD e HOLICK, 2012).

No Brasil, aproximadamente 60% dos adolescentes saudáveis apresentam valores insuficientes de 25(OH)D (PETERS et al., 2009) e 58% dos idosos não-institucionalizados moradores de São Paulo apresentam deficiência (LOPES et al. 2014). O mesmo é observado na população adulta brasileira, onde a média das concentrações séricas de 25(OH)D é insuficientes em homens e mulheres 48.4/51 nmol/L, respectivamente (MARTINI et al., 2013). Estas evidências sugerem que a deficiência desta vitamina, assim como a obesidade, é uma pandemia; sendo considerada um problema mundial de saúde pública (HOLICK, 2012; HOSSEIN-NEZHAD e HOLICK, 2013).

Figura 1. Tecido adiposo hipertrofiado. Fonte: Adaptado de De Souza e Martini, 2015. (autorização concedida)

1.2 AÇÃO DA VITAMINA D NO TECIDO ADIPOSEO

A função clássica estabelecida e mais elucidada da vitamina D refere-se à manutenção da homeostase do cálcio no metabolismo ósseo (SUDA et al., 2003; LEWIS et al., 2010). Concentrações deficientes de 25(OH)D, biomarcador da vitamina D, podem acarretar osteoporose e a osteomalácia em adultos, e o raquitismo em crianças. Nos últimos anos, o excesso de peso, em especial a obesidade, tem sido associada com frequência a deficiência de vitamina D (KAIDAR-PERSON et al., 2008; De SOUZA e MARTINI, 2015). Todavia, os mecanismos relativos a essa associação não são totalmente esclarecidos e sua causalidade ainda é controversa. Seria a deficiência de vitamina D um agente significativo na obesidade ou a obesidade seria determinante no déficit de vitamina D?

Estudos demonstraram que enzimas importantes no metabolismo da vitamina D, tais como a 24 e 25-hidroxilase, a 1 α -hidroxilase e a 1,25 dihidroxivitamina (OH) D são expressas no tecido adiposo (TRAYHURN et al., 2011; WAMBERG et al., 2013), sendo esse um dos fatores pelo qual a vitamina D pode atuar neste tecido. WAMBERG et al. (2013) demonstrou que ocorre diminuição na expressão do gene que codifica 25-hidroxilase (CYP2R1) e a 1 α -hidroxilase (CYP27B1) no tecido adiposo subcutâneo em indivíduos obesos. Por outro lado, este mesmo estudo observou aumento na expressão do gene que cataboliza a 24-hidroxilase (CYP24A1) vitamina D após a perda de peso, sugerindo que o tecido adiposo tem a capacidade de metabolizar a vitamina D e essa pode ser alterada dinamicamente durante a perda de peso e também na obesidade. Além disso, o receptor de vitamina D (VDR) igualmente atua de forma ativa no tecido adiposo. Estudos relataram que o VDR é expresso tanto no tecido adiposo humano (DING et al., 2013) quanto em cultura de células adiposas (TRAYHURN et al., 2011).

Alterações no VDR têm sido correlacionadas com o desenvolvimento e predisposição à obesidade em diversas fases da vida (SANTOS et al., 2012; BINH et al., 2011; YE et al., 2001). De fato, VASILOPOULOS et al. (2013) e FILUS et al. (2008) verificaram que polimorfismos no gene do VDR (Talq, Apal e Bsml) foram associados com o excesso de peso e a obesidade em adultos.

Adicionalmente, estudos observacionais (SANEI et al., 2013) e clínicos (PATHAK et al., 2014) relataram associação inversa entre concentrações séricas de 25(OH)D e medidas de adiposidade, como índice de massa corporal (IMC), circunferência da cintura e gordura corporal. Ademais, a deficiência de vitamina D foi relacionada com maior adiposidade visceral e tamanho dos adipócitos em mulheres (CARON-JOBIN et al., 2011). A origem das baixas concentrações de 25(OH)D tipicamente observadas em indivíduos obesos, ainda não é totalmente compreendida. No entanto, até o momento, as principais hipóteses sugeridas são:

1-Indivíduos obesos estão propensos a menor síntese de vitamina D em comparação com a população em geral, devido à menor prática de atividade física ou de lazer ao ar livre e uso de vestuário com menor exposição cutânea. Esses hábitos contribuem para diminuição na exposição solar, refletindo na síntese de vitamina D (NAKAMURA et al., 2015). Foi demonstrado que o IMC, percentual de gordura corporal, e hábito de tomar sol foram correlacionados com as concentrações séricas de 25(OH) D em adultos (KULL et al., 2009). Todavia, níveis de atividade física ou de lazer ao ar livre e exposição solar raramente são especificados, por isso é difícil definir conclusivamente sobre o efeito posterior em relação às concentrações séricas da vitamina D.

2- O sequestro de vitamina D pelo tecido adiposo é outro fator que pode contribuir para baixas concentrações séricas de 25(OH)D em indivíduos com excesso de peso (WORTSMAN et al, 2000; BLUM et al 2008). Tem sido proposto que ocorrem maior captação e armazenamento de vitamina D, devido à característica lipossolúvel desta vitamina, pelo tecido adiposo de indivíduos obesos em relação a indivíduos não obesos (WORTSMAN et al, 2000). Nesse estudo, com 38 indivíduos expostos à mesma quantidade de radiação UVB, foi demonstrado diminuição nas concentrações de 25 (OH)D em obesos, quando comparado com eutróficos (20.0 ± 3.4 ng/mL *versus* 33.9 ± 4.1 ng/mL, $p= 0.017$). O aumento de 25(OH) D na corrente sanguínea foi 57% menor nos obesos, mesmo com ambos os grupos apresentando concentrações similares do precursor da vitamina D₃ (7-dehidrocolesterol) na pele. Este resultado sugere que a capacidade de síntese cutânea de vitamina D₃ não é prejudicada pelo excesso de peso, e, portanto, não ocorre redução na eficácia da radiação ultravioleta B (UVB) em obesos. Propondo a redução na biodisponibilidade de 25(OH) na circulação devido ao sequestro pelo tecido adiposo (WORTSMAN et al. 2000).

Essa é a hipótese mais aceita e relatada na literatura, entretanto, estudo com 17 indivíduos obesos, cujas concentrações de 25(OH)D foram mensuradas no tecido adiposo, relatou que as concentrações de vitamina D₃ (colecalfiferol) no tecido adiposo foram correlacionadas positivamente quanto às concentrações séricas de 25(OH)D (BLUM et al, 2008). Esse estudo parece questionar a teoria do sequestro de vitamina D, pois indica o tecido adiposo como local de armazenamento, porém, isso especificamente não implica no sequestro dessa vitamina (BLUM et al, 2008).

3- Existe ainda a hipótese de "diluição volumétrica", DRINCIC et al. (2012), avaliando 686 indivíduos aparentemente saudáveis, mostrou que toda a variabilidade nas concentrações séricas de 25 (OH)D em obesos pode ser atribuída, essencialmente, a diluição volumétrica.

Após o ajuste para composição corporal, a diferença entre as concentrações séricas de 25(OH)D entre obesos e não obesos desapareceu. Este estudo indica que o tratamento da deficiência de vitamina D em obesos deve ser baseado na composição corporal.

4- Outro mecanismo que pode explicar as baixas concentrações de vitamina D na obesidade é referente à principal organela na produção de energia: a mitocôndria. Foi demonstrado em adultos com deficiência de vitamina D que a suplementação com colecalciferol aumentou a fosforilação oxidativa mitocondrial, sugerindo melhorias na função do músculo esquelético tais como melhora nos sintomas da fadiga muscular (SINHA et al., 2013). Isto ocorre devido à diminuição na concentração de cálcio intracelular, causado pela deficiência de vitamina D, ocasionando diminuição na fosforilação oxidativa mitocondrial (SINHA et al., 2013; BOUILLON e VERSTUYF, 2013). Complementariamente, as duas enzimas responsáveis pela ativação e inativação de 25(OH)D, a 1α -hidroxilase e a 24,25-hidroxilase respectivamente, estão localizadas na mitocôndria (BOUILLON e VERSTUYF, 2013). Dessa forma, estas evidências sugerem a associação entre deficiência de vitamina D, disfunção muscular e aumento no risco para obesidade.

5- Finalmente, o excesso de tecido adiposo compromete as concentrações séricas de 25(OH)D a partir da leptina, devido à importância da leptina na homeostase energética torna-se necessário compreender sua correlação com a vitamina D. É reconhecido que concentrações séricas de leptina estão associadas positivamente com o teor de gordura corporal (SCHWARTZ et al., 2000). Consequentemente, concentrações séricas de vitamina D e leptina foram negativamente associadas em obesos (MAETANI et al., 2009; GRETHEN et al., 2012). Neste mecanismo, a leptina estimula o fator de crescimento de fibroblastos 23 (FGF23), por sua vez, o FGF23 inibe a síntese renal de 1α -hidroxilase, e, portanto, dificulta a produção de 1,25 (OH)D, a forma ativa da vitamina D (KONG et al., 2013; BOUILLON et al., 2014).

Entretanto, os dados que suportam este complexo mecanismo entre a leptina, a vitamina D e a adiposidade permanecem inconclusivos.

Evidências robustas e elucidadas para as hipóteses do sequestro e da diluição volumétrica, seja de forma independente ou associada, indicam que estas são as melhores hipóteses para explicar as baixas concentrações de vitamina D em indivíduos com excesso de peso ou obesidade. Contudo, apesar de vários estudos confirmarem a correlação entre a obesidade e a deficiência de vitamina D, apenas estas evidências não são suficientes para determinar que a deficiência de vitamina D é causada pelo excesso de peso.

A causalidade entre obesidade e deficiência de vitamina D foi melhor explicada em um estudo de randomização Mendeliana com 42.024 indivíduos. Este estudo, conduzido por VIMALESWARAN et al. (2013), constatou que a cada 10% de aumento no IMC resultou em uma diminuição de 4,2% nas concentrações séricas de 25(OH)D, porém, alterações nas concentrações séricas de 25(OH)D não modificaram significativamente o IMC. Esses pesquisadores concluíram que deficiência de vitamina D não ocasionou a progressão da obesidade, contudo, a obesidade foi um componente causal na progressão da deficiência de vitamina D.

De fato, deficiência de vitamina D e a obesidade atingiram níveis epidêmicos em todo o mundo e pesquisas abordando estas duas condições aumentaram ao longo dos últimos anos (POURSHAHIDI, 2015). Consonantemente, a vitamina D está associada às alterações lipídicas, glicolíticas e inflamatórias e todas estas alterações são conhecidamente mediadas pela adiposidade.

1.3 A IMPORTÂNCIA DA VITAMINA D NA HOMEOSTASE DA GLICOSE E PERFIL LIPÍDICO

Após a identificação da presença do VDR (JOHNSON et al., 1994) e da 1α -hidroxilase (BLAND et al., 2004) nas células β pancreáticas, o papel da vitamina D na homeostase da glicose e resistência à insulina tornou-se alvo de estudos (PEECHAKARA e PITTAS, 2008). Em animais, foi demonstrado que a vitamina D aumenta a secreção de insulina mediada pela glicose (CADE e NORMAN, 1986), enquanto que *in vitro*, a 25(OH)D foi correlacionada com o aumento da expressão do receptor de insulina e o aumento da insulina mediada pelo transporte de glicose (MAESTRO et al., 2000). A vitamina D também pode aumentar indiretamente a ação da insulina através da regulação extracelular de cálcio, pois aumenta o fluxo de cálcio através das membranas celulares, ocasionando uma adequada manutenção do cálcio citosólico intracelular nas células β (BLAND et al., 2004).

Em conformidade com estes primeiros resultados, estudos observacionais evidenciaram uma associação inversa entre concentrações séricas de 25(OH)D e glicose sérica, resistência à insulina e prevalência de *diabetes mellitus* do tipo 2 (DM2) em diferentes faixas etárias e etnias (SCRAGG et al., 2004; FOROUHI et al., 2008; CHUNG et al., 2014). KHAN et al. (2013), avaliando resultados de 17 estudos prospectivos com uma média de seguimento de 10 anos em adultos aparentemente saudáveis, evidenciou-se que concentrações séricas de 25(OH)D foram inversamente associadas com o risco futuro de DM2 e síndrome metabólica.

Sabe-se que estudos clínicos podem indicar uma relação de causalidade, entretanto, a causalidade entre a deficiência de vitamina D e a homeostase da glicose, como mostram algumas revisões e meta-análise, ainda não está bem estabelecida. MITRI et al., (2011) Em uma revisão sistemática de estudos observacionais e clínicos, avaliando a ingestão de vitamina D e concentrações séricas de 25(OH)D, notou-se que a ingestão de vitamina D₃ >500 unidades internacionais (UI) por dia diminuiu o risco de DM2 em 13% em comparação com a ingestão de vitamina D₃ < 200UI/dia. Os indivíduos com maiores concentrações de 25(OH)D (> 25ng/mL) tiveram um risco 43% menor de desenvolver diabetes tipo 2 em comparação com aqueles com menores concentrações (< 14ng/mL). Em adição, neste estudo também foi relatado que apenas indivíduos com intolerância a glicose apresentaram melhora na resistência à insulina. Por outro lado, uma revisão sistemática e meta-análise de estudos clínicos, avaliando o efeito da suplementação de vitamina D₃ em adultos com e sem alterações nos níveis séricos de glicose, revelou que a suplementação de vitamina D não apresentou efeito sobre a homeostase da glicose ou na prevenção da DM2 (SEIDA et al., 2014).

Efeitos benéficos sobre a secreção e sensibilidade à insulina influenciam indiretamente o metabolismo lipídico (JORDE e GRIMNES, 2011), assim, além da adiposidade, o metabolismo da glicose também interage com o perfil lipídico. Do mesmo modo, estudos têm demonstrado que concentrações séricas adequadas de vitamina D repercutem positivamente sobre o perfil lipídico (JORDE e GRIMNES, 2011; SUN et al., 2015). A vitamina D pode atuar diretamente sobre os lipídicos séricos, uma vez que a vitamina D é considerada essencial para a manutenção de níveis adequados de apolipoproteína AI (ApoA-I), componente principal do HDL-colesterol (CARBONE et al., 2008).

Em um estudo transversal com 170 adultos britânicos sul-asiáticos (69 homens e 101 mulheres), sem história de diabetes ou outras doenças crônicas, foi observado que a concentração sérica de 25(OH)D foi um preditor independente no aumento da ApoA-1 (JONH et al., 2005). Semelhantemente, foi reportado que adultos com deficiência de vitamina D apresentam diminuição nas concentrações de ApoA-1 (AUWERX et al., 1992). Outro mecanismo pelo qual a vitamina D pode influenciar indiretamente o perfil lipídico está relacionado à ação desta vitamina sobre o paratormônio (PTH), estudo *in vitro* demonstrou que a supressão do PTH, ocasionada por um aumento nas concentrações séricas de 25(OH)D, reduziu a lipólise (ZEMEL et al., 2000).

Metanálise com 22 estudos transversais e 10 estudos de intervenção para identificar os efeitos da vitamina D sobre o perfil lipídico revelou que nos estudos transversais as concentrações séricas de 25(OH)D foram positivamente associadas com o HDL-c, resultando em concentrações favoráveis de LDL-c ou colesterol total e negativamente correlacionadas com o triglicerídeos (TG). Em contrapartida, em estudos de intervenção, os efeitos da suplementação de vitamina D₃ sobre o perfil lipídico apresentaram resultados contraditórios (JORDE e GRIMNES, 2011).

Recentemente, estudos transversais demonstraram resultados benéficos da vitamina sobre o perfil lipídico em diferentes faixas etárias e populações (KELISHADI et al., 2014; YASEIN et al., 2015; VITEZOVA et al., 2015; SUN et al. 2015). Similar efeito foi observado em estudo longitudinal com 1762 indivíduos acompanhados por 14 anos, onde foi identificada associação negativa entre as concentrações séricas de 25(OH)D e TG. Neste estudo, o aumento das concentrações séricas de 25(OH)D, durante o período de seguimento, foi concomitantemente acompanhado por diminuição nas concentrações séricas de TG (JORDE et al., 2010). Dessa forma, é razoável concluir que concentrações séricas de 25(OH)D podem atuar de forma benéfica sobre o perfil lipídico.

1.4 O PAPEL DA VITAMINA D NA INFLAMAÇÃO

Na obesidade, o tecido adiposo sofre um alargamento hipertrófico, ocasionando desequilíbrio do fluxo sanguíneo, levando à hipóxia, infiltração de macrófagos e inflamação (TRAYHURN, 2013). Estudos relatam que a obesidade também está associada à resposta inflamatória crônica de baixo grau, caracterizada pela produção anormal de citocinas, proteínas de fase aguda e ativação de vias de inflamatórias de sinalização (HOTAMISLIGIL, 2006; WELLEN e HOTAMISLIGIL, 2003). O TNF- α , a IL-6 e a PCR são algumas das citocinas que estão alteradas na inflamação mediada pela obesidade (BERG e SCHERER, 2005). Esta resposta inflamatória, predominantemente desencadeada no tecido adiposo, determina relação de causalidade entre a obesidade e doenças correlacionadas, resistência à insulina, DM2 e alterações no metabolismo lipídico (HOTAMISLIGIL, 2006; NISHIMURA et al., 2009).

Neste contexto, os efeitos da vitamina D no sistema imunitário adaptativo podem ser observados através da diferenciação de células T auxiliares (TH), proteína quinase ativada por mitógenos (MAPK), bem como no fator nuclear-kB (NF-kB) (HIGGINS et al., 2013). Assim, a vitamina D suprime o efeito autoimune através da regulação da diferenciação e da atividade de células T auxiliares, resultando no equilíbrio entre TH1/TH2 que favorece a redução no desenvolvimento da autoimunidade (CANTORNA e MAHON, 2004).

Foi demonstrado que ambas as formas, 1,25(OH)D e 25(OH)D, podem reduzir o lipopolissacarídeo (LPS) que é induzido pela produção das citocinas TNF- α e IL-6, provavelmente por inibir a ativação da p38MAPK em monócitos humanos (ZHANG et al., 2012).

Outra via pela qual a vitamina D atua na inflamação é através da sinalização de NF- κ B, a ativação do NF- κ B é essencial para o sinal de transdução de citocinas pró-inflamatórias nos adipócitos. Com a degradação da proteína I κ Ba inicia-se a ativação de NF- κ B (GAO e BING, 2011; CHEN et al., 2011). A 1,25(OH) $_2$ D pode aumentar a I κ Ba em pré-adipócitos humanos, impedindo a ativação do NF- κ B (GAO et al., 2013), como representado na **Figura 2**.

Figura 2. Ação da 1,25 dihidroxivitamina D₃ na resposta inflamatória.

Fonte: Adaptado de De Souza e Martini, 2015. (autorização concedida)

Estudos transversais apontam que deficiência de vitamina D está associada com concentrações séricas elevadas de marcadores inflamatórios, tais como IL-6, TNF- α , e PCR, tanto em indivíduos saudáveis quanto em obesos (BELLIA et al., 2013; LAIRD et al., 2014;).

AMER e QAYYAM (2012), avaliando 15.167 indivíduos do *National Health and Nutrition Exame Survey* (NHANES), observaram que concentrações séricas de 25(OH) D <53nmol/L foram inversamente associadas com a PCR ($\beta = -0.34$; $p < 0.001$), porém o mesmo não foi observado naqueles com 25 (OH) D superior a 53nmol/L ($\beta = 0.05$; $p = 0.07$). Em adição, estudo com 147 obesos mórbidos, após controle por fatores de confusão como idade, sexo, estação do ano e medidas de adiposidade, demonstrou que concentrações séricas de 25(OH)D também foram inversamente correlacionadas com a PCR ($r = -0.31$; $p = 0.043$), IL-6 ($r = -0.50$; $p = 0.003$) e TNF- α ($r = -0.61$; $p = 0.001$) (BELLIA et al, 2013).

Em estudos clínicos, as evidências relacionadas aos efeitos benéficos da vitamina D sobre os marcadores inflamatórios ainda são divergentes. Em adultos saudáveis com sobrepeso ou obesidade, a suplementação oral de D₃ de 4.000UI/dia durante 12 semanas não apresentou efeito nos marcadores inflamatórios (CARRILO et al., 2012), similarmente ao observado por CHANDLER et al.(2014) em Afro-Americanos, ambos os estudos eram randomizados, duplo-cego e controlados. Contraditoriamente, outros estudos randomizados demonstraram que a suplementação de vitamina D₃ diminui significativamente os marcadores inflamatórios em mulheres saudáveis (BISCHOFF-FERRARI et al., 2012), obesos (BELENCHIA et al, 2013) e diabéticos (KAMPMANN et al., 2014).

Estes resultados contraditórios sugerem que o aumento nas concentrações séricas de 25(OH)D pode diminuir de forma modesta a maioria dos marcadores inflamatórios, todavia, a eficácia da vitamina D nesta redução parece depender principalmente da doença estudada, pois algumas doenças são mais inflamatórias como obesidade e DM2, e das concentrações iniciais de 25(OH)D (CANNELL et al., 2014).

Considerando a ação da vitamina D sobre a adiposidade, homeostase da glicose, perfil lipídico e na inflamação, torna-se importante ponderar com atenção a atuação simultânea desta vitamina sobre estas alterações metabólicas. Sobretudo, porque essas alterações metabólicas podem ocorrer de forma concomitante (JUNG e CHOI, 2014).

1.5 JUSTIFICATIVA

O componente adiposo está presente como principal fator nas alterações do perfil metabólico em qualquer faixa etária. Neste âmbito, a vitamina D pode ter uma ação direta e indireta sobre a adiposidade e, conseqüentemente, sobre alterações metabólicas mediadas pela adiposidade. De acordo com as lacunas e complexidade concernentes à ação da vitamina D sobre estas condições metabólicas, o presente estudo visa avançar no conhecimento da relação entre as associações existentes entre a vitamina D, homeostase da glicose, perfil lipídico e inflamação mediados pela adiposidade.

2. OBJETIVOS

2.1 OBJETIVO GERAL

- Investigar e descrever as associações entre as concentrações séricas de 25(OH)D e perfil metabólico, mediadas pela adiposidade.

2.2. OBJETIVOS ESPECÍFICOS

- **Artigo 1:** Investigar a associação entre as concentrações séricas de vitamina D e marcadores inflamatórios em adultos brasileiros.
- **Artigo 2:** Avaliar as alterações na homeostase da glicose e marcadores lipídicos mediados pela deficiência de vitamina D e obesidade em adolescentes
- **Artigo 3:** Investigar as associações entre concentrações séricas de vitamina D, perfil lipídico e composição corporal em idosos espanhóis aparentemente saudáveis.

3. METODOLOGIA

A presente tese é composta por três diferentes populações, tendo como eixo central as inter-relações entre a obesidade, perfil metabólico e a vitamina D. Considerando que são artigos independentes com base de dados, análises estatísticas e populações distintas, o detalhamento de cada um dos métodos, resultados e conclusões estão inseridos no texto dos respectivos artigos aos quais se referem.

Uma breve descrição de cada estudo e suas respectivas populações encontra-se abaixo:

1. ISA - Capital 2008

O Inquérito de Saúde do município de São Paulo (ISA - Capital) é um estudo transversal com amostra representativa, tendo a participação de 3271 indivíduos. No ISA - Capital, a coleta de dados foi realizada em domicílio com entrevistadores treinados entre 2008-2009, com a coleta de dados dietéticos, amostra de sangue, pressão arterial e antropometria. Também foram coletados dados demográficos, socioeconômicos, estilo de vida (tabagismo, atividade física, etilismo), morbidade referida e dados de ingestão (CASTRO et al., 2009; SELEM et al., 2014).

No ISA – Capital, 2008, os domicílios foram selecionados aleatoriamente dentro de setores censitários, de modo a permitir a coleta de dados geral a partir de cerca de 1.661 homens e mulheres com idade superior a 12 anos.

Destes, 750 indivíduos participaram da coleta sanguínea e tiveram sua pressão arterial e medidas antropométricas igualmente coletadas (CASTRO et al., 2009).

No presente estudo (artigo 1), todos os indivíduos que tiveram uma amostra de sangue coletada e com idade >19 e <60 anos ($n= 301$) foram incluídos. Indivíduos em uso de suplementação de vitamina D, medicamentos anti-inflamatórios, os inibidores de absorção de gordura ou terapia antiretroviral foram excluídos do estudo ($n = 20$). Dessa forma, a amostragem final foi de 281 indivíduos.

Os questionários utilizados podem ser encontrados no Manual de avaliação do consumo alimentar em estudos populacionais: a experiência do inquérito de saúde em São Paulo (ISA) (UNIVERSIDADE DE SÃO PAULO, 2012)

2- HELENA Study

O estudo *Healthy Lifestyle in Europe by Nutrition Adolescence-* (HELENA) é caracterizado por ser um estudo multicêntrico transversal com o objetivo de obter dados confiáveis e comparáveis de uma amostra aleatória de 3.000 adolescentes europeus com idade entre 12.5 – 17.5 anos. Amplos dados de nutrição e parâmetros relacionados com a saúde foram coletados: ingestão alimentar, o conhecimento de nutrição e atitudes alimentares, escolhas e preferências alimentares, composição corporal, lipídios plasmáticos e perfil metabólico, estado nutricional relativos às vitaminas, função imunológica relacionada com o estado nutricional, atividade e aptidão física e genótipo (DE HENAUW et al, 2007; GONZÁLEZ-GROSS et al, 2008).

Os adolescentes participantes do estudo HELENA foram recrutados de forma estratificada, aleatória e com seleção de amostragem por agrupamento. Os critérios para seleção da cidade incluíam o equilíbrio geográfico e a presença de um experiente grupo de pesquisa. Os critérios de exclusão foram limitados a adolescentes que não eram capazes de falar a língua local, não estavam na faixa etária entre 12.5-17.5 anos, estavam participando simultaneamente em outro ensaio clínico e aqueles que haviam sofrido de infecção aguda 1 semana antes da visita.

Dessa forma, para o estudo apresentado nesta tese (artigo 2), uma subamostra de adolescentes foi utilizada (n= 1006). Esta subamostra corresponde aos selecionados para coleta de sangue em 10 cidades dos 9 países europeus participantes do HELENA: Atenas (Grécia), Dortmund (Alemanha), Ghent (Bélgica), Heraklion (Grécia), Lille (França), Pecs (Hungria), Roma (Itália), Estocolmo (Suécia), Viena (Áustria) e Zaragoza (Espanha).

Os questionários utilizados no estudo HELENA podem ser encontrados no Manual Operativo do estudo Helena (GONZÁLEZ-GROSS ET AL., 2013).

3. PHYSMED

O PHYSMED é um estudo multicêntrico transversal com o objetivo de avaliar a atividade física, o estado nutricional e a composição corporal em idosos não-institucionalizados de duas cidades Espanholas: Madrid e Palma de Mallorca.

No PHYSMED, foram recrutados por amostragem aleatória 433 indivíduos (57% mulheres) com idade acima dos 55 anos, não-institucionalizados, com ausência de doença física ou mental que poderiam limitar a participação nos testes de aptidão física ou a

capacidade de responder os questionários, com ausência de alcoolismo crônico ou dependência de drogas e sem ingestão de medicamentos provenientes de pesquisa clínica por pelo menos 1 ano.

Para o 3º artigo, foram selecionados os indivíduos com dados completos (n= 383) de concentração sérica de vitamina D (25 [OH] D), perfil lipídico, composição corporal, ingestão de vitamina D, atividade física e atividade física ao ar livre e ingestão alcoólica.

O recordatório 24 horas utilizado e os questionários de atividade física validados para idosos espanhóis encontram-se nos **ANEXO 1, 2 e 3**, respectivamente.

4. ASPECTOS ÉTICOS

As três pesquisas das quais decorrem os artigos apresentados nesta tese foram aprovadas pelos Comitês de Ética de cada Universidade a qual se derivam. Também foram observados os princípios da Declaração de Helsinki (HELENA STUDY e PHYSMED), assim como as exigências da resolução nº 196, de 10 de outubro, de 1996, do Conselho Nacional de Saúde (ISA - Capital-2008), **Anexos 4, 5 e 6**.

5. RESULTADOS E DISCUSSÃO

5.1 PRIMEIRO ARTIGO

Association between 25-hydroxyvitamin D and inflammatory biomarker levels in a cross-sectional population-based study, São Paulo, Brazil.

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Abstract

Besides the classic vitamin D function on bone homeostasis, there are evidences showing that adequate status of vitamin D can modulate inflammation. We hypothesized that higher plasma levels of 25 hydroxyvitamin D (25[OH]D) would correlate with lower plasma levels of pro-inflammatory cytokines, acute phase proteins and soluble adhesion molecules and higher plasma levels of anti-inflammatory cytokines. We included all adults (20y – 59y) of the population-based, cross-sectional study, Health Survey – São Paulo (HS-SP), conducted in São Paulo (Brazil) in the study (n=281). Anthropometric parameters, blood pressure measurements and a fasting blood sample were collected by trained fieldworkers. Serum 25(OH)D concentration, plasma inflammatory biomarker levels (C-reactive protein [CRP], interleukin [IL]-1 β , IL-6, IL-8, IL-10, tumor necrosis factor [TNF]- α , IL-12p70, adiponectin, monocyte chemoattractant protein [MCP]-1, soluble intercellular adhesion molecule [sICAM]-1 and soluble vascular cell adhesion molecule [sVCAM]-1), and plasma blood lipid parameters were evaluated. The prevalence of vitamin D inadequacy (<50nmol/L) was 65.5%. Inadequate subjects were younger, with lower BMI, systolic (SBP) and diastolic (DBP) blood pressures, triglyceride and total cholesterol levels as well as low density lipoprotein cholesterol (LDL-c), compared to individuals adequate for vitamin D status. After adjustment, plasma concentration of sICAM-1 was statistically higher among adequate subjects. Stratifying for BMI categories, a negative association was observed between plasma IL-6 and TNF- α levels and serum 25(OH)D concentration in normal weight subjects, while a negative association was detected between plasma adiponectin level and serum 25(OH)D concentration in overweight subjects.

The present findings suggest that BMI interacts with serum 25(OH)D levels, modulating inflammatory response and affecting plasma IL-6, TNF- α and adiponectin levels. These data indicate that BMI plays a determinant role in the vitamin D-inflammation axis.

Keywords: 25(OH)D, inflammatory biomarkers, sICAM-1, BMI and vitamin D status.

Introduction

Vitamin D insufficiency is a worldwide problem, observed both in countries with temperate climates and in equatorial countries [1]. Besides vitamin D classical role on bone homeostasis, observational data support an association between poor vitamin D status and chronic diseases such as diabetes, autoimmune disease and cardiovascular disease, all of which involve immune or inflammatory disfunctions [2].

In this sense, it has been postulated that vitamin D receptor (VDR) is expressed by macrophages and dendritic cells and inducible expressed by lymphocytes following activation, indicating an important role of vitamin D in the modulation of immune and inflammatory responses [3]. Moreover, the immune system is able to produce the enzyme 25-hydroxyvitamin D₃ 1- α -hydroxylase (CYP27B1), which converts 25-hydroxyvitamin D (25(OH)D) into the active form of vitamin D₃, 1,25-dihydroxyvitamin D [1,25(OH)₂D] [4]. The 1,25(OH)₂D reduces gene expression of proinflammatory mediators, such as TNF- α , IL-6 and monocyte chemoattractant protein (MCP)-1, since 1,25(OH)₂D leads to a reduction in nuclear translocation of nuclear factor- κ B (NF- κ B), in addition to the suppression of Toll like receptors (TLR)2 and TLR4 proteins and mRNA expression [5,6,7].

Besides vitamin D deficiency, obesity plays a central role in the development of several chronic diseases associated with a metabolic inflammatory state, termed metaflammation and defined as low-grade chronic inflammation, orchestrated by metabolic cells in response to excess nutrients and energy. The expansion of adipocytes or neighboring preadipocytes, caused by excess consumption of nutrients, triggers secretion of proinflammatory and chemotactic cytokines in this tissue [8, 9], leading to infiltration of a large number of immune cells, which display a proinflammatory state of activation, such as

M1 macrophages, mast cells, natural killer cells, $CD4^+$ T_H1 , $CD8^+$ effector T cells and B cells. These cells activate proinflammatory pathways and the secretion of elevated levels of proinflammatory cytokines, leading to systemic inflammation [9]. Also, this state of subclinical inflammation is characterized by a reduction in the concentration of anti-inflammatory markers, such as adiponectin [10].

In addition, several studies [11-13] and a meta-analysis [14] have demonstrated that the 25(OH)D concentration, a marker of vitamin D status, is inversely associated with body mass index (BMI) and adiposity, the reasons for which remain the subject of debate but may be multifactorial. Some of the hypotheses proposed include the sequestration of vitamin D_3 by adipocyte [15], lower intake of vitamin D from food and reduced exposure to sunlight [16].

Considering the possible role of vitamin D on the modulation of inflammation, we hypothesized that higher plasma levels of 25(OH)D would correlate with lower plasma levels of pro-inflammatory cytokines, acute phase proteins and soluble adhesion molecules and higher plasma levels of anti-inflammatory cytokines. Since vitamin D deficiency has become a public health problem, as have chronic diseases, the aim of this study was to investigate the association between eleven inflammatory biomarkers and 25(OH)D concentrations in a subsample of the cross-sectional population-based survey, taking into account the influence of BMI in this association.

Methods and materials

Subject and study design

Data was derived from the population-based survey Health Survey—São Paulo (HS-SP), a cross-sectional study of health and living conditions among a representative sample of individuals living in São Paulo, southeastern Brazil, in 2008. To compose the HS-SP sample, a two-stage cluster sampling of census tracts and households was performed. In the first stage, 70 census tracts were randomly selected from the totality of urban census tracts in the city of São Paulo, as the primary sampling unit. In the second stage, households were randomly selected within census tracts, in order to allow general data collection from about 1661 men and women aged more than 12 years [17,18]. Of this group, 750 individuals donated a blood sample and had their blood pressure and anthropometric measurements collected.

For the present study, all individuals that had a blood sample collected, and who aged >19 y and <60 y ($n=301$) were included. Minimum sample size of 300 was calculated based on prevalence of 0.5 with a sample error of 0.07 at a 5% significance level and a design effect of 1.5. Individuals in use of vitamin D supplementation, anti-inflammatory medication, fat absorption inhibitors or antiretroviral therapy were excluded from the study ($n=20$) - **Figure 1** shows the subject selection for the study. The study protocol (number 2001) was reviewed and approved by the Ethics Committee of the School of Public Health, University of São Paulo. All individuals signed the informed consent form.

Data collection and processing

Information on demographics and socioeconomic characteristics was obtained using structured questionnaires, carried out between 2008 and 2009, during the first home visit by trained fieldworkers. Participants were classified according to smoking habits (current smoker or non-smoker), skin color (white, black, “pardo” [brown], yellow or indigenous), practice of moderate physical activities (yes or no question) and alcohol consumption (current drinker or never drink).

Body weight, height and waist circumference, along with systolic and diastolic blood pressures were measured on the second home visit, carried out 2010 and 2011, by trained research assistants following standardized procedures. Body weight was measured using a set of automatic scales and waist circumference was measured at the midpoint between the iliac crest and the rib cage. Height was measured using a fixed stadiometer, with head, shoulders, buttocks and heels pressed back against the wall. BMI was calculated as $\text{weight (Kg)}/\text{height}^2 (\text{m}^2)$. Blood pressure was determined as the mean of 2 consecutive measurements, on the right arm, taken with an automatic device (Omron model HEM-712C, Omron Health Care, Inc, USA).

Figure 1- Flowchart of the sample selection for the study.

Biochemical Measurements

Blood samples were obtained by venipuncture after 12-hour overnight fasting and centrifuged, aliquoted and stored in a freezer at -80 °C until further analysis. Plasma high sensitivity C-reactive protein (CRP) concentration was measured by an automated immunoturbidimetric assay system (IMMAGE immunochemistry system) (Beckman Coulter Inc., Brea, CA, USA). Plasma interleukin (IL)-1 β , IL-6, IL-8, IL-10, tumor necrosis factor (TNF)- α , IL-12p70, adiponectin, monocyte chemoattractant protein (MCP)-1, soluble intercellular adhesion molecule (sICAM)-1 and soluble vascular cell adhesion molecule (sVCAM)-1 levels were measured using a Multiplex immunoassay (Milliplex, Merck Millipore, MA, USA).

Plasma triglyceride (TG), total cholesterol (T-C), HDL cholesterol (HDL-c) and LDL cholesterol (LDL-c) levels were measured on a Roche Modular Auto Analyzer using an enzymatic colorimetric assay (Roche Diagnostics GmbH, Mannheim, Germany). Serum 25(OH)D levels were measured by high-performance liquid chromatography (HPLC; Immundiagnostik AG, Bensheim, Germany). The coefficient of variance was 3.3 to 4.5%, adequate vitamin D status was defined as ≥ 50 nmol/L [19].

Statistical Analyses

Continuous variables with a normal distribution were expressed as means and standard deviation or otherwise, as median and interquartile range. Categorical variables were expressed as percentage. All variables were checked for normality of distribution, and a natural logarithmic transformation was applied for plasma CRP, IL-6, IL-8, IL-12, adiponectin and IL-1 β concentration to normalize the distribution of data.

Student's *t*-test was used to compare means of continuous variables for vitamin D status adequacy (being 50 nmol/L the cutoff point). Proportions among skin color(white and non-white), gender, smoking habits, alcohol consumption and practice of moderate physical activity were compared using the Chi-square test. Multiple linear regressions were used to test whether blood pressure, circulating lipids (total, LDL, HDL and VLDL cholesterol as well as triglycerides) and plasma inflammatory biomarker concentration varied for vitamin D status in the presence of confounder variables. After adjustment, the confounder variables that remained at the model were age, BMI and gender.

In order to test the interaction between serum 25(OH)D concentration and BMI categories, an interaction term (the product of BMI*25(OH)D) was tested in the model, including the confounder variables BMI, age and gender as covariates. Multiple linear regression was performed to examine the independent associations of BMI on serum 25(OH)D concentration, for this analysis BMI was stratified by categories (normal weight defined as BMI<25Kg/m² and overweight as BMI≥25Kg/m²) adjusted for age and gender.

An ANOVA test was used to test differences in serum 25(OH)D and plasma inflammatory biomarkers concentrations between seasons of blood sample collection, and this analysis was adjusted by age and skin color (white or non-white). All tests adopted a significance level of p<0.05 and were performed using STATA version 10.1 (StataCorp, Texas, USA).

Results

After applying the exclusion criteria, 281 adults remained at the study (**Figure 1**). The characteristics of the study population, stratified by vitamin D status, are shown in **Table 1**. Only 34.5% of the population evaluated had serum 25(OH)D concentration of over 50 nmol/L and 11.4% had serum 25(OH)D concentration over 75nmol/L. Participants considered inadequate were younger (p<0.001), had lower BMI (p=0.004), systolic (SBP) (p<0.001) and diastolic (DBP) blood pressures (p=0.026), triglycerides (p<0.001), total cholesterol (p<0.001), and LDL-c (p<0.001) levels in comparison to individuals adequate for vitamin D status.

After adjustment, plasma sICAM-1 concentration was statistically higher among participants with serum 25(OH)D concentration greater than 50 nmol/L ($p=0.013$).

Table 1. Characteristics of HS-SP 2008-2010 population among vitamin D status adequacy

	Adequate	Inadequate
n (%)	97 (34.5)	184 (65.5)
Age (years)	47 (34 - 52)	36 (24 - 46)*
BMI (Kg/m ²)	26.7 (24.1 - 30.0)	24.9 (21.8 – 28.4)*
SBP (mmHg)	125.4 ± 18.2	120.1 ± 18.9†
DBP (mmHg)	77.9 ± 13.4	75.5 ± 12.8†
Triglycerides (mg/dL)	141 (95 - 220)	93 (67 - 131) †
Total Cholesterol (mg/dL)	207 (190 - 234)	169 (145 – 194) †
HDL-c (mg/dL)	48.6 ±12.3	46.8 ± 11.9
LDL-c (mg/dL)	125 (105-151)	101 (79-121) †
C-reactive protein (mg/L)	2.2 (1.2-4.6)	2.0 (0.6-4.4)
Adiponectin (ng/mL)	6281 (3948-9028)	6434 (3919-9415)
IL-6 (pg/mL)	1.90 (1.32 – 2.90)	2.04 (1.19-2.88)
IL-1β (pg/mL)	0.34 (0.20 – 0.72)	0.41 (0.20 – 0.83)
IL-12(pg/mL)	3.29 (1.89-5.68)	4.26 (2.47 – 6.14)
IL-8(pg/mL)	2.83 (1.95 – 3.68)	2.35 (1.54 – 3.33)
IL-10(pg/mL)	16.4 ± 11.7	18.3 ± 11.1
TNF-α(pg/mL)	4.91± 1.95	4.83± 1.88
MCP-1(pg/mL)	223.6±103.6	205.4 ± 80.2
sICAM-1 (ng/mL)	166.4 ±71.1	137.9± 64.0 †
sVCAM-1 (ng/mL)	915.4±213.5	863.6± 240.7
Caucasians(%)	65.0	56.5
Female (%)	66.0	60.3
Smoke habits (%)	22.7	25.5
Alcohol consumption (%)	49.5	54.0
Physicalactivity (%)	12.4	11.2

Values are means ± Standard Deviation or median (interquartile range). T test for comparison of means.

* Student T test

† Multiple regression analysis, adjusted for age, BMI and gender. $p<0.05$.

Furthermore, when the differences in serum 25(OH)D concentration were analyzed between seasons, those subjects who had their blood sample collected in fall had higher 25(OH)D serum levels than those whose blood sample was collected in summer, and this was reflected by differences in plasma sICAM-1 levels, among the eleven inflammatory biomarkers (**Figure 2**).

Figure 2. Differences in serum 25(OH)D and plasma sICAM-1 concentration between season in Brazilian population.

Anova adjusted by age and skin color (white and non-white) Different letters indicate statistically significant differences between groups ($p < 0.05$)

Moreover, BMI was considered effect modifier of the association between serum 25(OH)D levels and adiponectin ($P=0.023$), IL-6 ($P=0.021$) and TNF- α ($P=0.005$) levels. Therefore, association analyses were performed separately in overweight and normal weight participants, considering a cut-off point of 25 Kg/m² (**Table2**). In participants who had their BMI below 25 Kg/m², when plasma 25(OH)D concentration was high, plasma IL-6 and TNF- α levels tend to be low ($p=0.009$ and $p=0.025$, respectively), shown in Figure 3. Nevertheless, in participants whose BMI was above 25 Kg/m², when serum 25(OH)D concentration was high, plasma adiponectin levels were low ($p=0.040$) (**Figure 3**).

Table 2. Association between 25(OH)D concentration (nmol/L) and inflammatory biomarkers plasma concentration stratified by BMI classification, HS-SP 2008-2010.

Plasma inflammatory biomarkers	BMI<25 (n=131)		BMI≥25 (n=147)	
	β	95%CI (β)	β	95%CI (β)
C-reactive protein (mg/L)	0.29	-0.17; 0.73	0.15	-0.19; 0.49
Adiponectin (ng/mL)*	0.08	-0.13; 0.30	-0.18	-0.35; -0.01†
IL-6 (pg/mL)*	-0.44	-0.77; -0.11†	0.04	-0.22; 0.29
IL-1β (pg/mL)*	-0.38	-0.81; 0.04	-0.10	-0.48; 0.28
IL-12 (pg/mL)*	-0.28	-0.62; 0.07	-0.04	-0.37; 0.29
IL-8 (pg/mL)*	-0.01	-0.22; 0.19	0.06	-0.13; 0.24
IL-10 (pg/mL)	-2.58	-7.03; 1.88	-0.99	-4.67; 2.68
TNF-α (pg/mL)	-0.81	-1.52; -0.11†	0.49	-0.13; 1.12
MCP-1 (pg/mL)	-7.34	-32.95; 18.26	4.59	-26.19; 35.37
sICAM-1 (ng/mL)	19.84	-2.58; 42.28	12.21	-11.00; 35.41
sVCAM-1 (ng/mL)	-13.54	-98.88; 71.80	30.85	-46.38; 108.08

BMI, body mass index; 95%CI, 95% confidence interval; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, vascular cell adhesion molecule-1; TNF-α, tumor necrosis factor alpha.

β—coefficient and CI from multiple regression analysis, adjusted for age and gender.

* Log_n transformed variables.

†P<0.05

Figure 3. Association among inflammatory biomarkers, BMI, and 25(OH)D levels in HS-SP 2008-2010 population.

Black plus symbols and gray triangle symbols represents observed values of BMI < 25 kg/m² and BMI > 25 kg/m², respectively. The lines represent coefficients of 25(OH)D obtained by multiple regression adjusted by sex and age. BMI-25(OH)D interaction are expressed as “P for interaction”, at the bottom of the graph, and are considered statistically significant when $P < 0.05$.

Discussion

Although the city of São Paulo is crossed by the Tropic of Capricorn (23°S), presenting relatively mild winters and abundant sunlight, in the present study, an elevated prevalence of 25(OH)D insufficiency was observed. Several recent studies have shown that vitamin D deficiency and insufficiency is also higher in countries with lower latitudes, such as countries in Southern Europe [2,20], South Asia, the Middle East [21-23], Australia [24] and the USA [25]. Maeda et al.[26] found that, despite abundant sunlight, different populations presented vitamin D deficiency or insufficiency in São Paulo. Besides age, habit-related sunlight exposure differences, season of the year and BMI are related to vitamin D serum concentration, confirming the relationship between environment and lifestyle factors in vitamin D status.

Conversely, inflammatory biomarkers, such as CRP, IL-6 and MCP have been inversely associated with serum 25(OH)D concentration [27-29]. Thus, in an effort to elucidate whether 25(OH)D modulates immune system response, a wider range of inflammatory biomarkers was assessed in the present study. Immune modulation of vitamin D has received increasing attention by the scientific community as evidence shows that vitamin D status has potent immunoregulatory effects, particularly in adipose tissue [30,31]. In this study, plasma concentrations of sICAM-1 were statistically higher in subjects with serum 25(OH)D concentrations of over 50 nmol/L when compared to subjects with inadequate vitamin D status. Conversely, an *in vitro* study of peripheral blood mononuclear cells obtained from healthy subjects and inflammatory bowel disease (IBD) patients showed that treatment with vitamin D derivatives, alone or in combination with LPS or TNF-alpha,

significantly decreased ICAM-1 levels both in healthy subjects and IBD patients [32]. Moreover, it should be noted that the Framingham Offspring study found no relationship between serum 25(OH)D concentration and plasma ICAM-1 levels [33]. Likewise, Jorde *et al.* [34] verified no effect of supplementation with vitamin D on biomarkers of inflammation, including sICAM-1, in overweight and obese subjects. Nevertheless, results on the effects of vitamin D supplementation remain conflicting, where this disparity may be due to the duration of supplementation or amount of vitamin D given, as both vary considerably across studies. In this sense, the differences observed in the present study may be related to the characteristics of the sample, since only adults not in use of anti-inflammatory drugs were evaluated, and it is possible that the effect of vitamin D status is more clearly seen when the immune system is stimulated.

Similar to our results, other studies have demonstrated variations on serum 25(OH)D concentration among different seasons, and in countries at different latitudes [35, 36]. In this sample, only sICAM-1 plasma concentration also varied according to the season of blood collection, reflecting the results found for serum 25(OH)D concentration. Thus, emphasizing the association found between vitamin D status and sICAM-1.

Stratifying the sample for BMI categories revealed a negative association between serum 25(OH)D concentration and plasma IL-6 and TNF- α levels in normal-weight subjects. Recently, similar findings were reported by Laird *et al.* in older Irish adults [37], by Kim *et al.* in overweight Koreans [38], and in a longitudinal study conducted by Ibero-Baraibar *et al.* [39] in middle-aged Spanish participants. In addition, some studies found that plasma TNF- α [40] or IL-6 [41] levels were negatively correlated with serum 25(OH)D concentrations.

Although it is well known that waist circumference (WC) is a better measurement of cardiometabolic risk, and, consequently, proinflammatory profile, than BMI alone [42], in this study we chose to use BMI as a confounding variable, because some of the subjects did not have their WC measured, and because a strong correlation between BMI and WC was found among those who had both measurements ($r^2 = 0.76$, $p < 0.001$). Thus, in this population the BMI may be a good predictor of cardiometabolic risk and central obesity.

The molecular mechanisms of the relationship between vitamin D status and some inflammatory biomarkers such as IL-6 and TNF- α are still not well understood, but an explanation may lie in the inhibitory properties of vitamin D on activation of the transcription factor nuclear factor-kappa B (NF- κ B). It has been shown that vitamin D up-regulates I κ B- α levels by increasing mRNA stability and decreasing I κ B- α phosphorylation. Increases in I κ B- α levels lead to a reduction in nuclear translocation of NF- κ B, thereby causing a decline in activity. Thus, vitamin D might regulate the release of the proinflammatory cytokines, which represents a downstream target of NF κ B activation [43]. Another biological explanation for this relationship is that the VDR is found in significant concentrations in the T lymphocyte and macrophage populations [44] and the 25(OH)D-1- α -hydroxylase, enzyme responsible for the final hydroxylation step in the synthesis of active vitamin D, is expressed by activated macrophages [45]. Furthermore, the 24-hydroxylase, an enzyme that degrades the 1,25 (OH) $_2$ D is also expressed in macrophages/monocytes, suggesting that in the immune system vitamin D has a paracrine role [46]. Our results suggest that both factors (normal weight and higher serum vitamin D concentration) may have contributed to lower serum concentrations of TNF- α and IL-6 in normal weight individuals.

Regarding adiponectin, a negative association was found between plasma adiponectin levels and serum 25(OH)D concentration, but only in overweight subjects.

By contrast, previous studies have identified significant positive correlations between serum 25(OH)D concentration and total plasma adiponectin levels in obese patients with vitamin D sufficiency and vitamin D insufficiency [47,48]. Further studies are needed to better clarify whether, and the mechanisms by which, BMI alters the association between plasma adiponectin levels and serum 25(OH)D concentration.

A poor vitamin D status is frequently observed in obese individuals [49,50]. However, in the present study, although statistically significant difference in BMI was observed among vitamin D adequacy, this significance was lost when the model was adjusted for age and gender. Furthermore, when individuals were stratified in normal weight and overweight, an association between vitamin D and cytokines IL-6, TNF- α and adiponectin was identified. Some researchers have reported that less outdoor activities is an important confounding factor in obese individuals, as they tend to practice less outdoor activity and thus have lower sunlight exposure [51]. Other investigators suggest a direct association, claiming vitamin D can be sequestered and stored in adipose tissue, which has a higher storage capacity in obesity, subsequently decreasing the bioavailability of vitamin D in circulation [52]. Finally, causality between vitamin D deficiency and BMI was better explained in a Mendelian randomization study performed by Vilmaleswaran et al.[53], that postulated obesity as a causal factor in the development of vitamin D deficiency and not the inverse.

The main limitation of this study was its cross-sectional design which precludes inference of causality. Another limitation was the sample size, which became smaller than initially planned after applying the exclusion criteria, and might have limitations in order to perform robust regression analysis. However, because 11 inflammatory biomarkers were analyzed, several inflammatory pathways were investigated giving a wider context of the relationship between vitamin D and inflammation. Moreover, these findings suggest and

confirm the hypothesis that BMI interacts with serum 25(OH)D, modulating inflammatory response, affecting plasma IL-6, TNF- α and adiponectin levels. However, future studies are necessary to further investigate the mechanisms by which the BMI-serum 25(OH)D concentration interaction modulates inflammation.

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5.2 SEGUNDO ARTIGO

Hypovitaminosis D in adolescents: an independent association of adiposity and glucose homeostasis

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Abstract

Context and Objectives- Vitamin D deficiency may be an important factor of obesity-linked metabolic complications as alteration in insulin secretion, type 2 diabetes mellitus and alteration on lipids levels. However, studies examining this relationship are limited and have provided inconsistent results. Therefore, we investigated alterations in glucose homeostasis and lipids levels that could be mediated by vitamin D deficiency and obesity.

Participants and Methods- A subsample of 1009 adolescents (470 males) from the HELENA cross sectional study aged 12.5 to 17.49 years was analysed. Fasting blood samples were taken and analyzed for concentrations of 25-hydroxyvitamin D [25(OH)D], glucose, insulin levels and lipid profile. Body composition, the homeostasis model assessment insulin resistance (HOMA-IR) index and the quantitative insulin sensitivity check index (QUICKI) were also assessed.

Results- The 25(OH)D concentrations differed according to BMI status in relation to glucose parameters. Waist circumference was significantly lower in the vitamin D sufficient group ($R^2=.079$ $p<0.02$). 25(OH)D was independently and positively associated with the QUICKI index ($R^2=.13$ $p< 0.001$) and negatively associated with BMI ($R^2=-.10$ $p< 0.05$). The 25(OH)D concentrations were lower in the highest tertile of LDL/HDL ($p<0.05$). Increase in BMI was associated with a 1.93 times increase of vitamin D deficiency in adolescents (95% CI= 1.03 to 3.62; $p= 0.040$).

Conclusion: Glucose homeostasis markers, lipid profile and body composition were favorable at subjects without vitamin D deficiency, suggesting that better vitamin D status may have an important role in glucose homeostasis and body composition in adolescents.

Introduction

There is increasing evidence that adolescents is a group at risk for hypovitaminosis D (<75 nmol/L), notably those with overweight (1). Body composition measures such as BMI and fat mass have been associated with vitamin D deficiency in young people (2). Accordingly, the prevalence of vitamin D deficiency increases with increasing adiposity, from 29% of fat mass in overweight, to 34 % and 49% in obese and severely obese children, respectively (1). In this way, vitamin D status appears to be an important factor in the obesity-linked metabolic complications as alteration on glucose, insulin secretion and type 2 diabetes mellitus (3).

Some studies have shown an association between low serum 25-hydroxyvitamin D [25(OH)D] and glucose metabolism parameters in children and adolescents (4,5). Observational studies showed that 25(OH)D was inversely correlated with homeostatic model assessment insulin resistance (HOMA-IR) and fasting glucose; and positively correlated with insulin and quantitative insulin-sensitivity check *index* (QUICKI) in obese and non-obese adolescents (6,7). However, studies researching the relationship between vitamin D deficiency and insulin resistance in obese adolescents showed controversial results, mainly after adjustments for body composition (8).

Besides markers of glucose homeostasis, BMI and adiposity, serum lipid concentrations (9,10) could be also related to 25(OH)D concentrations. Both vitamin D deficiency (<50 nmol/l) and insufficiency (<75 nmol/l) were reported to increase total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and triglycerides in adults, but in adolescent the findings are still contradictory (9,10).

It is recognized that adiposity plays a key role in the development of metabolic diseases and low vitamin D concentrations could be directly and indirectly involved, however to date no studies assessed the associations between IR and vitamin D deficiency regardless of obesity and lipid alterations in a representative sample of European adolescents. Thus, with the increase of metabolic disorders in young people and its influence in adulthood, we prompted to evaluate alterations in glucose homeostasis that could be modulated by vitamin D deficiency independently of adiposity and lipid alterations.

Material and Methods

Study design and study sample

The present study sample was derived from the cross-sectional multi-centre Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study (n=3528) carried out in adolescents (12. 5 – 17. 5 years) in ten European cities (Athens, Heraklion, Dortmund, Ghent, Lille, Pécs, Rome, Stockholm, Vienna and Zaragoza). General HELENA procedures, characteristics and inclusion criteria can be found elsewhere (11). The protocol was approved by The Human Research Committee of Athens, Bonn, Ghent, Lille, Heraklion, Rome, Pecs, Vienna and Zaragoza. This study was performed following the ethical guidelines of the Declaration of Helsinki 1964 (revision of Edinburgh 2000). Informed consent was obtained from the adolescents and their parents. Within the sampling procedure, one third of the adolescents were fixed to participate in the blood sampling. For the present study, a subsample of 1006 adolescents (470 males and 536 females) had valid data on 25(OH)D concentrations.

Specimen collection and biochemical analyses

Blood samples were collected in the early morning after a 12-h overnight fast. The stability of samples during transport and storage in the HELENA study was reported previously (12). Plasma 25(OH)D was analyzed by ELISA using a kit (OCTEIA 25-Hydroxy Vitamin D) from Immunodiagnostic System (Germany) and measured with a SunriseTM Photometer by TECAN (Germany). The cut-off for vitamin D was adopted according to The Society for Adolescents Health and Medicine that defines >75 nmol/l (>30ng/mL) as sufficient', 50–74.99 nmol/L (20–29.99ng/mL) as insufficient and less than 50 nmol/L (<20 ng/mL) as deficient (13).

Serum TC, TG, HDL-c, LDL-c, and glucose were measured on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) with enzymatic methods. ApoB and apoA1 were measured in an immunochemical reaction with a BN II analyser (Dade Behring), according to the manufacturer's instructions. TC/HDL-c, LDL-c/HDL-c, Non HDL-c/HDL-c, TG/HDL-c and apoB/apoA1 ratios were calculated. Glucose was measured with an enzymatic method on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany), using the manufacturer's reagents and instructions. Insulin was measured from heparin plasma by a solid-phase two-site chemiluminescent immunometric assay with an Immulite 2000 analyser (DPC Biermann GmbH, Bad Nauheim, Germany). The HOMA-IR index was also calculated as: $\text{insulin } [(\mu\text{LU/mL}) \times \text{glucose (nmol/L)} / 22.5]$. The quantitative insulin sensitivity check index (QUICKI) was calculated as: $[(\log(\text{fasting insulin } \mu\text{U: mL}) + \log(\text{fasting glucose mg: dL}))]$. Intra-assay CV for all blood variables was 3.9 % and interassay CV was 4.3 %.

Dietary assessment

The mean daily calcium and vitamin D intakes were estimated from two non-consecutive 24h-recalls by HELENA-DIAT software¹⁴. Vereecken et al. (14) demonstrated that HELENA-DIAT is a validate instrument for an interviewer-administered 24h-recall for all nutrients, including vitamin D and calcium. The German Food Code and Nutrient Data Base (BSL version II.3.1.2005) was used for dietary data and The multiple Source Method (MSM) was provided by German Institute of Human Nutrition for corrected between and within-person variability¹⁵. Underreporters were excluded, considering the estimated basal metabolic rate <0.96 (16).

Anthropometric measurements and physical activity

Anthropometric measurements were taken following a standardized protocol as previously described (17). Weight and height were measured in underwear and barefoot using an electronic scale (Type SECA 861, UK, precision 100 g, range 0-150 kg) and a stadiometer (Type SECA 225, UK precision 0.2 cm, range 70-200 cm). BMI was calculated as (kg / m^2) and was categorized as described by Cole et al. (18). Waist circumference with a non-elastic tape was used to measure to the nearest 0.1 cm. Intra-observer technical errors of measurement in all the skinfold thickness measure were smaller than 1 mm and reliability greater than 95%. For skinfolds, inter-observer reliability was higher than 90% (19). Fat mass, fat free mass and body fat % were calculated using skinfold thickness from Slaughter's equation (20).

A detailed description of physical activity (PA) measurement in the HELENA study has been published elsewhere (21). Briefly, a uni-axial accelerometer (GT1M, Actigraph, Pensacola, FL) was used to assess PA. Adolescents were instructed to wear the accelerometer for 7 consecutive days during waking hours except when engaged in water-based activities. The time sampling interval (epoch) was set at 15 seconds. The time engaged in moderate-to-vigorous PA (MVPA) and vigorous PA was defined by the cutoff points of ≥ 2000 counts/minute and ≥ 4000 counts/minute, respectively. Sedentary time was defined using the standard cut-off point of ≤ 100 counts/minute. Additionally, the time spent in at least moderate intensity activity was calculated as the total time spent in moderate and vigorous physical activity (MVPA, min/d), which was included in the present study.

Statistical analysis

All variables were checked for normality of distribution by Kolmogorov-Smirnov test. All biochemical variables were required transformation to account for skewness, and then were underwent log transformation. Mann-Whitney U was performed to evaluate the differences among all variables between male and female and Kruskal-Wallis test to evaluate the differences according to vitamin D status. Descriptive values are shown as mean and standard deviation or median and interquartile range (non normal distribution). Spearman's correlation coefficients were computed between all variables and 25(OH)D concentrations.

Analysis of covariance (ANCOVA) were conducted to compare the association between vitamin D status (deficiency and sufficiency) and body composition, lipid profile and glucose homeostasis markers; controlling for age, gender, physical activity and latitude.

Post hoc analyses were conducted with Bonferroni adjustment (**Figure 2a and b**).

Two stepwise multiple linear regressions were performed to find associations between 25(OH)D concentrations and each measure of body composition as well as glucose homeostasis markers, in both, vitamin D was entered as dependent variable. More specifically, the first linear regression was performed with all body composition measures as covariates following for age, gender, latitude, physical activity, vitamin D and calcium intake and TC. The second was performed with all glucose homeostasis parameters as covariates following for age, gender, latitude, physical activity, vitamin D and calcium intake, TC, BMI and fat free mass. Finally, multiple logistic regression analysis was also performed to find an association between vitamin D deficiency and lipid profile, body composition and glucose parameters. All statistical analyses were performed using the SPSS software package, version 22.0 (SPSS, Chicago, IL, USA). For all analysis $p < 0.05$ was considered as significant.

Results

Table 1 presents 25(OH)D concentrations differed according to BMI status in relation to glucose parameters. In non-overweight adolescents (BMI $<25\text{kg/m}^2$), those who had sufficient 25(OH)D concentrations had better glucose homeostasis parameters (glucose, HOMA-IR and QUICKI) than adolescents who had deficient 25(OH)D concentrations ($p < 0.01$). In overweight adolescents no differences in glucose homeostasis parameters depending on 25(OH)D status could be observed. Lipid profile did not show any significant difference between overweight and non-overweight subjects in relation to vitamin D status. Nevertheless, ApoA-I showed borderline differences inside the overweight group, those at sufficient 25(OH)D concentrations had higher ApoA-I than subjects at a deficient 25(OH)D ($p < 0.05$).

Differences between lipid profile and vitamin D status was observed, 25(OH)D levels were lower in the highest tertile of lipids profile parameters being significant for LDL/HDL ($p<0.05$), data not shown.

Figure 1 shows the Spearman's correlation between 25(OH)D concentration and glucose metabolism measurements by gender. Glucose, Insulin and QUICKI score were correlated with 25(OH)D concentrations, but only in girls.

Table 1. Biochemical characteristics according to BMI and vitamin D status

	BMI<25 (non-overweight)			BMI>25 (overweight and obese)		
	Vitamin D deficiency (<75 nmol/L) n=682	Vitamin D sufficiency (≥75 nmol/l) n = 186	<i>p</i>	Vitamin D deficiency (<75 nmol/L) n= 118	Vitamin D sufficiency (≥75 nmol/l) n= 20	<i>p</i>
Glucose (mg/dL)	91.1 ± 7.3	89.2 ± 6.6	<0.001	91.8 ± 7.7	91.3 ± 6.5	0.78
Insulin (µIU/mL)	9.5 ± 6.3	8.4 ± 4.7	0.01	15.4 ± 13.3	13.7 ± 5.9	0.91
HOMA- IR	2.1 ± 1.5	1.8 ± 1.1	0.00	3.6 ± 3.4	3.1 ± 1.3	0.93
QUICKI	0.350 ± 0.029	0.356 ± 0.028	0.00	0.327 ± 0.025	0.328 ± 0.024	0.93
Triglycerides (TG) (mg/dL)	66.3 ± 31.2	68.6 ± 31.4	0.62	86.9 ± 52.1	78.1 ± 35.9	0.55
Totalcholesterol(TC) (mg/dL)	160.3 ± 27.2	160.1 ± 28.6	0.71	162.5 ± 29.3	166.0 ± 26.6	0.55
HDL-c (mg/dL)	55.9 ± 10.5	54.9 ± 10.5	0.20	50.0 ± 10.3	53.8 ± 11.4	0.14
LDL-c (mg/dL)	93.3 ± 24.8	94.1 ± 24.1	0.51	100.1 ± 25.8	101.1 ± 17.3	0.72
VLDL (mg/dL)	13.2 ± 6.2	13.7 ± 6.2	0.62	17.3 ± 10.4	15.6 ± 7.1	0.55
Non HDL-c (mg/dL)	104.3 ± 25.2	105.2 ± 25.5	0.80	112.4 ± 27.2	112.1 ± 18.4	0.82
Non HDL/HDL ratio	1.9 ± 0.6	1.9 ± 0.5	0.15	2.3 ± 0.7	2.1 ± 0.3	0.48
TC/ HDL ratio	2.9 ± 0.6	2.9 ± 0.5	0.15	3.3 ± 0.7	3.1 ± 0.3	0.48
LDL/HDL ratio	1.7 ± 0.6	1.7 ± 0.5	0.13	2.0 ± 0.7	1.9 ± 0.3	0.53
TG/HDL ratio	1.2 ± 0.7	1.3 ± 0.6	0.33	1.8 ± 1.3	1.5 ± 0.8	0.34
ApoA-I (g/L)	1.5 ± 0.21	1.5 ± 0.23	0.72	1.4 ± 0.22	1.5 ± 0.29	0.05
ApoB (g/L)	0.6 ± 0.15	0.6 ± 0.15	0.91	0.7 ± 0.16	0.6 ± 0.11	0.73
ApoB/ApoA1 ratio	0.43 ± 0.12	0.43 ± 0.11	0.82	0.50 ± 0.15	0.45 ± 0.06	0.15

BMI, body mass index; HOMA-IR, homeostasis model assessment insulin resistance index; QUICKI, quantitative insulin sensitivity check index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; ApoA-I, apolipoprotein AI; ApoB, apolipoprotein B. Characteristics values are unadjusted, data are presented as mean ± SD.

Mann Whitney Test was performed to evaluate the difference between variables according to BMI and vitamin D status

Figure 1. Spearman's correlation between 25(OH)D concentration and glucose metabolism measurement by gender. (a) male, (b) female.

After controlling for age, gender, latitude and physical activity the Ancova analysis revealed that waist circumference was significantly lower in vitamin D sufficient group ($p=0.025$), **Figure 2a** Glucose and insulin concentrations were also lower in the vitamin D sufficient group after controlling for age, gender, latitude, BMI, fat free mass and physical activity ($p=0.021$ and 0.043 , respectively). In the other hand, the insulin sensitivity (QUICKI index) was higher in vitamin D sufficient group ($p<0.01$), after controlling for the same confounders variables, **Figure 2b**. Adolescents with insulin resistance, HOMA-IR values greater than 3.16 or QUICKI less than 0.328, had low 25(OH)D concentration (59.7 vs 53.8 nmol/L and 60.1 vs 54.5 nmol/l, both $p < 0.01$; respectively)

Figure 2a. BMI and Waist circumference according to vitamin D status controlling for age, gender, latitude and physical activity.

Figure 2b. Glucose, Insulin and QUICKI score according to vitamin D status controlling for age, gender, latitude, BMI, fat free mass and physical activity.

*Glucose and Insulin were performed as log variable.

The first multivariate linear regression analysis showed an independent and negative association between 25(OH)D concentrations and BMI ($\beta = -0.10$; $p < 0.05$) when age, gender, latitude, physical activity, vitamin D and calcium intake and TC were considered. The second multivariate linear regression analysis showed an independent and positive association between 25(OH)D concentrations with insulin sensitivity ($\beta = 0.20$; $p < 0.001$) when age, gender, latitude, physical activity, vitamin D and calcium intake, TC, fat free mass and BMI were considered. The first model explains almost 10% of the variance and the second may explain 13%, **Table 2**.

Table 2. Relationship between BMI or QUICKI with 25(OH)D.

	Body Composition *				Glucose Metabolism **		
	β	R^2	p		β	R^2	p
Model 1		0.093				0.123	
Latitude	0.30		0.000	Latitude	0.32		0.000
Age	0.11		0.014	QUICKI	0.21		0.000
Model 2		0.104				0.133	
Latitude	0.31		0.000	Latitude	0.34		0.000
Age	0.11		0.012	QUICKI	0.20		0.000
BMI	-0.10		0.020	Age	0.10		0.024

BMI, body mass index; QUICKI, quantitative insulin sensitivity check index; β , standardized regression coefficients.

Vitamin D entered as log transformed variable

* All body composition measure entered as covariates

** All variables related to metabolism glucose entered as covariates

In the **Table 3**, logistic regression showed that increasing BMI was associated with an increase of 1.93 times odds vitamin D deficiency in adolescents (95% CI= 1.03 to 3.62; $p = 0.040$). In this way, adolescents in the higher median glucose levels had 1.39 times odd vitamin D deficiency than those in lower median glucose levels (95% CI= 1.01 to 1.93; $p < 0.05$).

Table 3. Logistic regression analysis between vitamin D deficiency and body composition and glucose metabolism.

	β	OR (95% CI)	<i>p</i>
Body composition*			
BMI	0.660	1.93 (1.03 to 3.62)	0.04
FFM	0.182	1.20 0.74 to 1.92)	0.45
FM	-0.219	0.80 (0.50 to 1.26)	0.34
Glucose Metabolism**			
HOMA-IR	-.098	0.90 (0.73 to 1.12)	0.37
QUICKI	3.528	34.0 (0.00 to 13.75)	0.51
Glucose	0.335	1.39 (1.01 to 1.93)	0.04
Insulin	-0.065	0.93 (0.58 to 1.50)	0.78

BMI, body mass index; FFM, fat free mass; FM, fat mass; HOMA-IR, , homeostasis model assessment insulin resistance index; QUICKI, quantitative insulin sensitivity check index.

*Age and gender entered in the model.

** Only glucose homeostasis variables entered in the model

Discussion

There are no universal accepted blood 25(OH)D thresholds to define adequacy in adolescents. We should take into account that adolescence is a period of growth, being crucial for bone mass development, in which vitamin D status should follow recommendations for increasing bone mineral content (BMC). The literature has shown that concentrations of 25(OH)D > 80 nmol/l can be considered optimal for increasing BMC. Moreover, the cut-off of 50 nmol/L has been mostly proposed for maintaining bone mass, but not for its development (22,23). Thus, the cut-off of 75 nmol/L was chosen for analysing the HELENA data.

Our main finding showed that non-overweight adolescents with sufficient vitamin D levels had lower glucose, insulin levels and HOMA-IR than those who had vitamin D deficiency. A similar trend was observed in overweight and obese, but no statistical significance was noted. The physiological mechanism by which vitamin D could contribute to glucose control in overweight and non-overweight subjects is still unclear. One possible mechanism occurs because vitamin D receptor gene (VDR), vitamin D binding protein (DBP) and vitamin D 1 α hydroxylase (CYP1 α) could modify insulin secretion inducing to insulin resistance and deregulation of glucose homeostasis (24). The positive effects of vitamin D supplementation on insulin and glucose metabolism have been postulated in studies with young people, suggesting that the correction of an inadequate vitamin D status may be important for the treatment of obesity and its associated insulin resistance (25).

Prospective studies and systematic reviews revealed that low 25(OH)D concentrations are associated with type 2 diabetes risk and metabolic syndrome (MS) (26). Our results showed a positive correlation between 25(OH)D and insulin sensitivity by QUICKI index. Moreover, a negative correlation between 25(OH)D and fasting glucose and insulin was found. Similar results were reported in Asian (5) and US adolescents (27). Even after adjustment for adiposity parameters, glucose and insulin levels remained significantly lower in vitamin D sufficient subjects while insulin sensitivity remained higher in the same subjects. In addition, linear regression showed that insulin sensitivity index was positively associated with 25(OH)D concentrations and lower 25(OH)D concentrations were related to highest glucose levels. The inverse association between 25(OH)D and IR was significant in all participants, including non-overweight. This results show that the relationship between 25(OH)D concentration and glucose homeostasis may occurs independently of body composition and lipids levels.

Some studies also found a positive association between serum 25(OH)D concentration and insulin sensitivity (5,28). The cohort study *Prospective Metabolism and Islet cell Evaluation* (PROMISE) revealed that this inverse association with risk of MS is partly driven by the vitamin D association with glucose homeostasis (29). In addition, the correction of 25(OH)D concentration may improve the insulin sensitivity, mainly in obese adolescents (25). Thus, this interaction between 25(OH)D and glucose metabolism may be explained by the fact that vitamin D can enhance insulin responsiveness for glucose transport (30) and insulin sensitivity by directly stimulating the insulin receptor expression. Also, vitamin D has an indirect effect by regulating extracellular calcium concentration and influx through cell membranes (31).

Additionally, adiposity, an increasing global problem in children and adolescents, is another influencing factor for both vitamin D status and abnormal glucose homeostasis. Previous results showed that the relationship between these parameters remained significant after adjustments (27,32). In accordance, our findings showed a negative correlation between waist circumference and 25(OH)D, and BMI exhibit an independent and negative association with 25(OH)D after taking into account some confounders. Moreover, in the unadjusted logistic regression, we found that vitamin D deficiency is associated with BMI increase. Our results highlights that relationship between vitamin D concentration and body composition may be independent of lipids levels. Observational studies have shown a high prevalence of vitamin D deficiency in obese subjects (33,34), but few studies are performed in adolescents. Data from 58 obese adolescents revealed a 1% increment in total body fat mass corresponded to a decrease of 1.15 ± 0.55 nmol/l 25(OH)D concentrations (35). A Mendelian randomized study suggested that obesity may be a causal factor of vitamin D deficiency, but the effect of lower 25(OH)D over BMI increase tends to be small (36).

Furthermore, the possible mechanism and the causality by which obesity affects vitamin D levels is still uncertain. The most accepted mechanisms trying to explain the inverse correlation between adiposity and vitamin D is the sequestration of vitamin D by adipocytes, elevated storage of vitamin D appears in the larger-than-normal fat depots prior to conversion into 25(OH)D (37,38).

Cross-sectional and longitudinal studies demonstrated that 25(OH)D concentrations have a favorable effect on lipid profile, indicating a relationship between vitamin D and lipids (39). It has been suggested that vitamin D may alter lipid profile through regulatory action that increases the activity of lipoprotein lipase in adiposity (40,41). However, interventional studies comparing vitamin D group *versus* placebo group obtained contradictory results (42,43). In our sample, only LDL/HDL ratio showed significant differences in relation to 25(OH) levels. No relation was found with the other lipid variables. Similarly, a study with black and Caucasian American children using a cut-off of <50nmol/L for 25(OH)D did not find difference in lipid parameters between vitamin D deficient and non-deficient group (44).

Strengths of the HELENA study are the strict standardization of the field work, centralized blood analysis, and a sampling protocol which permits the analysis of relationship between variables. The main limitation of this study is its cross-sectional design which cannot evaluate the causality in these findings and this results should be applied only in similar population. Moreover, it was not possible to evaluate PTH levels and is speculated that the status of both vitamin D and PTH need to be considered for optimal evaluation of the impact of vitamin D status on glucose metabolism.

Conclusion

In apparently healthy adolescents, markers of glucose homeostasis were associated with 25(OH)D, independently of adiposity and lipid profile.

Insulin sensitivity and a lower levels of blood glucose and insulin were associated to higher 25(OH)D concentrations. Moreover, adiposity measures (BMI and WC) were negatively associated with 25(OH)D concentrations. These findings highlight the importance of further vitamin D prospective and interventional studies with specific focus on lipid profile and glucose homeostasis in adolescents,

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5.3 TERCEIRO ARTIGO

Body Composition and blood lipid profile are independently associated with 25(OH) D concentration in Spanish elderly. The PHYSMED study

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Abstract

Introduction: Studies have shown that vitamin D deficiency increased risk for lipid metabolism disorders. However, studies examining this relationship in elderly are limited and have provided inconsistent results. **Objective:** The purpose of this study was to assess associations between vitamin D, blood lipids and body composition in apparently healthy Spanish elderly. **Methods:** A cross-sectional multicentre study was carried out in a subsample of 383 participants (58.2% females) aged of 55-88 years. Fasting blood samples analyzed to determine serum concentrations of 25(OH)D, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG) and total cholesterol (TC). Body composition parameters (fat mass, fat free mass) were obtained by bioimpedance, waist circumference (WC) was measured three times to the nearest 0.1 cm using a non-stretch measuring tape. To assess vitamin D intake, two non-consecutive 24h diet recalls were obtained. Statistical analyses were performed using the SPSS software package, version 22.0. **Results:** BMI, fat mass and total fat mass were lower in vitamin D sufficient subjects than vitamin D insufficient and deficient subjects, but this difference was not significant (for all $p > 0.05$). Those with vitamin D adequate levels also showed lower TC/HDL ratio than those had inadequate (insufficient or deficient) vitamin D levels ($p = 0.04$). It was found a significant association between 25(OH)D and BMI, waist circumference, total muscle mass, TC/HDL-c ratio, HDL and TG (for all $p \leq 0.05$) after controlling for some confounders. The unadjusted logistic regression shown that those who have no adequate HDL levels ($< 40 \text{ mg/dl}$) have 1.7 times higher odds than vitamin D deficiency than those who have HDL levels adequate ($> 60 \text{ mg/dl}$) (95% CI= 1.10 to 2.85 $p = 0.017$) and waist circumference was negatively associated with vitamin D status odds ratio of 0.98 (0.96 to 1.00; $p = 0.04$).

Conclusion: Vitamin D concentration was positive and independently correlated with HDL-c and total muscle mass, as well as negative correlated with WC, LDL-c/HDL-c and TC/HDL-c independently of age, gender and some confounders.

Key words: vitamin D, BMI, lipid profile, HDL-c and elderly.

Introduction

Low vitamin D concentration is an increasing public health problem around the world¹, including the Mediterranean countries². Elderly people are especially at risk because with aging there is an important change in lifestyle like less solar exposure and less outdoor activity³. In the last years, some diseases like metabolic syndrome⁴, coronary heart disease⁵, stroke⁵ and certain cancers^{1,6} have been related to lower vitamin D status. Serum 25-hydroxyvitamin D [25(OH)D] is significantly associated with lipid metabolism^{7,8} and studies have shown that individuals with vitamin D deficiency have an increased risk for lipid metabolism disorders^{4,9}.

Some studies have shown that serum 25(OH)D levels are negatively associated with low-density lipoprotein cholesterol (LDL-c), triglycerides (TG) and total cholesterol (TC) and positively associated with high-density lipoprotein cholesterol (HDL-c)^{10,11}.

On the other hand, numerous studies have confirmed the inverse correlation between 25(OH)D and adiposity. In fact, overweighted and obese people tend to have low levels of 25(OH)D^{12,13}. Clinical practice guidelines as those from the American Endocrinology Society have suggested that obese individuals should take 2-3 times more vitamin D than normal-weight individuals. However, they recognize that more studies are needed to clearly justify this decision¹⁴.

All in all, aging, hypovitaminosis D, lipid alterations and body composition are powerfully linked. The present study is part of the PHYSMED study which was designed to analyse lifestyle, dietary intake and biomarkers in well-defined physically active and sedentary non-institutionalized active and non-active Spanish elderly. To our knowledge, data on this relationship taking into account the large number of confounders that can have an effect in this association are, until now, scarce for elderly-

Thus, the main aim of this study was to investigate the associations between vitamin D, blood lipids and body composition in apparently healthy Spanish elderly living in two cities (Madrid and Palma de Mallorca).

Material and methods

Subjects and study design

The PHYSMED study is a cross-sectional multicentre study performed in non-institutionalized elderly people focused on evaluating the relationship between vitamin D and body composition and lipids profile. This study was conducted in two different cities in Spain: Madrid and Palma de Mallorca. The study was performed according to the principles established in the Declaration of Helsinki and approved by the Ethical Committee of the Technical University of Madrid (Spain). All participants signed an informed consent.

The exclusion criteria were: age under 55, being institutionalized, suffering from a physical or mental illness which would have limited their participation in the physical fitness tests or their ability to respond the questionnaires, chronic alcoholism or drug addiction and intake of drugs for clinical research over the past year. The participants were recruited through sport federations, sport facilities and municipal clubs located at Madrid and Mallorca and a total of 433 subjects (57% women) aged from 55 to 88 years participated in the study. Vitamin D concentration [25(OH)D] was assessed in 383 (58.2 % women), who composed the final sample for this study.

Specimen collection and biochemical analyses

Fasted (12 h) blood samples were collected from each participant and blood lipid profile was analysed in Madrid and Palma de Mallorca, at the biochemical laboratory of the High Sports Council and of the Hospital Palma de Mallorca, respectively. The extraction was performed by standard venipuncture on vacuum Vacutainer® tubes. The tubes were immediately placed on ice and after coagulum formation the sample was centrifuged at 3,000 rpm for 10 minutes. Serum was separated in eppendorfs of 1 mL and it was processed to analyse total cholesterol (TC), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c), VLDL and triglycerides (TG) using the Beckman AU400 analyser (Beckman AU400, Beckman Instruments, Ltd., Bucks, UK) by calorimetric method. Intra-assay and inter-assay coefficients of variation were 0.79% and 1.2%, 1.83% and 2.5%, 0.70% and 1.66%, 0.87% and 1.45%, for TC, LDL, HDL and TG, respectively. Vitamin D (25(OH)D) analysis was centralized at High Sports Council Laboratory in Madrid (Spain). Therefore, serum samples from Palma de Mallorca were dry-ice shipped to Madrid and analyzed together with those serum samples stored in Madrid at -80°C to measure vitamin D, using an electrochemoluminescence immunoassay running in an E411 analyzer (Roche Diagnostics, Switzerland). According to Holick et al ¹⁴, deficiency was considered at (< 20 ng/ml), insufficiency (20- 29.9 ng/ml) and sufficiency (> 30 ng/ml).

Season and Latitude

In order to include the season as a co-variable, the date of blood sample collection was considered. Samples collected from October to March were classified as "autumn-winter samples" and those collected from April to September were classified as "spring-summer samples".

The latitude of each city was obtained from <http://www.aemet.es>. Latitudes of the involved cities were: Palma de Mallorca (39° 34' N) and Madrid (40° 24' N).

Anthropometric measurements

Height was measured to the nearest millimetre using a mobile stadiometer (SECA 213, Germany), with the subject's head in the Frankfurt plane. Weight, body composition (fat mass, fat free mass, total fat mass, total muscular mass) were assessed by means of a bioimpedance analysis (TANITA Corp, BC-418MA, Tokyo, Japan) in standardized conditions. BMI was calculated as weight (kg)/height² (cm) and BMI groups were established as normal-weight (<18.5-24.9), overweight (25–29.9) and obese ($\geq 30 \text{ kg/m}^2$). Waist circumference (WC) was measured three times to the nearest 0.1 cm using a non-stretch measuring tape (Rosscraft SRL Mercosur, Buenos Aires, Argentina) by a trained ISAK anthropometrist.

Physical activity, smoking and alcohol habits

Physical activity was assessed with the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ). MLTPAQ is a one-year recall questionnaire and it was carried out by trained interviewers. It has been validated for both Spanish men and women and consists of a total of 67 items^{15, 16}. However, for this study, only outdoor activities (a total of 43 items) were considered. All outdoor activities were added together and divided between 365 days to obtain minutes per day.

Additionally, regular time doing physical activity expressed by minutes per day was assessed by means of the EXERNET questionnaire, a validated questionnaire in Spanish elderly¹⁷. EXERNET questionnaire also contained questions about general information, smoking and alcohol habits. Participants could answer less than 5 cigarettes per day, between 5 to 10 cigarettes per day, between 10 to 15 cigarettes per day, between 15 to 20 cigarettes per day and more than one package per day. Moreover, subjects were asked if they drink alcohol regularly and they could answer yes or no.

Physical fitness test

A multi-component battery of physical fitness tests validated for Spanish elderly¹⁸ was used to measure: lower and upper body strength by the chair stand test, agility and dynamic balance by the 8-foot up-and-go test, aerobic endurance by the 6-min walk test¹⁹ and handgrip strength with a handgrip dynamometer (Takei TKK 510, Tokyo, Japan, range 5-100 kg, precision 0.1 kg) from each subject. The handgrip strength was assessed for both hands in a standing position. All tests were performed twice, except the 6-min walk test, and the best score was retained. Then, the results of physical fitness tests were divided into quartiles and stratified by gender and age. The score for each test ranged from 0 (worst) to 3 (best) points. Scores of each test were added together and the maximum possible was 12 points. Our population was divided into two groups: low fitness (0-6 points) and high fitness (7-12 points).

Vitamin D intake

To assess vitamin D intake, two non-consecutive 24h dietary recalls were obtained from the subjects by well-trained dieticians that administered the recalls. A computer program was used to convert food into nutrients (ALIMENTA®; NUCOX, Palma, Spain) based on Spanish^{20,21} and European²² food composition tables, and complemented with food composition data available for Majorcan food items²³.

Statistical analysis

All statistical analyses were performed using the SPSS software package, version 22.0 (SPSS, Chicago, IL, USA). All variables were checked for normality of distribution using the Kolmogorov-Smirnov test. The 25(OH)D, TC, and LDL-c values showed normal distribution. Independent sample *t*-test and one-way Anova (for normally distributed variables); Mann-Whitney U and Kruskal-Wallis test (for non-normally distributed variables) were performed to evaluate the differences in these parameters according to vitamin D status.

Pearson's correlation coefficients were computed between 25(OH)D concentrations and body composition and lipid profile adjusted by age. Two analysis of covariance (ANCOVA) were conducted to verify the association of body composition and lipid profile on 25(OH)D concentration controlling by age, gender, physical activity, city, outdoor physical activity, season of blood collection, vitamin D intake and alcohol intake. The first was performed considering vitamin D (continuous) as dependent variable and the second was performed considering vitamin D (categorized in deficiency, insufficiency and sufficiency) as independent variable. *Post hoc* analyses were conducted with Bonferroni adjustment.

Stepwise multiple linear regression was used to find associations between serum 25(OH)D concentrations and some factors including each measure of body composition. Separate stepwise multiple linear regression models were built to evaluate the association between serum 25(OH)D concentrations (dependent variable) and body composition and blood lipid profile as independent variables. In the same way, in both multiple linear regressions potential confounders were also included (age, gender, physical activity, city, outdoor physical activity, season, vitamin D intake, alcohol). These variables factors were included because city, season, and alcohol intake were different between 25(OH) D concentrations. Physical and outdoor activity, smoking habits and vitamin D intake were different between body composition or between lipid profile parameters.

Finally, multiple logistic regression analysis was also performed including to find an association between vitamin D deficiency risk and lipid profile and body composition. For all analysis $p < 0.05$ was considered as significant.

Results

Among 30.5% of study participants had vitamin D deficiency, of which 32.7% were women. Vitamin D insufficiency was found in 37.3% subjects and only 32.1% of subjects had vitamin D sufficiency level. In addition, the mean of 25(OH)D concentration was 25.8 ± 10.1 ng/mL, which was higher in subjects from Palma than subjects from Madrid (23.6 vs. 28.12 ng/mL, $p < 0.01$). Vitamin D concentrations did not show difference between men and women. The subject characteristics and blood parameters according to vitamin D status are presented in **Table 1**.

Subjects with vitamin D deficiency (< 20 ng/ml) had significantly higher TC/HDL-c ratio (3.9 ± 0.8 vs. 3.7 ± 0.8 ; $p < 0.05$) than vitamin D sufficiency subjects (> 30 ng/ml), subjects with vitamin D deficiency also showed a borderline elevated LDL/HDL (2.5 ± 0.6 vs. 2.3 ± 0.7 ; $p = 0.05$) (**Table 1**).

Table 2 shows Pearson's correlation coefficients between 25(OH)D concentration and body composition and blood lipids In females, waist circumference ($r = -0.169$), TC/HDL-c ($r = -0.223$) and LDL/HDL ($r = -0.226$) were negatively correlated with 25(OH)D (all $p < 0.05$). In males, waist circumference was negatively correlated with 25(OH)D ($r = -0.176$; $p > 0.05$). On the other hand, HDL-c was positively correlated with 25(OH)D ($r = 0.201$; $p > 0.05$), but only in females

Table 1. Body composition and lipid profile by vitamin D status

Variables	Vitamin D status			P
	Deficient (< 20 ng/ml, n =117)	Insufficient (20- 29.9 ng/ml, n =143)	Sufficient (> 30 ng/ml, n=123)	
City (Madrid) ^a	81 (69.2 %)	68 (47.6 %)	49 (39.8%)	0.001
Age **	68.4 ± 7.0	65.9 ± 6.7	66.0 ± 6.0	0.004
Gender (male) ^a	44 (37. 6%)	59 (41.3%)	57 (46.3%)	0.38
BMI ^b	27.0 (18.7- 39.1)	27.2 (17- 41.2)	26.4 (19.4- 41.8)	0.45
Waist circumference (cm) ^b	90.0 (65.0-124.7)	89.2 (61.3-121.3)	88.3(64- 114.6)	0.34
Fat free mass (kg) ^b	44.0 (34.1-71.9)	45.2 (34.5- 76.7)	45.0 (33.9- 76.5)	0.55
Fat mass (kg) ^b	21.9 (11.1- 45.7)	21.7 (7.3-44.2)	21.9 (6.4-48.7)	0.85
Total fat mass (%) ^b	33.5 (17.6- 45.8)	32.7 (12.1- 49.3)	31.8 (10.1- 49.8)	0.41
Total muscle mass (kg) ^b	41.3 (32.5- 68.8)	43.1 (32.5- 68.8)	42.7 (32.3- 72.9)	0.43
TC (mg/dL) ^a	207.0 (131-305)	214.0 (138-333)	207 (112-351)	0.35
TG (mg/dL) ^b	95.5 (33-239)	91.0 (45-274.1)	82.2 (44.5-199.6)	0.38
HDL-c (mg/dL) ^b	53.4 (31.5-88.8)	55.0 (20-98)	57.2 (27.7-92)	0.08
LDL-c (mg/dL) ^a	134.4 (71.4-218.8)	137.7 (73- 225.9)	132 (64.9-249.5)	0.23
VLDL-c (mg/dL) ^b	19.0 (7-47.8)	18.1 (9-54.8)	16.4 (8.9-39.9)	0.36
TC/HDL ratio ^b	3.9 (2.5-6.2)	3.8 (2.3-7.9)	3.5 (2.3-6.5)	0.04
LDL/HDL ratio ^b	2.5 (1.2-4.2)	2.4 (1.1- 5.1)	2.2 (1.1- 5.1)	0.05
PA(hs/wk) ^b	2.0 (0-15.2)	3.0 (0- 20)	3.0 (0- 20)	0.50
PA outdoor (min/d) ^b	82.4 (0- 352.3)	76.6 (0- 353.21)	76.7 (0- 322.7)	0.78
Smoking (yes)	7 (6%)	9 (6.3%)	12 (9.8%)	0.44
Alcohol (yes)	52 (44.4 %)	73 (51%)	67 (54.5%)	0.26
Season (spring-summer)	58 (49.6 %)	67 (46.9%)	44 (35.8%)	0.70
Vitamin D intake (µg) ^b	1.7 (0- 94.0)	1.6 (0- 265.9)	1.7 (0.03- 255.4)	0.40

BMI, body mass index; PA, physical activity; TC, total cholesterol; TG, triglycerides.

Data are presented as median (min- max) or value (%).

Comparisons between vitamin D status were analysed by One Way Anova (a) or Kruskal-Wallis test (b), according to the normality of the variables.

** value presented as mean (SD).

Table 2. Pearson correlation coefficients between 25(OH)D levels and body composition and lipid profile.

Variables	25(OH)D concentration	
	Male (r)	Female (r)
Fat free mass (kg)	0.012	-0.087
Fat mass (kg)	-0.042	-0.105
Total fat mass (%)	-0.032	-0.101
Total muscle mass (kg)	0.016	-0.071
Waist circumference (cm)	-0.176**	-0.169**
BMI (Kg/m ²)	-0.061	-0.116
TC (mg/dL)	0.155	-0.023
TG (mg/dL)	-0.070	-0.090
HDL-c (mg/dL)	0.152	0.201**
LDL- c (mg/dL)	0.143	-0.092
VLDL-c (mg/dL)	-0.072	-0.091
TC/HDL ratio	-0.018	-0.223 **
LDL/HDL ratio	0.011	-0.226 **

**Correlations was significant at the $p < 0.05$ level (two-tailed).

The ANCOVA analysis performed with vitamin D as dependent variable revealed that there is a significant association of BMI ($p = 0.04$), waist circumference ($p = 0.004$), TC/HDL-c ratio ($p = 0.026$), HDL-c ($p = 0.001$) (**Figure 1a and 1b**) and triglycerides (TG) ($p = 0.014$) and 25(OH)D concentrations after controlling for age, gender, physical activity, city, outdoor physical activity, season of blood collection, vitamin D intake and alcohol intake.

Figure 1a. Lipids profile according to 25(OH)D concentrations in Spanish Elderly.

HDL-c group was considered in two groups inadequate levels (< 40mg/dl) and adequate levels(> 60mg/dl). TC/HDL-c ratio was considered in two groups :Low risk (<3.5 mg/dl for men;<3.4 mg/dl for women), higher risk (≥ 3.5 mg/dl for men; ≥ 3.4 mg/dl for women)

Figure 1b. Body composition measurement according to 25(OH)D concentrations in Spanish Elderly.

*Waist circumference was considered cutoff for European people: Men > 94 cm and women >80cm. Adapted from Zimmet e Alberti (2006).

The ANCOVA analysis with vitamin D as independent variable and controlling for the same confounders, found a significant association of vitamin D status (deficiency, insufficiency and sufficiency) and HDL-c, Total cholesterol/HDL, LDL/HDL (all $p < 0.05$) (Figure 2a, 2b), but only a borderline association was observed with body composition parameter as fat mass in tertiles and BMI (both $p = 0.05$).

Figure 2. LDL/HDL ratio, TC/HDL ratio (a) and HDL-c (b) according to vitamin D status in Spanish Elderly.

In the association between 25(OH)D concentrations and body composition the linear regression showed that city, season, physical activity, waist circumference and total muscle mass were independently associated with 25(OH)D concentrations after adjustment for age, gender, outdoor physical activity, vitamin D intake, alcohol intake and smoking habits, **Table 3.**

In the same way, **Table 4** shows that city, physical activity, season, alcohol consumption and HDL-c also were significant and independent associated with serum 25(OH)D levels. As shown in the models, city, physical activity, and HDL-c were correlated positively with 25(OH)D concentration and season was negatively correlated, explaining 11% of the variance.

Table 3. Multivariate linear regression analysis of the association between serum 25(OH)D concentrations and body composition

	β	R^2	p
Model 1			
City	0.281		0.000
Physical Activity	0.186	0.103	0.000
Waist circumference	-0.125		0.012
Model 2			
City	0.267		0.000
Physical Activity	0.161		0.003
Waist circumference	-0.187	0.116	0.001
Total muscle mass (Kg)	0.128		0.026
Model 3			
City	0.261		0.000
Physical Activity	0.155		0.004
Waist circumference	-0.169	0.126	0.003
Total muscle mass (Kg)	0.122		0.033
Season	-0.101		0.044

β , standardized regression coefficients.

All body composition measure entered in the model as covariates.

Table 4. Multivariate linear regression analysis of the association between serum 25(OH)D concentrations and lipid profile.

	β	R^2	p
Model 1			
City	0.286		0.000
Physical Activity	0.188	0.104	0.000
HDL-c	0.144		0.004
Model 2			
City	0.276		0.000
Physical Activity	0.180		0.001
HDL-c	0.135	0.11	0.007
Season	-0.112		0.025

β , standardized regression coefficients;
All lipids profile measurement entered in the model as covariates

The unadjusted logistic regression revealed that those who have inadequate HDL-c levels (<40mg/dl) have 1.7 times higher odds for vitamin D deficiency than those who have adequate HDL-c levels (>60mg/dl) (95% CI= 1.10 to 2.85 $p= 0.017$) and LDL/HDL ratio was inversely associated to vitamin D status (95% CI= 0.473 to 0.888, $p =0.07$). The waist circumference was negatively associated with vitamin D status odds ratio of 0.98 (0.96 to 1.00; $p= 0.04$), **Table 5**.

Table 5. Logistic regression analysis of vitamin D deficiency status in Spanish elderly.

	β	OR (95% CI)	<i>p</i>
Body composition			
Waist circumference	-0.019	0.981 (0.963 to 1.00)	0.049
Lipid Profile			
HDL-c groups	0.574	1.776 (1.107 to 2.851)	0.017
LDL-c/HDL-c ratio	-0.434	0.648 (0.473 to 0.888)	0.007
TC/HDL-c ratio	0.570	1.769 (1.123 to 2.785)	0.014

All controlling for age and gender; each variable was entered in the analysis alone.

Vitamin D status was considered: deficiency <30 ng/ml and sufficiency $\geq$ 30 ng/ml)

HDL-c group was considered in two groups inadequate levels (< 40mg/dl) and adequate levels (> 60mg/dl). TC/HDL-c was considered in two groups: low risk (<3.5 mg/dl for men; <3.4 mg/dl for women), higher risk ($\geq$ 3.5 mg/dl for men; $\geq$ 3.4 mg/dl for women).

Discussion

Some studies have reported low vitamin D status in European elderly²⁴⁻²⁶. This fact, together with weight gain and ageing is correlated to cardiometabolic condition^{10,27}. In our sample, almost 68% show low 25(OH) serum (deficiency or insufficiency) concentrations and TC/HDL-c, important cardiovascular parameters, was significantly lower in vitamin D sufficient subjects.

The relationship between vitamin D and lipid profile has been reported⁹, but only few studies have investigated this relationship in apparently healthy European elderly^{7,28-30}. A significant negative correlation was found between 25(OH)D concentrations and LDL/HDL, TC/HDL and a significant positive correlation with HDL, all in females.

Similar results were found in the Framingham population¹⁰, in Norwegians¹¹, US citizens with cardiovascular diseases³¹ and Japanese men³². The positive correlation between 25(OH) D and HDL-c was reported in some cross-sectional studies, included in European⁹. It has been postulated that vitamin D could influence directly and indirectly lipid metabolism. One of the mechanisms, reported in an in vitro study, is through the suppression of PTH secretion that may reduce lipolysis³³. Another possible mechanism is the increase of calcium absorption increasing the formation of insoluble calcium-fatty complexes in the intestine, thus reducing the absorption of fatty acids. Consequently, this may lead to a reduction in total cholesterol and LDL-c levels³⁴. Vitamin D also can indirectly influence lipid metabolism through its role in insulin sensitivity and insulin secretion³⁵.

Results of the two ANCOVA showed that after controlling for some confounder factors, HDL, TC/HDL, LDL/HDL and TG were associated with vitamin D concentrations. Besides, multiple linear regression showed that HDL-c was positive and independently correlated with 25(OH)D concentrations. In the studies of Kim et al. (2008)³¹ and Jorde et al (2010)¹¹, both with more than 8000 subjects, similar findings after extensive adjustments for confounders like age, gender, sample month collected and BMI were observed. In addition, TG was also related to vitamin D concentrations^{11,28,30}.

Our results also showed that those who have higher values in the LDL/HDL ratio and the TC/HDL-c ratio had 0.64% and 1.76% more odds of vitamin D deficiency, respectively. On the other hand, subjects with inadequate HDL-c levels had 1.77 % more odds of vitamin D deficiency. It is recognized that vitamin D may also affect lipid levels, since vitamin D is considered essential for the maintenance of adequate levels of apolipoprotein A-I, a major component of HDL-c, consequently to modify TG and LDL-c levels³⁶. These results confirm that vitamin D concentrations may exert a beneficial role in lipid profile.

Body Composition

The association between 25(OH)D concentrations and obesity has been reported, moreover, low vitamin D status has been related to higher body fat in cross-sectional, genetic and interventional studies^{12,13,37}. Our results shown a negative correlation between 25(OH)D concentrations and waist circumference. After controlling (for age, gender, physical activity, city, outdoor physical activity, season blood collection, vitamin D intake and alcohol intake), higher BMI and waist circumference were related to lower 25(OH)D concentrations. Recently, studies have shown inverse associations between 25(OH)D concentrations and body composition measurements among various ethnic groups around the world^{4,38,39}. A Mendelian randomization study performed with 42,024 adults evidenced that each 1 kg / m² of BMI was associated with a reduction of 1.15% in 25(OH)D concentrations³⁷. Furthermore, an interventional study with data from 17,614 healthy adults showed pronounced differences across BMI categories; obese subjects and overweight subjects had serum 25(OH)D levels on average 19.8 nmol/L and 8.0 nmol/L lower than normal weight subjects, respectively⁴⁰. The most recognized possible mechanisms that may explain the inverse correlation between vitamin D and adiposity measures is the sequestration of vitamin D by adipocyte. Elevated storage of vitamin D occurs in the larger-than-normal fat depots prior to conversion into 25(OH)D⁴¹, mainly because vitamin D is a fat-soluble molecule causing decreased the bioavailability this vitamin in the circulation.

The linear regression showed that waist circumference was negative and independently associated and total muscle mass was positive and independently associated with 25(OH)D. A receptor for 1,25 dihydroxyvitamin D (1,25(OH)₂D)⁴², and genotypic variations for this receptor have been associated with differences in muscle strength, due to vitamin D may have beneficial effect on muscle^{43,43}.

Additionally, Mawer et al. (1972) have shown that 25(OH)D is stored 20% in muscle and 33% in fat, featuring the muscle as another reservoir of vitamin D in humans⁴⁵.

We also found that higher WC was associated with 0.98% more odds of vitamin D deficiency. One of the main mechanisms by which vitamin D may act in human adipose tissue is via the expression of vitamin D-metabolizing enzymes such as 25-hydroxylase CYP2J2, CYP27B1^{46,47}. This capacity to metabolize vitamin D locally was demonstrated when, after weight loss in obese subjects, plasma 25(OH)D increased and expression levels of 25-hydroxylase (CYP2J2) and 1 α -hydroxylase (CYP27B1) declined at subcutaneous adipose tissue of these subjects⁴⁸. All evidence suggests that 25(OH)D and its metabolites could be a target for adipose tissue, through the endocrine system as well as autocrine/paracrine actions of vitamin D^{47,49}.

Finally, in this study, both regressions showed that city, season and physical activity were also predictors of 25(OH) D concentrations. Studies have shown that sun exposure and physical^{50,51} activity were correlated to better vitamin D status in older people; moreover the photosynthesis of vitamin D₃ is dependent of some factors as latitude and season⁵². This studies confirm our data because of subjects from Palma de Mallorca (a seaside city) can be more exposed to solar radiation, probably because their go to the beach more had higher levels of 25(OH)D concentrations than subjects from Madrid.

The strengths of this study were the use of objective measure physical performance, the sampling procedure and the strict standardization of the field work among the cities involved in the study avoided to a great extent of confounding bias.

The main limitation of this study was not possible to evaluate PTH levels and consider its impact on adiposity and lipogenesis. Also, this study has a cross-sectional design, thus the inference of causality cannot be evaluate.

Conclusion

Our findings showed that vitamin D concentration was positive and independently correlated with HDL-c and total muscle mass, as well as negative and independently correlated with WC, LDL/HDL and TC/HDL independently of age, gender and some confounders. In our study, the body composition and lipid profile have a significant influence on vitamin D concentration. Subjects with higher vitamin D concentration had better lipid profile and better body composition (lower WC and BMI and higher total muscle). There are a number of properly designed randomized trials regarding vitamin D status but few in Spanish elderly. Therefore, these studies are necessary for further elucidate and clarify these associations and lead to better clinical practice aiming at decreasing hypovitaminosis D and its comorbidities in this population.

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6. CONSIDERAÇÕES FINAIS

Com os resultados dos artigos que integram essa tese, verificou-se que a adiposidade interage com as concentrações séricas de vitamina D, possivelmente modulando tanto a resposta inflamatória quanto a homeostase da glicose e o perfil lipídico. Entretanto, essa interação também pode ocorrer de forma independente da composição corporal.

Em adultos, nossos resultados demonstraram que em indivíduos eutróficos as concentrações séricas vitamina D foram associadas negativamente com alguns biomarcadores inflamatórios, sugerindo que esta vitamina pode ser relevante no perfil inflamatório, mesmo sem a presença do excesso de peso. Este resultado confirma a provável ação da vitamina D na inibição da ativação do NF- κ B, regulando a liberação de citocinas pró-inflamatórias.

A associação das concentrações séricas de vitamina D com o IMC e/ou com a CC ocorreu independentemente da faixa etária e do gênero, entretanto, a modulação com o perfil metabólico (homeostase da glicose, perfil lipídico e inflamação) foi ressaltada em indivíduos sem excesso de peso. Este fato é relevante, pois demonstra que a vitamina D pode e deve ser um fator coadjuvante na prevenção das complicações metabólicas decorrentes do excesso de peso, contudo, também possui uma ação importante no perfil metabólico de indivíduos sem excesso de peso, inclusive adolescentes.

Adicionalmente, nos adolescentes, a deficiência de vitamina D foi associada com o aumento no IMC, corroborando que esse é um grupo de risco para ambas as epidemias e possivelmente o crescimento global dessas epidemias estão correlacionados.

Finalmente, nossos resultados ratificam e aprofundam o conhecimento sobre a importância da manutenção de concentrações adequadas de vitamina D em diferentes populações e fases da vida, demonstrando que a vitamina D pode estar associada de forma independente com as principais complicações metabólicas. Assim, os artigos apresentados fornecem evidências que podem contribuir na formulação de intervenções e ações eficazes na prevenção e controle da deficiência e insuficiência de vitamina D no que concerne a composição corporal e fatores correlatos.

7. REFERÊNCIAS (introdução e metodologia)

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ANEXOS

ANEXO I. RECORDATÓRIO DE 24 HORAS DO ESTUDO PHYSMED

imFine
Grupo de Investigación
en Nutrición, Ejercicio
y Estilo de Vida Saludable

ciberobn
Centro de Investigación Biomédica en Red
Fisiopatología de la Obesidad y Nutrición

DETERMINANTES DE RIESGO DE PRIMEROS EVENTOS CARDIOVASCULARES

SUBPROYECTO 02. ANTIOXIDANTES Y ESTRÉS OXIDATIVO

Primer recordatorio 24 horas

NÚMERO

Nombre: _____

Apellidos: _____

Dirección: _____

Población: _____ C

Código Postal: Teléfono: _____

Fecha en que se realiza la entrevista: / 2014

Hora

de la entrevista: :

Nombre del entrevistador: _____

8	8	0	0	0			
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RECUERDO DE 24 HORAS

CODIGO: _____

FECHA ____/____/____

ALIMENTOS Y SUPLEMENTOS CONSUMIDOS					
LUNES	MARTES	MIERCOLES	JUEVES	VIERNES	SÁBADO
DOMINGO1					
DESAYUNO	ALIMENTOS Y SUPLEMENTOS (ingredientes del menú)		Cantidad (g) o tamaño de las porciones		
Hora de inicio:					
Hora de finalización:					
Lugar:					
Menú:					
MEDIA MAÑANA					
Hora de inicio:					
Hora de finalización:					
Lugar:					
Menú:					
COMIDA					
Hora de inicio:					
Hora de finalización:					
Lugar:					
Menú:					
MERIENDA					
Hora de inicio:					
Hora de finalización:					
Lugar:					
Menú:					

8	8	0	0	0			
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CENA		
Hora de inicio:		
Hora de finalización:		
Lugar:		
Menú:		
ENTRE HORAS		
Hora de inicio:		
Hora de finalización:		
Lugar:		
Menú:		

¹ Tachar el día de la semana al que corresponde la encuesta

8	8	0	0	0			
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9. Indique cuánto tiempo dedica a cada una de las siguientes actividades:

	Caminar	Estar sentado	Tareas del hogar
Menos de 1 hora	(1) <input type="text"/>	<input type="text"/>	<input type="text"/>
Entre 1 y 2 horas	(2) <input type="text"/>	<input type="text"/>	<input type="text"/>
Entre 2 y 3 horas	(3) <input type="text"/>	<input type="text"/>	<input type="text"/>
Entre 3 y 4 horas	(4) <input type="text"/>	<input type="text"/>	<input type="text"/>
Entre 4 y 5 horas	(5) <input type="text"/>	<input type="text"/>	<input type="text"/>
Más de 5 horas	(6) <input type="text"/>	<input type="text"/>	<input type="text"/>

10. ¿Toma medicación de manera habitual?

Si ☐ (1)

No ☐ (0)

En caso afirmativo indique cuál (grapar a esta hoja los folletos):

Nombre del medicamento	Laboratorio fabricante	Nombre principio activo	Cantidad (g, mg, ml)	Dosis que toma	Frecuencia (día, semana, mes)

11A. ¿Actualmente fuma?

Si ☐ (1)

No ☐ (0)

11. B ¿Ha fumado?

Si ☐ (1)

No ☐ (0)

12A. ¿Cuántos cigarrillos al día?

- Menos de 5 cigarrillos (1)
 Entre 5 y 10 cigarrillos (2)
 Entre 10 y 15 cigarrillos (3)
 Entre 15 y 20 cigarrillos (4)
 Más de una cajetilla diaria (5)

12B. ¿Cuántos cigarrillos fumaba al día?

- Menos de 5 cigarrillos (1)
 Entre 5 y 10 cigarrillos (2)
 Entre 10 y 15 cigarrillos (3)
 Entre 15 y 20 cigarrillos (4)
 Más de una cajetilla diaria (5)

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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- 13A. ¿Toma bebidas alcohólicas de manera habitual (incluye cerveza y vino)?

Si ☐ (1) No ☐ (0)

Page 10

- 13B. ¿Toma bebidas alcohólicas de manera habitual (incluye cerveza y vino)?

Si ☐ (1) No ☐ (0)

Page 10

- 14A. ¿Cuánta cantidad tomas? _____

72

- 14B. ¿Cuánta cantidad tomabas? _____

Page 10

15. ¿Vive sólo? Si ☐ (1) No ☐ (0)

Page 10

16. ¿Con quién?

Cónyuge (1)
 Hijo/a (2)
 Hermano/a (3)
 Otro (4)

Indicar cuál:

11

17. ¿Tiene su casa ascensor? Si ☐ (1) No ☐ (0)

10

18. ¿En qué piso vive? _____

11

19. ¿Qué estudios tiene?

No sabe leer ni escribir	(1)
Estudios primarios	(2)
Estudios secundarios	(3)
Estudios universitarios	(4)

11

20. ¿Cuál es su nivel de renta actual?

¿Renta propia? Si ☐ (1) No ☐ (0)

10

Menos de 600 €/mes		(1)
Entre 600 y 900 €/mes		(2)
Más de 900 €/mes		(3)

10

CUESTIONARIO DE SALUD EUROQOL-5D (EQ-5D)

Marque con una cruz la respuesta de cada apartado que mejor describa su estado de salud en el día de hoy.

21. MOVILIDAD:

No tengo problemas para caminar		(1)
Tengo algunos problemas para caminar		(2)
Tengo que estar en la cama		(3)

11

22. CUIDADO PERSONAL:

No tengo problemas con el cuidado personal		(1)
Tengo algunos problemas con el cuidado personal		(2)
Soy incapaz de ocuparme de mi cuidado personal		(3)

10

[illegible]

23. ACTIVIDADES COTIDIANAS:

(p.e. trabajar, estudiar, hacer las tareas domésticas, actividades familiares o durante el tiempo libre).

No tengo problemas para realizar mis actividades cotidianas ☐ (1)

Tengo algunos problemas para realizar mis actividades cotidianas	(2)
--	-----

Soy incapaz de realizar mis actividades cotidianas		(3)
--	--	-----

24. DOLOR / MALESTAR:

No tengo dolor ni malestar ☐ (1)

Tengo moderado dolor o malestar	(2)
---------------------------------	-----

Tengo mucho dolor o malestar		(3)
------------------------------	--	-----

25. ANSIEDAD O DEPRESIÓN:

No estoy ansioso o deprimido ☐ (1)

Estoy moderadamente ansioso o deprimido	(2)
---	-----

Estoy muy ansioso o deprimido	(3)
-------------------------------	-----

26. Comparado con mi estado general de salud durante los dos últimos 12 meses, mi estado de salud hoy es:

Mejor (1)

Igual	(2)
-------	-----

Peor		(3)
------	--	-----

[illegible]

27. Para ayudar a la gente a describir lo bueno o malo que es su estado de salud hemos dibujado una escala parecida a un termómetro en la cual se marca con un 100 el mejor estado de salud que se pueda imaginar y con un 0 el peor estado de salud que se pueda imaginar.

Nos gustaría que nos indicara en esta escala, en su opinión, lo bueno o lo malo que es su estado de salud en el día de hoy.

Por favor, dibuje una línea desde el casillero donde dice "Su estado de salud hoy" hasta el punto del termómetro que en su opinión indique lo bueno o lo malo que es su estado de salud en el día de hoy.

El mejor estado de salud imaginable

100

90

80

70

60

50

40

30

20

10

0

El peor estado de salud imaginable

ANEXO III- QUESTIONARIO DE ATIVIDADE FISICA DE MINESSOTA

**DETERMINANTES DE RIESGO DE PRIMEROS EVENTOS
CARDIOVASCULARES
SUBPROYECTO 02. ANTIOXIDANTES Y ESTRÉS OXIDATIVO.**

CUESTIONARIO SOBRE ACTIVIDAD FÍSICA

Nombre: _____

Apellidos: _____

Dirección: _____

Población:

Código Postal:

Teléfono: _____

Fecha en que se realiza la entrevista: / / 2014

Hora de la entrevista: :

Nombre del entrevistador: _____

[illegible]

ACTIVIDAD FÍSICA

A continuación encontrará un cuadro con un listado de actividades físicas y unas columnas con períodos de tiempo de realización de las mismas (semana, mes, trimestre y año). Cada columna está dividida en días y minutos. La forma de rellenar el cuestionario es la siguiente:

1. Se lee atentamente cada actividad una a una y cuando se encuentre una que se haya realizado durante la última semana, con números claros y sin salirse del recuadro, se rellenan las casillas correspondientes a los días y minutos.

2. Seguidamente se repite la misma acción para el último mes, el último trimestre y el último año.

Ha de tener en cuenta que si ha realizado alguna actividad la última semana supone también que la ha realizado el último mes, trimestre y año.

Para asegurar la uniformidad de la información recogida consideramos que:

- cada piso de escaleras = ½ min
- una vuelta en esquí acuático = 5 min
- un set de tenis individual = 20 min
- un set de tenis de dobles = 15 min
- golf 9 hoyos = 90 min

Ejemplo:

Una persona que:

- Durante la última semana ha ido a caminar media hora cada día menos el fin de semana, tiene que anotar un 5 en la columna de días de práctica a la semana y 30 en minutos/día de práctica. Si durante el último año también ha ido a caminar pero durante 2 meses en el verano no ha hecho esta actividad, tendrá que anotar 200 en la columna de días de práctica al año y 30 en minutos/día de práctica.
- Durante la última semana ha subido 2 veces al día 2 pisos por la escalera, tiene que anotar un 7 en la columna de días de práctica a la semana y 2 a minutos/día de práctica. Si esta actividad la repite todo el año, tendrá que anotar 365 en la columna días de práctica al año y 2 en minutos/día de práctica.

[illegible]

ANEXO IV-APROVAÇÃO DO COMITÊ DE ÉTICA -ESTUDO ISA-CAPITAL **COMITÊ DE ÉTICA EM PESQUISA - COEP**Faculdade de Saúde Pública
Universidade de São Paulo

OF.COEP/420/10

7 de dezembro de 2010.

Prezada Professora,

O Comitê de Ética em Pesquisa da Faculdade de Saúde Pública da Universidade de São Paulo - COEP **analisou**, em sua **10ª/10 Sessão Ordinária**, realizada em **03/12/2010**, de acordo com a Resolução n.º 196/96 do Conselho Nacional de Saúde - CNS e suas complementares, o protocolo de pesquisa n.º 2001, intitulado **"HOMOCISTEÍNA, POLIMORFISMOS DO GENE MTHFR, FATORES DIETÉTICOS E RISCO CARDIOVASCULAR: ESTUDO DE BASE POPULACIONAL - ISA-CAPITAL"**, área temática **GRUPO III**, sob responsabilidade da Professora Doutora **Dirce Maria Lobo Marchioni**, considerando **HOMOLOGADO** o Termo de Consentimento Livre e Esclarecido re-encaminhado conforme as recomendações da Comissão Nacional de Ética em Pesquisa em seu ofício CONEP/CNS/MS n.º 3023/10.

Cabe lembrar que, conforme a Resolução CNS 196/96, são deveres do(a) pesquisador(a):
1. Comunicar de imediato qualquer alteração no projeto e aguardar manifestação deste Comitê de Ética em Pesquisa para dar continuidade à pesquisa; 2. Manter sob sua guarda e em local seguro, pelo prazo de 5 (cinco) anos, os dados da pesquisa, contendo fichas individuais e todos os demais documentos recomendados pelo COEP, no caso eventual auditoria; 3. Comunicar formalmente a este Comitê quando houver o encerramento da pesquisa; 4. Elaborar e apresentar relatórios parciais e final; 5. Justificar perante o COEP interrupção do projeto ou a não publicação dos resultados.

Atenciosamente,

Claudio Leone
Professor Titular

Coordenador do Comitê de Ética em Pesquisa - FSP/USP

Ilm.ª Sr.ª
Prof.ª Dr.ª **Dirce Maria Lobo Marchioni**
Departamento de Nutrição
Faculdade de Saúde Pública da USP

ANEXO V- APROVAÇÃO DO COMITÊ DE ÉTICA- ESTUDO HELENA

Fax env. por: 976488338

Grupo Estudio HELENA

15/06/06

11:33

Pg: 22/32

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)
Avda. Gómez Laguna, 25 planta 3
50009 Zaragoza

ZARAGOZA

(11)

HELENA

Dr. Luis Alberto Moreno Aznar
E.U. Ciencias de la Salud
Universidad de Zaragoza
C/ Domingo Miral s/n
50009 Zaragoza

Zaragoza, 17 de febrero de 2006

Ref Estudio PI 06/01

En la reunión del CEIC de Aragón celebrada el día 15 de febrero de 2006 (acta 03/2006) se evaluó el proyecto de investigación titulado "Estilos de vida saludables en Europa a través de la nutrición en adolescentes europeos de 13-16 años. Estudio transversal (HELENA-CSS)", que usted presentó como investigador principal.

Tras revisar la documentación aportada este Comité decide aprobar la realización de dicho proyecto. Le adjunto el certificado de aprobación y la lista de asistentes a la reunión del día 15 de

Reciba un cordial saludo,

María González Minjós *

Secretaria del CEIC de Aragón

Fax env. por: 976400338

Grupo Estudio HELENA

15/06/06

11:33

Pg: 23/32

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)Avda. Gómez Laguna, 25 planta 3
50009 Zaragoza

Dña. María González Hinjos, Secretaria del Comité Ético de Investigación Clínica de Aragón,

CERTIFICA

Que el proyecto de investigación titulado “Estilos de vida saludables en Europa a través de la nutrición en adolescentes europeos de 13-16 años. Estudio transversal (HELENA-CSS)”.

Investigador Principal: **Dr. Luis Alberto Moreno Aznar**

Ha sido evaluado por este Comité y tras la revisión de la documentación aportada, este CEIC resuelve **AUTORIZAR** la realización del estudio, según consta en el acta 03/2006, de 15 de febrero de 2006, y en este sentido se informa al Investigador Principal del proyecto.

Lo que firmo en Zaragoza, a 17 de febrero de 2006.

María González Hinjos

ANEXO VI-APROVAÇÃO DO COMITÊ DE ÉTICA- ESTUDO PHYSMED

INFORME QUE EMITE EL COMITÉ DE ÉTICA DE LA UNIVERSIDAD POLITÉCNICA DE MADRID

A petición del Vicerrector de Investigación de la Universidad Politécnica de Madrid, D. Roberto Prieto López, de un dictamen sobre los aspectos éticos del

Proyecto **“Determinantes de riesgo de primeros eventos cardiovasculares. Un estudio coordinado de casos y controles anidado de la cohorte PREDIMED. Subproyecto 02. Antioxidantes y estrés oxidativo.”**, cuyo investigador principal es D^a Marcela Gonzalez Gross, del Departamento de Salud y Rendimiento Humano/ImFine, de la Facultad de Ciencias de la Actividad Física y del Deporte (INEF), tras los pertinentes análisis, estudios y consultas, la Comisión emite el siguiente informe:

En la información remitida queda reflejado que, por un lado, se informa a las personas participantes de manera comprensible de los objetivos del estudio y de las pruebas a las que van a ser sometidas. Todas ellas serán mayores de edad con plena capacidad para firmar el consentimiento informado. Por otro lado, el responsable del proyecto asegura conocer los procesos y protocolos relativos a la protección de datos,

Por tanto, no hay inconveniente en **informar favorablemente la propuesta**, recomendando el efectivo cumplimiento de los compromisos declarados.

Y para que así conste, firmo el presente informe en Madrid a 21 de mayo de dos mil trece.

El Presidente del Comité

Vicente Ortega Castro

El Secretario del Comité

Julián Cabrera Quesada

ANEXO VII

ARTIGO DE REVISÃO PUBLICADO NO JOURNAL OF OBESITY AND METABOLIC
RESEARCH

REVIEW ARTICLE

Year : 2015 | Volume : 2 | Issue : 3 | Page : 161–166

The role of Vitamin D in obesity and inflammation at adipose tissue

Wyslenny Nascimento de Souza, Ligia Araujo Martini

Department of Nutrition, School of Public Health, University of São Paulo, Brazil Ave. Dr. Arnaldo, 715. Zip code: 01248 904, São Paulo, Brazil

Correspondence Address:

Wyslenny Nascimento de Souza

Ave. Prof Mello de Moraes, 1235, BL G Apto 409. Zip Code: 05508 030, São Paulo
Brazil

Abstract

The prevalence of obesity and vitamin D deficiency has increased in the last decade, becoming pandemics. In obesity, macrophage accumulation occurs in the adipose tissue. This is associated with a low-grade chronic inflammation and leads to the release of inflammatory cytokines. Vitamin D was found to have anti-adipogenic activity and may exert immunoregulatory effects as well as reduce the adipose tissue inflammation. Recent studies suggest that vitamin D and vitamin D receptor (VDR) play an important role in adipose tissue whereas the expression of genes of the 25-hydroxyvitamin D 1 α -hydroxylase (CYP27B1) and VDR were demonstrated in human adipocytes. Growing evidence suggests vitamin D also plays a role in the type of preadipocytes, and the proneness to the inflammatory process.

How to cite this article:

de Souza WN, Martini LA. The role of Vitamin D in obesity and inflammation at adipose tissue. J Obes Metab Res 2015;2:161-166

8- ARTIGO PUBLICADO NA NUTRITION RESEARCH

Original Research

Association between 25-hydroxyvitamin D and inflammatory biomarker levels in a cross-sectional population-based study, São Paulo, Brazil ☆☆☆☆

Wysllenny N. de Souza, Marina M. Norde, Érica Oki, Marcelo M. Rogero, Dirce M.L. Marchioni, Regina M. Fisberg, Lígia A. Martini *

Nutrition Department, School of Public Health, University of São Paulo, Ave. Dr. Arnaldo, 715, 01246-904 São Paulo, Brazil

ARTICLE INFO

Article history:

Received 27 May 2015

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Accepted 21 October 2015

Keywords:

25(OH)D

Inflammatory biomarkers

sICAM-1

BMI

Vitamin D status

ABSTRACT

Besides the classic vitamin D function on bone homeostasis, there are bodies of evidence showing that adequate status of vitamin D can modulate inflammation. We hypothesized that higher plasma levels of 25-hydroxyvitamin D (25(OH)D) would correlate with lower plasma levels of proinflammatory cytokines, acute-phase proteins, and soluble adhesion molecules and higher plasma levels of anti-inflammatory cytokines. We included all adults (age, 20–59 years) of the population-based, cross-sectional study, Health Survey-São Paulo, conducted in São Paulo (Brazil) in the study (n = 281). Anthropometric parameters, blood pressure measurements, and a fasting blood sample were collected by trained fieldworkers. Serum 25(OH)D concentration, plasma inflammatory biomarker levels (C-reactive protein, interleukin [IL]-1 β , IL-6, IL-8, IL-10, tumor necrosis factor [TNF] α , IL-12p70, adiponectin, monocyte chemoattractant protein-1, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1), and plasma blood lipid parameters were evaluated. The prevalence of vitamin D inadequacy (<50 nmol/L) was 65.5%. Inadequate participants were younger, with lower body mass index (BMI), systolic and diastolic blood pressures, triglyceride, and total cholesterol levels as well as low-density lipoprotein cholesterol, compared with individuals adequate for vitamin D status. After adjustment, plasma concentration of soluble intercellular adhesion molecule-1 was statistically higher among adequate participants. Stratifying for BMI categories, a negative association was observed between plasma IL-6 and TNF- α levels and serum 25(OH)D concentration in

Abbreviations: 1,25(OH) $_2$ D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein cholesterol; I κ B- α , NF- κ B inhibitor α ; IL, interleukin; LDL-c, low-density lipoprotein cholesterol; MCP-1, soluble monocyte chemoattractant protein-1; NF- κ B, nuclear factor κ B; SBP, systolic blood pressure; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF- α , tumor necrosis factor α .

* This study was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant Nos. 2012/20401-7, 2013/01741-4, and 2013/01740-8).

** W.N.S., M.M.N., and E.O. were responsible for analysis, interpretation, and drafting of the manuscript. M.M.R., L.A.M., D.M.L.M., and R.M.F. provided valuable advice and performed critical review of the manuscript. All authors read and approved the final manuscript.

* The authors have no conflicts of interest to declare.

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<http://dx.doi.org/10.1016/j.nutres.2015.10.006>

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ANEXO IX- COMPROVANTE DE SUBMISSÃO AO
JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

Date: 01/03/2016
To: "Wyslenny Nascimento Souza" lenny.souzaa@gmail.com
From: "JCEM" jcem@endocrine.org
Subject: JCEM / Submission Confirmation for Hypovitaminosis D in adolescents: an independent association of adiposity and glucose homeostasis

Dear Dr. Souza,

Your submission entitled "Hypovitaminosis D in adolescents: an independent association of adiposity and glucose homeostasis" has been received by The Journal of Clinical Endocrinology & Metabolism

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is <http://jcem.edmgr.com/>.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to this journal.

The Journal Office

The Journal of Clinical Endocrinology & Metabolism
jcem@endocrine.org

CURRÍCULO LATTES

Wyslenny Nascimento de Souza

Endereço para acessar este CV: <http://lattes.cnpq.br/1875754904954640>

Última atualização do currículo em 29/10/2015

Possui graduação em Nutrição pelo Centro Universitário Maurício de Nassau (Uninassau) e mestrado em Ciências dos Alimentos pela Universidade de São Paulo (USP). Atualmente é doutoranda em Nutrição em Saúde pública pela Universidade de São Paulo, tendo realizado um doutorado sanduíche no IMFINE research group na Universidad Politécnica de Madrid. . **(Texto informado pelo autor)**

Identificação

Nome Wyslenny Nascimento de Souza

Nome em citações bibliográficas SOUZA, W. N.;Souza, Wyslenny Nascimento de;Nascimento de Souza, W;De Souza, WN

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Formação acadêmica/titulação

2014 Doutorado em andamento em Nutrição em Saúde Pública.
Universidade de São Paulo, USP, Brasil.
com **período sanduíche** em Universidad Politécnica de Madrid (Orientador: Marcela González-Gross).
Orientador: Ligia Araujo Martini.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.

2012 Doutorado em andamento em Nutrição em Saúde Pública.
Universidade de São Paulo, USP, Brasil.
Título: Efeitos calcêmicos e hormonais da suplementação da vitamina D em adolescentes com excesso de peso,
Orientador: Ligia Araujo Martini.

2009 - 2011 Mestrado em Ciências dos Alimentos (Conceito CAPES 7).
Universidade de São Paulo, USP, Brasil.
Título: Ingestão de Vitamina K por grupos de diferentes faixas etárias, Ano de Obtenção: 2011.
Orientador: Marilene De Vuono Camargo Pentead.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.
Grande área: Ciências Agrárias

2005 - 2008 Graduação em Nutrição.
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Título: Perfil Nutricional de Pacientes Portadores de Valvulopatias Internados no Pronto Socorro Cardiológico de Pernambuco.
Orientador: Marina de Moraes Vasconcelos Petribú.

Lúgia Araújo Martini

Bolsista de Produtividade em Pesquisa do CNPq - Nível 2 - CA SN - Saúde Coletiva e Nutrição

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Identificação

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Nome em citações bibliográficas MARTINI, LA;Martini, L. A.;Martini, Lúgia A.;Martini, Lúgia Araújo;Araujo Martini, Lúgia;MARTINI, LÚGIA

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Formação acadêmica/titulação

- 1994 - 1998** Doutorado em Nutrição (Conceito CAPES 6).
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Título: Influência dos fatores dietéticos sobre a massa óssea de pacientes litiascos, Ano de obtenção: 1998.
Orientador: Profa Dra Ita Pfefferman Heilberg.
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Palavras-chave: dieta; densidade mineral óssea; litíase renal; hipercalcúria.
Grande área: Ciências da Saúde / Área: Nutrição / Subárea: Bioquímica da Nutrição.
Setores de atividade: Cuidado À Saúde das Pessoas.
- 1991 - 1993** Mestrado em Nutrição (Conceito CAPES 6).
Universidade Federal de São Paulo, UNIFESP, Brasil.
Título: Avaliação da ingestão de nutrientes litogênicos em pacientes litiascos, Ano de Obtenção: 1993.
Orientador: Prof Dr. Nestor Schor.
Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, Brasil.
Palavras-chave: dieta; litíase renal.
Grande área: Ciências da Saúde / Área: Nutrição / Subárea: Análise Nutricional de População.
Setores de atividade: Nutrição e Alimentação.
- 1985 - 1989** Graduação em Nutrição.
Centro Universitário São Camilo - Campus Pompeia, SAO CAMILO, Brasil.

Pós-doutorado e Livre-docência

- 2008** Livre-docência.
Universidade de São Paulo, USP, Brasil.
Título: Metabolismo ósseo: uma abordagem nutricional, Ano de obtenção: 2008.
Palavras-chave: Avaliação Nutricional; densidade mineral óssea; doença óssea.
- 1998 - 2001** Pós-Doutorado.
Jean Mayer Human Nutrition Research Center On Aging At Tufts University.
Bolsista do(a): Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, Brasil.
Grande área: Ciências da Saúde / Área: Nutrição / Subárea: Bioquímica da Nutrição / Especialidade: Biodisponibilidade de Nutrientes.
Grande Área: Ciências da Saúde / Área: Nutrição / Subárea: Bioquímica da Nutrição / Especialidade: Interação Gene Nutriente.