Universidade de São Paulo
Faculdade de Saúde Pública

**Relação entre estado nutricional da vitamina D
e pressão arterial em adultos residentes na
cidade de São Paulo**

Vivian Cristina Garcia

Dissertação apresentada ao Programa de
Pós-Graduação em Nutrição em Saúde
Pública para obtenção do título de Mestre
em Ciências.

Área de Concentração: Nutrição em Saúde
Pública.

Orientadora: Profa. Dra. Lígia A. Martini

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“Há pessoas que transformam o sol numa simples mancha amarela, mas há aquelas que fazem de uma simples mancha amarela, o próprio sol”

Pablo Picasso

RESUMO

Garcia VC. Relação entre estado nutricional da vitamina D e pressão arterial em adultos residentes na cidade de São Paulo [Dissertação de Mestrado] São Paulo. Faculdade de Saúde Pública da USP; 2011.

Introdução – A baixa concentração sérica de vitamina D tem sido associadas com a hipertensão no mundo todo. A hipovitaminose D tem sido observada mesmo na nossa população. **Objetivo** – Investigar as

concentrações séricas de vitamina D e sua associação com a pressão arterial (PA) em indivíduos adultos residentes na cidade de São Paulo.

Métodos – Para esta dissertação foram desenvolvidos dois artigos. Na revisão (artigo 1), foram selecionados artigos indexados nas bases de dados Pubmed, Lilacs e Medline, incluindo estudos realizados no Brasil. O artigo original (artigo 2) descreve o estudo transversal realizado com 332 adultos, 65% mulheres, onde foi avaliada a associação entre vitamina D, paratormônio intacto (PTHi) e PA. Foram feitas: aferição da PA e coleta de medidas antropométricas e amostras sanguíneas. A concentração sérica de 25(OH)D₃ foi mensurada pela técnica de cromatografia líquida de alta eficiência (HPLC). O valor médio de 2 medidas de PA foi considerado para as análises. Os participantes foram divididos em 3 grupos: (1) PA normal; (2) PA elevada; (3) PA normal pelo uso de medicação. A insuficiência de vitamina D foi considerada quando 25(OH)D₃ ≤ 75 nmol/L e o PTHi elevado quando > 65 pg/mL. A relação entre vitamina D, PTHi e PA foi ajustada pelo índice de massa corpórea (IMC), circunferência da cintura (CC) e perfil lipídico. **Resultados** – Na revisão foi enfatizada a relação da vitamina D com doenças cardiovasculares, considerando, inclusive, os diferentes mecanismos fisiológicos propostos. No artigo original, observou-se idade média e desvio padrão de 50 (15) anos, IMC 29 (6) kg/m² e CC 97 (13) cm. Entre os indivíduos avaliados, 75% tinham sobrepeso ou obesidade. PA média foi 129/80 (18/11) mmHg. A concentração média de cálcio sérico foi 9,3 (0,5) mg/dL, PTHi 40,8 (18,7) pg/mL e vitamina D 55,8 (17,1) nmol/L. O

PTHi elevado e a insuficiência de vitamina D estiveram presentes em 12% e 86% da amostra, respectivamente. Não foram observadas diferenças nas prevalências de insuficiência de vitamina D e PTHi elevado entre os grupos de PA. Não foram observadas associações entre vitamina D e PA. Entretanto, uma correlação positiva foi observada entre PTHi e PA sistólica ($r=0,168$; $p=0,002$) e diastólica ($r=0,168$; $p=0,002$), IMC ($r=0,125$; $p=0,023$), CC ($r=0,172$; $p=0,002$) e % de massa gorda ($r=0,158$; $p=0,004$). O PTHi manteve-se correlacionado com a PA mesmo após realização dos ajustes.

Conclusão – A associação entre PTH e pressão arterial, observada por este estudo, acrescenta novas informações com relação ao envolvimento do metabolismo da vitamina D na regulação da pressão arterial. Mais estudos são necessários para esclarecer as vias metabólicas existentes entre metabolismo do PTH, da vitamina D e da pressão arterial.

Descritores: Vitamina D, Paratormônio, Hipertensão arterial sistêmica

ABSTRACT

Garcia VC. Relationship between vitamin D status and blood pressure in adults living at Sao Paulo city. [Master in Science] Sao Paulo. School of Public Health. University of Sao Paulo; 2011.

Background – Low vitamin D has been associated with hypertension worldwide. Hypovitaminosis D has also been observed in our population.

Objective – To evaluate whether vitamin D status are related to blood pressure (BP) in adults living at Sao Paulo city.

Methods – For this dissertation, two articles were developed. In review (article 1), articles indexed in database Pubmed, Lilacs and Medline were selected, including Brazilian studies. Original article (article 2) describe cross-sectional study performed with 332 adults, 65% women, that evaluate the association between vitamin D, intact parathyroid hormone (iPTH) and BP. Anthropometric measurements, BP and a fasting blood sample were obtained. Serum concentration of 25(OH)D₃ was measured by high-performance liquid chromatography (HPLC) technique. Mean value of two measures of BP was considered to analysis. Participants were divided in three categories of blood pressure: (1) normal blood pressure; (2) high blood pressure; (3) normal blood pressure by medication. Vitamin D insufficiency was defined by 25(OH)D₃ ≤ 75 nmol/L, high iPTH > 65 pg/mL. The relationship between vitamin D, iPTH and BP were adjusted for body mass index (BMI), waist circumference (WC), blood lipids.

Results – In review, the relationship of vitamin D with cardiovascular disease was emphasized considering the different physiological mechanisms proposed. In the original article, mean age and standard deviation was 50 (15) years, BMI 29 (6) kg/m², WC 97 (13) cm. Overweight and obesity was present in 75% of individuals. Mean BP was 129/80 (18/11) mmHg. Mean serum calcium concentration was 9.3 (0.5) mg/dL, iPTH 40.8(18.7) pg/mL and vitamin D 55.8 (17.1) nmol/L. Elevated iPTH and vitamin D insufficiency was present in

12% and 86% of the sample, respectively. No differences were observed on prevalence of vitamin D insufficiency and high iPTH among blood pressure groups. No significant association was observed between BP and vitamin D. However, a positive correlation was observed between iPTH and systolic ($r=0.168$; $p=0.002$) and diastolic BP ($r=0.168$; $p=0.002$), BMI ($r=0.125$; $p=0.023$), WC ($r=0.172$; $p=0.002$) and %FM ($r=0.158$; $p=0.004$). The iPTH remained correlated with BP even with adjustments. **Conclusion** – The association between PTH and blood pressure observed in this study adds a new piece of information in literature regarding the involvement of the vitamin D metabolism with blood pressure. More studies are necessary to clarifying the metabolic pathways existing between PTH, vitamin D and blood pressure.

Key Words – Essential hypertension, Vitamin D, Parathyroid hormone

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1. INTRODUÇÃO

1.1 VITAMINA D – METABOLISMO E AVALIAÇÃO

A vitamina D foi descoberta inicialmente como um fator anti-raquítico. É o principal fator necessário para o desenvolvimento e manutenção do tecido ósseo, e para manter a homeostase normal do cálcio e do fósforo. Além disso, evidências recentes têm sugerido o envolvimento desta vitamina em diversos processos celulares, incluindo efeitos na diferenciação e proliferação celular, na secreção hormonal, no sistema imune e em diversas doenças crônicas não transmissíveis (DARWISH e DELUCA, 1993; DELUCA, 1988; REICHEL e cols, 1989; PEREIRA e cols, 2002; FORD e cols, 2005; LIU e cols, 2005).

É encontrada em duas formas: como ergocalciferol (vitamina D₂) produzida pelas plantas, e como colecalciferol (vitamina D₃) produzida por tecido animal através da síntese cutânea sob ação da luz ultravioleta (290 a 310nm) no 7-deidrocolesterol na pele humana (MILLER e PORTALE, 1999). Estima-se que 80 a 90% da vitamina D corpórea é adquirida pela síntese cutânea, e o restante pela ingestão de alimentos que contenham esta vitamina (HOLICK, 1999). A vitamina D é um pró-hormônio biologicamente inativo, que para se tornar ativo deve passar por duas sucessivas hidroxilações, primeiro no fígado, no carbono 25, formando a 25-hidroxivitamina D [25(OH)D₃] denominada calcidiol, depois em outros tecidos como na próstata, tecido mamário e no cólon, mas principalmente nos rins, no carbono 1, formando a forma ativa da vitamina D a 1,25-diidroxivitamina D [1,25(OH)₂D₃] conhecida como calcitriol (MILLER e PORTALE, 1999; HOLICK, 1999).

Fatores como latitude, estação do ano e período do dia exercem influência sobre a produção cutânea de vitamina D. Durante o verão, o 7-diidrocolesterol cutâneo é mais eficientemente convertido a pré-vitamina D₃. A síntese cutânea da vitamina D é maior em regiões de baixa latitude devido

a maior exposição aos raios UVB. (WEBB e cols, 1988). Contudo, reduzidas concentrações de vitamina D plasmática também são observadas em países ensolarados (LIPS e cols, 2001). O uso de filtro solar, a quantidade de melanina na pele, tipos de vestimentas e elevados níveis de poluição podem reduzir a exposição cutânea aos raios UVB e como consequência ocorre diminuição na síntese da vitamina D.

Por outro lado, as fontes dietéticas naturais de vitamina D são limitadas. As principais fontes alimentares, óleo de fígado de bacalhau, salmão, sardinha e fígado não fazem parte do hábito alimentar da população brasileira. De acordo com as recomendações dietéticas propostas pelo Departamento de Agricultura dos Estados Unidos (USDA), em 2010, a ingestão de vitamina D deve atingir 15 µg/d para crianças e adultos, e 20 µg/d para adultos maiores de 70 anos. Dados recentes mostram que o consumo alimentar de vitamina D em nosso país é inferior a recomendação, principalmente em idosos. Em adultos com mais de 40 anos, de todas as regiões brasileiras, a ingestão média de vitamina D foi 1,8 µg/d nos homens e 1,9 µg/d nas mulheres (PINHEIRO e cols, 2009). Em 143 adolescentes saudáveis, de ambos os sexos, a ingestão média foi 3,6 µg/d (PETERS e cols, 2006). Em mulheres idosas com osteoporose, a ingestão média foi 4,2 µg/d (GENARO e cols, 2006). Observa-se também que as médias de ingestão são semelhantes entre as diversas faixas etárias, denotando a ausência de fontes alimentares. Como agravante, a fortificação de alimentos com vitamina D não é obrigatória no Brasil.

O nível individual ou o estado nutricional de vitamina D é mensurado por meio da concentração sérica da 25(OH)D₃. A forma biologicamente ativa da vitamina D, 1,25(OH)₂D₃, não é indicada para este propósito devido a razões como: a) as concentrações séricas da 1,25(OH)₂D₃ são rigidamente mantidas em valores normais; b) a concentração sérica de 25(OH)D₃ é aproximadamente 100 vezes maior em relação à concentração de 1,25(OH)₂D₃ e c) a hidroxilação da 25(OH)D₃ a 1,25(OH)₂D₃ ocorre em diversos tecidos, suprimindo as necessidades locais (MOSEKILDE, 2005).

Entretanto, as concentrações séricas consideradas adequadas ou não ainda são muito discutidas. GRANT e HOLICK (2005), a partir de uma revisão, propuseram que valores de 25(OH)D₃ abaixo de 80 e 50 nmol/L sejam considerados como insuficiência e deficiência, respectivamente.

Adicionalmente, HOLLIS em 2005, considera que a concentração ótima de vitamina D seria aquela necessária para manter o paratormônio (PTH) em concentrações adequadas, visto que a deficiência de vitamina D leva a diminuição do cálcio sérico que, em consequência, estimula as glândulas paratireóides a liberarem o PTH, culminando na elevação da reabsorção renal e óssea do cálcio. Neste sentido, vários estudos têm encontrado um platô de absorção de cálcio e concentrações adequadas de PTH, com concentrações séricas de 25(OH)D₃ próximas a 75 nmol/L (CHAPUY e cols, 1997; DAWSON-HUGHES, 2004; HEANEY, 2004; TANGPRICHA e cols, 2002; VIETH e cols, 2003).

Corroborando com estes pontos de corte, DAWSON-HUGHES e cols em 2005, propuseram níveis ótimos de 25(OH)D₃ para a redução de risco de fraturas – 50 a 80 nmol/L, ou em média 75 nmol/L.

Estudos recentes demonstram presença de hipovitaminose D em vários estágios de vida, mesmo em países ensolarados (LIPS e cols, 2001; HOLICK e cols, 2005; SARAIVA e cols, 2005). GENARO e cols (2007) e PETERS e cols (2009), observaram que 80% das mulheres na pós-menopausa e 59% de adolescentes saudáveis, residentes no estado de São Paulo, apresentavam insuficiência de vitamina D, segundo os pontos de corte de GRANT e HOLICK (2005).

1.2 VITAMINA D E PRESSÃO ARTERIAL

A hipertensão arterial sistêmica, principal responsável pelas doenças cardiovasculares, implica custos médicos e socioeconômicos elevados, decorrentes principalmente de suas complicações e é considerada responsável por cerca de 7,1 milhões de mortes por ano no mundo

(CHOBANIAN e cols, 2003). Segundo a Sociedade Brasileira de Hipertensão (2010), em 2007, aproximadamente 309 mil mortes foram causadas por doenças cardiovasculares e a prevalência estimada de hipertensão arterial sistêmica é acima de 30%, além das altas taxas de hospitalização.

Dados do INTERSALT Study, importante estudo sobre fatores de risco e controle de hipertensão, com mais de 10.000 indivíduos de diversos países, mostram pressão arterial sistólica e diastólica positivamente associada com a distância do equador (INTERSALT COOPERATIVE RESEARCH GROUP, 1988).

Analisando a população acima de 20 anos que participou do NHANES III, SCRAGG e cols, 2007, encontraram pressão arterial sistólica e diastólica, 3,0 e 1,6 mmHg respectivamente menores no maior quintil [$25(\text{OH})\text{D}_3 \geq 85,7$ nmol/L] quando comparados ao menor quintil de vitamina D [$25(\text{OH})\text{D}_3 \leq 40$ nmol/L]. Adicionalmente, MARTINS e cols, 2007, encontraram que, em adultos americanos, a prevalência de hipertensão é 30% maior no menor quartil quando comparada ao maior quartil de vitamina D. A redução de 2 a 3 mmHg na pressão arterial sistólica está associada a uma redução de 10 a 15% na mortalidade por doença cardiovascular (MARTINI & WOOD, 2008).

Recentemente, GIOVANNUCCI e cols, 2008, avaliaram a associação entre as concentrações séricas de $25(\text{OH})\text{D}_3$ e o risco de doença coronariana nos indivíduos (homens) que participaram do Health Professionals Follow-up Study. Homens com deficiência de vitamina D (≤ 15 ng/mL ou 37 nmol/L) apresentam risco significativamente maior de desenvolver infarto do miocárdio quando comparado àqueles com níveis suficientes de vitamina D (≥ 30 ng/mL ou 75 nmol/L) (RR 2,09; 95% CI 1,24-3,54).

A relação negativa entre concentrações séricas de vitamina D e hipertensão foi recentemente demonstrada por pesquisadores da Harvard University (FORMAN e cols, 2007). Os autores avaliaram a associação independente entre $25(\text{OH})\text{D}_3$ medida e o risco de hipertensão por meio de dados prospectivos de dois estudos de coorte: Nurses Healthy Study, realizado com mulheres, e Healthy Professional Follow-up Study, realizado

com homens, ambos com seguimento de 4 a 8 anos. Aos 4 anos de seguimento, o risco relativo para homens com reduzidos níveis de $25(\text{OH})\text{D}_3$ sérica desenvolverem hipertensão foi 6,13 (95% CI 1,00 a 37,80), enquanto que nas mulheres foi 2,67 (95% CI 1,05-6,97). Após 8 anos de seguimento, nos homens o risco relativo foi 3,53 (95% CI 1,02-12,3) e nas mulheres 1,7 (CI 0,92-3,16).

Desde 1987, LIND e cols, através de estudo duplo-cego, placebo-controlado, observaram redução na pressão arterial de 39 indivíduos hipertensos com a suplementação de vitamina D. Essa redução também foi ressaltada em mulheres idosas suplementadas com cálcio e vitamina D (PFEIFER e cols, 2001).

Em outro ensaio clínico, foi observado que administração de $1,25(\text{OH})_2\text{D}_3$ reduziu a pressão arterial, além da atividade da renina plasmática e dos níveis de angiotensina II (KIMURA e cols, 1999). Estudos experimentais demonstraram que a $1,25(\text{OH})_2\text{D}_3$ inibe a expressão do gene da renina no aparelho justaglomerular (LI e cols, 2002), e bloqueia a proliferação de célula vascular muscular lisa (VSMC) (CARTHY e cols, 1989). São vários os estudos que apontam concentrações séricas de $1,25(\text{OH})_2\text{D}_3$ inversamente associados com pressão arterial ou atividade da renina plasmática em normotensos e hipertensos (RESNICK e cols, 1986; BURGESS e cols, 1990; IMAOKA e cols, 1991; LIND e cols, 1995; KRISTAL-BONEH e cols, 1997). Assim a relação vitamina D - hipertensão pode ocorrer via sistema renina-angiotensina e função vascular. Além disso, a $1-\alpha$ hidroxilase, enzima envolvida na conversão da $25(\text{OH})\text{D}_3$ a $1,25(\text{OH})_2\text{D}_3$, tem expressão em diversos tecidos como: células endoteliais, VSMC, além das células renais (MERKE e cols, 1989 e ZEHNDER e cols, 1999), sugerindo um efeito parácrino da $25(\text{OH})\text{D}_3$ independente dos níveis circulantes de $1,25(\text{OH})_2\text{D}_3$. ZHOU e cols, 2008, demonstraram uma regulação do sistema renina-angiotensina através da suplementação de $1,25(\text{OH})_2\text{D}_3$ em ratos knockout -/- para enzima $1-\alpha$ hidroxilase.

2. HIPÓTESE

Indivíduos com baixas concentrações de vitamina D apresentam valores de pressão arterial sistólica e diastólica maiores do que indivíduos com concentrações normais de vitamina D.

3. OBJETIVO

Investigar a associação entre pressão arterial e metabolismo de vitamina D em indivíduos adultos de ambos os sexos com pressão arterial normal e alterada.

4. METODOLOGIA

4.1. DELINEAMENTO DO ESTUDO

Trata-se de um estudo transversal.

4.2. ÁREA ESTUDADA E TAMANHO AMOSTRAL

A amostra foi composta de adultos e idosos de ambos os sexos frequentadores do Centro de Saúde Paula Souza, participantes de Campanhas de Prevenção de Diabetes Mellitus (realizadas no campus da FSP-USP ou em outras unidades da rede pública de saúde) e alguns participantes do ISA-SP, estudo multicêntrico, transversal e de base populacional.

Os participantes foram abordados pessoalmente pelas nutricionistas que auxiliaram na coleta de dados do projeto.

Todos os participantes selecionados foram atendidos no Centro de Saúde Paula Souza, localizado na Faculdade de Saúde Pública da Universidade de São Paulo.

O presente estudo é parte do projeto “Relação entre estado nutricional da vitamina D e síndrome metabólica em adultos residentes na região metropolitana de São Paulo” aprovado pela FAPESP (07/52420-2).

- *Tamanho Amostral*

Para calcularmos o tamanho amostral foi utilizada a estimativa da prevalência de cerca de 20% de hipertensão na população brasileira (PASSOS, 2006). Desta forma, fixando o nível de significância de 5% e o poder do teste em 90%, obtém-se um tamanho amostral de 174 indivíduos

(LEVINE, 2000). Assim, a amostra total considerando os critérios de exclusão, mais as possíveis perdas ou recusas na participação do estudo foi de 400 indivíduos.

No presente estudo, foram excluídos os adolescentes, gestantes e lactantes, indivíduos que tinham ou relataram ter doenças crônicas que, potencialmente alteram o metabolismo da vitamina D ou, estão diretamente ligadas a hipertensão (insuficiência renal crônica, osteoporose, câncer, diabetes, insuficiência cardíaca, infarto, angina, doença coronária congestiva e isquemia cardíaca) e negros. Também foram excluídos indivíduos com valores séricos de cálcio, fósforo e creatinina fora da faixa de referência, indivíduos com valores discrepantes de vitamina D e paratormônio, e aqueles que utilizam suplementos e cálcio e/ou vitamina D ou multivitamínicos.

4.3. COLETA DE DADOS

Para este estudo os participantes realizaram:

- Avaliação antropométrica: peso, estatura, circunferência da cintura e impedância bioelétrica (obtenção do percentual de massa magra e massa gorda).
- Coleta de sangue: vitamina D sérica [25(OH)D₃], cálcio sérico, paratormônio sérico, fósforo sérico, creatinina sérica, glicose sérica de jejum, colesterol total e frações e triacilgliceróis.

4.3.1. Avaliação Antropométrica e da Composição Corporal

- Peso e Estatura

Para avaliação do peso corporal foi utilizada uma balança eletrônica do tipo plataforma, com capacidade para 150 kg, sensibilidade de 100 gramas, da marca TANITA®. Os indivíduos foram pesados com roupas leves

e descalços, posicionados em postura ereta, com os pés inteiramente compreendidos na plataforma da balança, de forma paralela, com braços ao longo do corpo e olhar no horizonte.

Para a aferição da estatura foi utilizado um estadiômetro com escala em milímetros, da marca Seca bodymeter 208[®], a ser fixado na parede. Para esta aferição os indivíduos ficaram de pés juntos, calcanhares encostados na parede, em postura ereta, olhando para frente, sem flectir ou estender a cabeça. O ápice da orelha e o canto externo do olho ficaram em linha paralela, formando um ângulo reto com a barra do estadiômetro. A leitura, em centímetros, foi efetuada depois que a barra horizontal do estadiômetro foi abaixada e apoiada sobre a cabeça.

Estes dados foram utilizados para calcular o Índice de Massa Corporal (IMC), definido como massa corporal em quilos dividido pela estatura em metro elevada ao quadrado (kg/m^2), e classificados de acordo com os critérios propostos pela Organização Mundial de Saúde (WHO 1995). Todos os dados foram coletados por pesquisadores de campo previamente treinados.

- Avaliação da Composição Corporal

As avaliações de massa magra e gordura corporal foram realizadas através da análise de impedância bioelétrica, utilizando um aparelho Quantum BIA – 101Q da marca RJL-101 (Detroit, MI), tetrapolar, com apresentação digital dos valores de resistência (R) e reatância (X_c).

As medidas de impedância bioelétrica foram tomadas do lado direito do indivíduo, que se manteve deitado em posição supina, com os braços abertos em ângulo de 30° em relação ao seu corpo. As pernas não tiveram contato entre si. Calçados e meias foram retirados, e durante o teste o examinado manteve-se imóvel.

O aparelho consta de dois pares de eletrodos compostos por um cabo preto e outro vermelho, onde cada cabo apresenta duas pinças de conexão, uma de cor vermelha e outra de cor preta. No primeiro par, um eletrodo foi

fixado para a entrada da corrente elétrica (injetor) sobre a superfície dorsal do pulso do braço e outro eletrodo para saída da corrente elétrica (detector) foi colocado sobre a terceira falange proximal. O segundo par de eletrodos foi posicionado na superfície anterior do tornozelo de mesmo lado ao colocado no braço, na superfície dorsal do terceiro osso metatarso. Após a colocação dos cabos, o analisador foi ligado e os valores de R e Xc registrados.

Utilizando o software RJL systems Cyprus 1.2 C Body Composition Analysis, quando se aplicam os valores de R, Xc, peso corporal, estatura, sexo e idade do examinado, são apresentados os valores percentuais de massa gorda (%MG) e massa magra (%MM).

Além disso, foi aferida a medida da circunferência da cintura com fita métrica flexível e não extensível, da seguinte forma: a fita métrica foi posicionada sobre o ponto médio entre o último arco costal e a crista ilíaca do indivíduo em pé, e a leitura foi feita no momento da expiração.

Todos os dados foram coletados por pesquisadores de campo previamente treinados.

4.3.2. Coleta de Sangue

Após 12hs de jejum foi realizada coleta de 20 mL de sangue venoso, por profissionais especializados, e com a utilização de materiais descartáveis. O sangue foi particionado em alíquotas em microtubos e imediatamente estocados a uma temperatura de -80 °C até a análise bioquímica.

Metodologia para as dosagens laboratoriais:

- *Vitamina D sérica* [25(OH)D₃]: realizada pela técnica de cromatografia de alta eficiência (high-performance liquid chromatography – HPLC). Resumidamente, a técnica consiste na precipitação e extração da fase sólida em colunas C₁₈. O eluente é evaporado por nitrogênio,

suspendido em fase móvel, e depois injetado no sistema HPLC. Os cromatogramas são detectados por detector UV, e os resultados quantificados por calibrador sérico [25(OH)D₃] e calculado pela integração da área do pico. Valores de 25(OH)D₃ abaixo de 75 nmol/L foram considerados como insuficiência.

- *Cálcio sérico*: método colorimétrico em espectrofotômetro – Kits comerciais Bioclin® ou similar. Valor de referência: 8,5 a 10,5 mg/dL.

- *Paratormônio intacto sérico*: pelo método eletroquimioluminescência – Kit comercial Roche®. Valor de referência: 15 a 65 pg/mL.

- *Fósforo sérico*: realizada por método colorimétrico – Kits comerciais Bioclin® ou similar. Valor de referência: 2,5 a 4,8 mg/dL.

- *Creatinina sérica*: método calorimétrico em espectrofotômetro – Kits comerciais Celm® ou similar. Valores de referência: abaixo de 1,5 mg/dL.

- *Glicose sérica de jejum*: realizada por método calorimétrico em espectrofotômetro – Kits comerciais Celm® ou similar. Valores de referência: 70 a 100 mg/dL.

- *Colesterol Total e frações e Triacilgliceróis*: método colorimétrico em espectrofotômetro – Kits comerciais Celm® ou similar. Valores normais estabelecidos de acordo com as III Diretrizes Brasileiras de Dislipidemias e Prevenção da Aterosclerose.

4.3.3. Aferição da Pressão Arterial

A pressão arterial foi obtida por meio de aparelho automático (Omron model HEM-712C, Omron Health Care, Inc, USA). Três medidas de pressão arterial foram realizadas com adequação do manguito à circunferência braquial. Após cinco minutos de repouso na posição sentada, com o braço

direito apoiado na altura do átrio esquerdo, o manguito foi automaticamente insuflado, além do valor de oclusão do pulso braquial. Foi fixado um intervalo de cinco minutos entre cada medida. Para os valores finais de pressão sistólica e diastólica (em mmHg), foram considerados aqueles que representam a média aritmética das duas últimas medidas. Valores acima de 140 e/ou 90 mmHg foram considerados alterados de acordo com as VI Diretrizes Brasileiras de Hipertensão Arterial (SBH, 2010). Também foi observado o uso de medicação anti-hipertensiva.

Para retirar a possível interferência do uso da medicação anti-hipertensiva, a amostra foi dividida em três categorias: 1) pressão arterial normal: indivíduos com valores de pressão arterial abaixo de 140 e 90 mmHg; 2) pressão arterial elevada: indivíduos com valores de pressão arterial acima de 140 e/ou 90 mmHg; e 3) pressão arterial normal pelo uso de medicação anti-hipertensiva: encaixam-se nesse grupo indivíduos com valores de pressão normal devido ao uso de medicamentos.

4.4. ANÁLISE ESTATÍSTICA

Os dados estão apresentados como média e desvio padrão. As variáveis avaliadas, pelo teste Kolmogorov-Smirnov, que não apresentaram distribuição normal (PTH, IMC, triacilglicerol e HDL-colesterol) foram transformadas em logaritmo buscando a normalidade. Para avaliar as diferenças existentes entre os grupos de pressão arterial foi realizado a análise de variância (ANOVA).

Para verificar a associação entre as variáveis do estudo foi aplicado o teste de correlação de *Pearson*. Buscando avaliar o quanto a associação entre vitamina D, PTH e pressão sanguínea foi interferida por outras variáveis confundidoras, dois modelos foram usados: modelo 1 – ajustado pelo perfil lipídico (colesterol total, LDL-colesterol e triacilgliceróis) e IMC; modelo 2 – ajustado pelo perfil lipídico e pela circunferência de cintura.

Considerando a possível interferência da utilização de medicação anti-hipertensiva sobre os dados analisados, as mesmas análises de correlação foram realizadas após a retirada dos indivíduos que faziam uso desse tipo de medicamentos. Finalmente, para melhor verificar a associação existente entre o PTH e a pressão arterial, foram excluídos os indivíduos com níveis de PTH elevado de toda amostra.

O modelo de regressão linear multivariado, com critério de seleção stepwise, foi utilizado para avaliação da 25(OH)D₃ e do PTH como variáveis preditoras para os níveis de pressão arterial sistólica e diastólica.

Os dados do presente estudo foram avaliados com o auxílio do *software Statistical Package for the Social Sciences* (SPSS), versão 17.0. O valor de significância considerado foi de 5%, ou seja, $p < 0,05$.

4.5. ASPECTOS ÉTICOS

Este trabalho foi aprovado pelo comitê de ética em pesquisa (COEP) da Faculdade de Saúde Pública da Universidade de São Paulo de acordo com os requisitos do CNS 196/96. Protocolo de pesquisa 1893 (Anexo 1).

Todos os indivíduos que concordaram em participar e assinaram o Termo de Consentimento Livre e Esclarecido (Anexo 2) foram convidados a comparecer ao Centro de Saúde Paula Souza, localizado na Faculdade de Saúde Pública – Av. Dr. Arnaldo 715, em dia e horário pré-estabelecido.

Neste local foram realizadas as coletas de sangue, medidas antropométricas, de composição corporal, e aferição da pressão arterial. Após a coleta sanguínea, foi oferecido o café da manhã.

5. RESULTADOS

Artigo 1:

Vitamin D and Cardiovascular Disease

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Review

Vitamin D and Cardiovascular Disease

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Abstract: Vitamin D insufficiency/deficiency has been observed worldwide at all stages of life. It has been characterized as a public health problem, since low concentrations of this vitamin have been linked to pathogenesis of several chronic diseases. Several studies have suggested that vitamin D is involved in cardiovascular diseases and have provided evidence that it has a role in reducing cardiovascular disease risk. It may be involved in regulation of gene expression through the presence of vitamin D receptors in various cells, regulation of blood pressure (through renin-angiotensin system), and modulation of cell growth and proliferation including vascular smooth muscle cells and cardiomyocytes. Identifying correct mechanisms and relationships between vitamin D and such diseases could be important in relation to patient care and healthcare policies.

Keywords: vitamin D, cardiovascular disease, hypertension.

1. INTRODUCTION

The main function of vitamin D relates to the development and maintenance of bone tissue. It is responsible for maintaining calcium and phosphorus homeostasis. Vitamin D insufficiency/deficiency has been observed worldwide at all stages of life. It has been characterized as a public health problem, since low concentrations of this vitamin has been linked to the pathogenesis of several chronic diseases with cardiovascular risk factors, such as hypertension, heart failure, atherosclerosis and peripheral arterial disease [1-3]. Following the discovery of the presence of vitamin D receptors (VDR) in many cells, including cardiomyocytes [4], vascular smooth muscle cells (VSMC) [5] and endothelium [6], several mechanisms have been proposed to explain the relationship between vitamin D and the development of cardiovascular disease. Such mechanisms include involvement of vitamin D in the angiotensin-renin system [7] and proliferation and growth of VSMC [8].

2. PHYSIOLOGY OF VITAMIN D

Vitamin D is found as ergocalciferol (vitamin D₂) produced by plant and as cholecalciferol (vitamin D₃) produced by animal tissue. It is also produced by exposure to ultraviolet-B (290 to 310 nm) in 7-dehydrocholesterol, which is present in human skin [9]. It has been estimated that 80 to 90% of vitamin D is acquired by means of cutaneous synthesis and the remainder through the diet [10]. Vitamin D prohormone is biologically inactive, and it becomes active through conversion to its major form 25-hydroxyvitamin D (25(OH)D) in the liver: this metabolite is used to classify vitamin D status. Following this, the hormonal form of vitamin D (1,25-dihydroxyvitamin D [1,25(OH)₂D₃] or calcitriol) is produced in other tissues like prostate, breast, colon and especially the kidneys, through 1- α -hydroxylase [9,10]. This metabolite production is controlled by the serum parathyroid hormone (PTH), calcium and phosphorus concentrations.

The effects of $1,25(\text{OH})_2\text{D}_3$ are mediated by VDR, which are present in many cells. At the nucleus of the target cells, $1,25(\text{OH})_2\text{D}_3$ associated with VDR binds to the retinoic X receptor (RXR), thereby forming heterodimers. These work on vitamin D response elements, hence initiating the cascade of molecular interactions that will modulate the transcription of the specific gene [11]. Thus, exceedingly low concentrations of $25(\text{OH})\text{D}$ can result in failure of this metabolic cascade and alter gene expression.

Individuals' vitamin D levels or their nutritional status regarding vitamin D are measured according to the plasma levels of $25(\text{OH})\text{D}$. The biologically active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$) is unsuitable for this purpose for reasons such as: a) the rigid maintenance of plasma levels of $1,25(\text{OH})_2\text{D}_3$ at normal concentrations, even with low plasma concentrations of $25(\text{OH})\text{D}$ (except in cases of chronic kidney disease and in the presence of high fibroblast growth factor-23 concentrations); b) plasma $25(\text{OH})\text{D}$ levels are approximately 100 times greater than those of $1,25(\text{OH})_2\text{D}_3$; and c) hydroxylation of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}_3$ occurs in various tissues, thereby covering local needs [12].

In 2005, Hollis [13] considered that the optimal level of vitamin D that would be required to maintain parathyroid hormone (PTH) at appropriate levels. It is known that vitamin D deficiency leads to decreased serum calcium, which consequently stimulates the parathyroid glands to release PTH, thereby increasing renal reabsorption and bone calcium levels. In this regard, several studies have found a plateau of calcium absorption and adequate PTH levels, with $25(\text{OH})\text{D}$ levels close to 30 ng/mL (75 nmol/L) [14-18]. However, the adequate levels of $25(\text{OH})\text{D}$ for non-calcemic disorders has still not been established.

2.1. SOURCES OF VITAMIN D

Factors such as latitude, season and time of day influence the cutaneous synthesis of vitamin D. During the summer, 7-dehydrocholesterol in the skin is more efficiently converted to previtamin D. Cutaneous synthesis

of vitamin D is higher in low-latitude regions because of greater exposure to sunlight [19]. The use of sunscreen, the amount of melanin in the skin, types of clothing and high levels of air pollution may reduce skin exposure to UVB and result in decreased synthesis of vitamin D. Another important factor leading to hypovitaminosis D is changes in lifestyle, such as reduction of outdoor activities.

There are limited numbers of natural dietary sources of vitamin D. Not all countries have regulations requiring food fortification and this leads to low consumption. Table 1 demonstrates the vitamin D content in selected foods. A recent review [20] showed the dietary requirements for adequate vitamin D nutritional status. For example, for 97.5% of the population aged 20-40 years, a mean intake during the winter of 8.7 µg/d would be needed in order to achieve 25(OH)D serum level greater than 25 nmol/L, and 41.1 µg/d for a 25(OH)D level of 80 nmol/L. The same dietary requirements have been observed among the elderly [21].

Table 1. Vitamin D₂ and D₃ content in selected foods, adapted from USDA national nutrient database for standard reference, Release 22.

Foods (common portion sizes)	Vitamin D content (µg)	
	Vitamin D ₂	Vitamin D ₃
Salmon, cooked (155g)	0.0	36.1
Tuna, canned in oil (85g)	0.0	5.7
Sardines (24g)	0.0	4.8
Liver, beef cooked (85g)	0.0	1.0
Top sirloin, beef cooked broiled (85g)	0.0	0.2
Whole milk with vitamin D fortification (244g)	0.0	1.3
Whole milk, without vitamin D (244g)	0.0	0.1
Butter (5g)	0.0	1.5
Mushrooms, portabella, grilled(121g)	0.3	0.0
Mushrooms, portabella, grilled, exposed to UV light (121g)	13.1	1.0
Mushrooms, shiitake, cooked (72g)	0.7	0.1
Vegetables (kale, broccoli, spinach, tomato, carrots and lettuce) (100g)	0.0	0.0

However, studies have demonstrated that several populations do not attain these dietary intake levels [22,23].

3. EPIDEMIOLOGICAL AND OBSERVATIONAL EVIDENCE

Cardiovascular diseases are the leading cause of death worldwide. According to the World Health Organization (WHO), these diseases affect 17.1 million people around the world and deaths occur predominantly in low and middle-income countries, almost equally in men and women. A reduction of 2 to 3 mmHg in systolic blood pressure is associated with a reduction of 10 to 15% in mortality from cardiovascular disease [24].

Recently, Giovannucci *et al.* [25] assessed the association between serum 25(OH)D and risk of coronary disease among men who participated in the Health Professionals Follow-up Study. Men with vitamin D deficiency (≤ 15 ng/ml or 37 nmol/L) were at significantly increased risk of developing myocardial infarction, compared with those with sufficient levels of vitamin D (≥ 30 ng/mL or 75 nmol/L) (RR 2.09; 95% CI: 1.24-3.54).

Analyzing the population of NHANES III, Melamed *et al.* [26] observed that the prevalence ratio of peripheral arterial disease for the lowest 25(OH)D quartile, compared with the highest quartile (< 44.5 and ≥ 73.8 nmol/L, respectively) was 1.80 (95% CI: 1.19-2.74). A similar situation has been observed in other studies [27,28] on the population of NHANES III, evaluating the associations between serum 25(OH)D and coronary heart disease, heart failure, stroke and peripheral arterial disease.

Comparing vitamin D status among more than 3000 subjects, over a seven-year follow-up period, Pilz *et al.* [29] found that patients with severe vitamin D deficiency [25(OH)D < 25 nmol/L] had a risk of dying from heart failure or sudden cardiac death that was three to five times greater than among patients with optimal levels of vitamin D [25(OH)D ≥ 75 nmol/L]. In patients who had already had heart failure, low serum calcitriol concentrations were associated with critical end-stage outcomes [30].

Evaluating data from InCHIANTI, a prospective cohort study on aging, it was observed that participants who were in the lowest quartile of serum 25(OH)D (≤ 26.25 nmol/L) were at higher risk of all-cause mortality (HR 2.11, 95% CI: 1.22-3.64, $p=0.007$) and mortality from cardiovascular disease (HR 2.64, 95% CI: 1.14–4.79, $p=0.02$), compared with those in the highest quartile (≥ 66.25 nmol/L) [31].

Among adolescents [32], it was also demonstrated that low 25(OH)D levels were strongly inversely associated with cardiometabolic risk factors (systolic blood pressure and plasma glucose concentrations).

The involvement of vitamin D insufficiency with hypertension has also been demonstrated. Investigating the population over the age of 20 years who participated in NHANES III, Scragg *et al.* [3] found systolic and diastolic pressures that were respectively 3.0 and 1.6 mmHg lower in highest quintile of 25(OH)D (≥ 85.7 nmol/L), compared with the lowest quintile of vitamin D (25(OH)D ≤ 40 nmol/L). In the Nurses Health Study and the Healthy Professional Follow-up Study, the negative relationship between serum levels of vitamin D and hypertension was also demonstrated [33]. After four years of follow-up, the relative risk for men with low levels of serum 25(OH)D to develop hypertension was 6.13 (95% CI: 1.00-37.80), while for women it was 2.67 (95% CI: 1.05-6.97). After eight years of follow-up, the relative risk for men was 3.53 (95% CI: 1.02-12.3) and for women, 1.7 (95% CI: 0.92-3.16).

4. PROPOSED MECHANISMS FOR VITAMIN D IN CARDIOVASCULAR DISEASE

The mechanisms underlying the role of vitamin D in the prevention of heart disease remain incompletely explained. However, the mechanisms hypothesized involved the presence of VDR in various cells and its possible modulation of the expression of several genes. 1,25(OH)₂D₃ may interfere in the cascade of reactions and consequent functional capacity of certain cells.

Such mechanisms include vitamin D as a negative regulator for renin and an inhibitor of cell proliferation and growth.

4.1. ANGIOTENSIN-RENIN SYSTEM

Inappropriate activation of the renin-angiotensin system may represent a major risk factor for hypertension and, consequently, for cardiovascular diseases. Several studies have indicated that serum levels of $1,25(\text{OH})_2\text{D}_3$ are inversely associated with blood pressure or plasma renin activity in normotensive and hypertensive subjects [34-38]. In an experimental study with wild-type mice, the research group of Yan Chun Li [7] showed that inhibition of $1,25(\text{OH})_2\text{D}_3$ synthesis led to an increase in renin expression, whereas $1,25(\text{OH})_2\text{D}_3$ injection led to renin suppression in the juxtaglomerular apparatus, independently of parathyroid hormone and calcium metabolism [39]. The same group [40] also demonstrated, in cell cultures, that $1,25(\text{OH})_2\text{D}_3$ directly suppressed renin gene transcription by means of a VDR-dependent mechanism. Elucidating this mechanism, a study found that $1,25(\text{OH})_2\text{D}_3$ suppressed renin gene expression in part by blocking the formation of the cyclic AMP response element [41]. These data suggest that vitamin D analogs and supplements may potentially be agents for controlling renin production and blood pressure.

Corroborating this hypothesis, Fryer *et al.* [42] evaluated the effects of paricalcitol and calcitriol on renin expression in C57/BL6 mice and showed that paricalcitol produces significant dose-dependent reductions in renin/GAPDH expression and calcitriol produced renin suppression. Additionally, Zhou *et al.* [43] demonstrated regulation of the renin-angiotensin system through supplementation of $1,25(\text{OH})_2\text{D}_3$ in $1-\alpha$ hydroxylase knockout mice free of enzyme.

4.2. ROLE OF VITAMIN D IN CARDIAC TISSUE

Few *in vitro* and *in vivo* studies have evaluated the role of vitamin D in cardiac tissue. Carthy *et al.* [8], demonstrated *in vitro* that $1,25(\text{OH})_2\text{D}_3$

blocked the proliferation and growth of VSMC. In a recent study on administration of vitamin D analogues in cells cultures, Wu-Wong *et al.* [5] observed regulation of the expression of IGF1, Wilms tumor 1 and TGF β , which are three genes that are known to modulate cell proliferation. In addition, they observed downregulation of the expression of natriuretic peptide precursor B and thrombospondin 1, which inhibit cell proliferation. However, another study by the same group [44] suggested that elevated phosphorus affects VDR-mediated gene expression in human VSMC, and therefore the effect is not limited to VDR.

Since VSMC is modulated by VDR, some studies have pointed towards its involvement in the endothelium. A study performed to evaluate endothelial function by brachial artery flow mediated dilatation in 23 asymptomatic vitamin D-deficient subjects found a positive correlation between endothelial function and 25(OH)D ($r=0.45$; $p=0.001$) [45].

With regard to the action of vitamin D on cardiomyocytes, a study [46] confirmed the presence of VDR, and that 1,25(OH) $_2$ D $_3$ affected the growth, proliferation and morphology of murine cardiac myocytes (HL-1 cells) in cultures. The cells were treated with 1,25(OH) $_2$ D $_3$, and increased expression of myotrophin with decreasing expression of atrial natriuretic peptide and c-myc were observed. Furthermore, the 1,25(OH) $_2$ D $_3$ treatment also increased the expression and nuclear localization of the VDR in these cardiomyocytes. Another study [47] showed that 1,25(OH) $_2$ D $_3$ treatment in a model of hypertensive rats subjected to a high-salt diet resulted in lower heart weight, myocardial collagen levels, left ventricular diameter and cardiac output, thus suggesting that it had an important preventive role in relation to the development of cardiac hypertrophy and consequent congestive heart failure. Corroborating these findings, it was found that paricalcitol supplementation in Dahl salt-sensitive rats that were also fed a high-salt diet attenuated the cardiac hypertrophy [48]. In Sprague-Dawley rats, maternal vitamin D deficiency led to increased left ventricle volume, greater cardiomyocyte numbers and size, and a higher proportion of mononucleated cardiomyocytes in the offspring at four weeks of age [49].

5. VITAMIN D SUPPLEMENTATION STUDIES

In 2009, Zittermann *et al.* [50] conducted a double-blind, placebo-controlled trial in which 12 months of supplementation of 83.3µg of vitamin D was supplied to 200 women who had started a weight reduction program. They found that the group of supplemented women had greater decreases in PTH levels, triacylglycerides and tumor necrosis factor- α (TNF- α). Another important point was that weight loss did not differ between the vitamin D and placebo groups. Additionally, a study investigated whether vitamin D was associated with cytokine production [51]. It was found that vitamin D supplementation increased the anti-inflammatory cytokine production, such as IL-10, in patients with heart failure.

A study on patients with predialysis chronic kidney disease showed that oral administration of alfacalcidol was associated with reduced risk of cardiovascular disease [52].

However, a randomized, double-blind, placebo-controlled trial on the population of the Women's Health Initiative [53], which was administered 1,000 mg elemental calcium carbonate and 10µg of vitamin D₃ daily, or placebo, found no reduction in mortality due to cardiovascular disease, but the hazard ratios trended in the direction of reduced risk. As an incidental finding, the daily amount of vitamin D₃ in this study, like in other studies using similar amounts of vitamin D supplementation [54], did not find any additional benefits. Furthermore, Bolland *et al.* [55] reported that calcium supplementation above the recommended levels in specific populations (elderly people or individuals with previous cardiovascular events) might increase the risk of cardiovascular events.

In a double-blind, placebo-controlled study in 1987, Lind *et al.* [56] observed reductions in the blood pressure of 39 hypertensive patients with vitamin D supplementation. This reduction was also highlighted in another study on older women supplemented with calcium and vitamin D [57]. Another trial observed that administration of 1,25(OH)₂D₃ reduced blood pressure, as well as plasma renin activity and angiotensin II levels [58].

On the other hand, Thierry-Palmer *et al.* [59] increased the supply of vitamin D in the diet of salt-sensitive rats that were administered a high-salt diet and observed an increase in serum 25(OH)D, but their hypertension was not alleviated. These findings could suggest that there is a potential difference in the effects on the vitamin D endocrine system between salt-induced hypertension and essential hypertension.

Table 2 highlights the cardiovascular effects of vitamin D supplementation.

Table 2. Cardiovascular effects of vitamin D supplementation.

Study	Population	Supplementation type, dose and period	Main outcomes
Kimura <i>et al.</i> , 1999 [58]	Case report on a 42-year-old man.	Oral administration of 0.2 µg of calcitriol	↓ blood pressure, plasma renin activity and levels of angiotensin II.
Pfeifer <i>et al.</i> , 2001 [57]	148 women 70 years of age or older (74 patients were in calcium group and 74 patients were in vitamin-D-calcium group).	1,200 mg of CaCO ₃ or 1,200 mg of CaCO ₃ + 20 µg of cholecalciferol	In vitamin-D-calcium group ↑ in 25(OH)D of 72% and ↓ in serum PTH of 17%, and in systolic blood pressure of 9.3%, heart rate ↓ 5.4%.
Schleithoff <i>et al.</i> , 2006 [51]	123 patients with congestive heart failure randomized into D(+) group and D(-) group	D(+) group received 50 µg of vitamin D ₃ + 500 mg of Ca/d and D(-) group received placebo + 500 mg of Ca/d for 9 months	In D(+) group ↓ PTH, ↑ IL-10 and TNFα remained constant. In D(-) group ↑ TNFα. The survival rate did not differ significantly between groups.
Zittermann <i>et al.</i> , 2009 [50]	200 women who started a program to reduce weight (100 patients were in vitamin D group and 100 patients were in placebo group).	83.3 µg/d of cholecalciferol for 12 months	↓PTH, triacylglycerides and TNF α.
Sigiura <i>et al.</i> , 2009 [52]	665 patients with predialysis chronic kidney disease (107 patients in alfacalcidol treatment group and 558 in non-treatment group).	0.25–0.5 µg/d of alfacalcidol for 24 weeks	Lower incidence of cumulative cardiovascular events in alfacalcidol treatment group.
LaCroix <i>et al.</i> , 2009 [53]	36,282 participants in <i>Women's Health Initiative</i> (18,176 postmenopausal women were in vitamin D group and 18,106 were in placebo group).	1,000 mg CaCO ₃ + 10 µg/d of cholecalciferol for 7 years	No reduction in cardiovascular mortality.

6. CONCLUSION

Hypovitaminosis D has been observed worldwide and several studies have demonstrated a strong association between vitamin D status and cardiovascular diseases. There are few food sources of vitamin D, and the lack of food fortification in some countries, associated with low cutaneous synthesis, intensifies vitamin D insufficiency. Moreover, the understanding of the exact mechanisms through which 25(OH)D or the active form 1,25(OH)₂D₃ regulate the renin-angiotensin system and cell proliferation and growth (such as VSMC and endothelium cells) remains incomplete. In this regard, identifying correct relationships between vitamin D status and cardiovascular disease is an important matter that could contribute towards prevention of such diseases. In the meanwhile, health professionals should be aware of the potential negative implications of vitamin D insufficiency and make recommendations for their patients to improve their vitamin D status.

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Artigo 2:

**Parathyroid Hormone, Vitamin D and Blood Pressure: Is There a
Link in Individuals Living in a Sunny Country?**

Artigo Original

A ser submetido

**PARATHYROID HORMONE, VITAMIN D AND BLOOD PRESSURE:
IS THERE A LINK IN INDIVIDUALS LIVING IN A SUNNY
COUNTRY?**

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ABSTRACT

Low vitamin D has been associated with hypertension, a major-risk factor for cardiovascular disease, and both insufficiency and deficiency of vitamin D has been described in sunny countries. We evaluated the relationship between vitamin D, intact parathyroid hormone (iPTH) and blood pressure (BP) in 332 adults (62% women). Anthropometric measurements, BP and a fasting blood sample were obtained. Serum concentration of 25(OH)D₃ was measured by high-performance liquid chromatography (HPLC). BP was measured at rest and mean of two measures was considered to analysis. Participants were divided in three categories of blood pressure: (1) normal blood pressure; (2) high blood pressure; (3) normal blood pressure by medication. Vitamin D insufficiency was defined by 25(OH)D₃ ≤ 75 nmol/L, high iPTH > 65 pg/mL. The relationship between vitamin D, iPTH and BP were adjusted for BMI, waist circumference (WC), blood lipids. Mean age was 50(15) years, BMI 29(6) kg/m², WC 97(13) cm. Overweight and obesity was present in 75% of individuals. Mean BP was 129/80(18/11) mmHg. Mean serum calcium was 9.3(0.5) mg/dL, iPTH 40.8(18.7) pg/mL and vitamin D 55.8(17.1) nmol/L. Elevated iPTH was present in 12% and vitamin D insufficiency in 86% of the sample. No differences were observed on prevalence of vitamin D insufficiency and high PTH among blood pressure groups. No significant association was observed between BP and vitamin D. A positive correlation was observed between iPTH with systolic (r=0.168; p=0.002) and diastolic BP (r=0.168; p=0.002), BMI (r=0.125; p=0.023), WC (r=0.172; p=0.002) and %FM (r=0.158; p=0.004). The iPTH remained correlated with BP even with adjustments. The present study adds a new piece of information in literature regarding the involvement of the calcemic hormones in the regulation of blood pressure.

Key Words – Essential hypertension, Vitamin D, Parathyroid hormone

INTRODUCTION

Hypertension remains a major-risk factor for cardiovascular disease (CVD). About one billion people worldwide suffer from hypertension and this may be responsible for approximately 7.1 million deaths per year [1]. Recently, The Brazilian Society of Hypertension [2] published that, in 2007, around 309 thousand deaths were caused by CVD and prevalence of hypertension was estimate above 30%. According with World Health Organization [3], CVDs are the leading cause of death globally and by 2030, 23.6 million people will die from this illness.

There are many evidences that vitamin D is involved in several mechanisms in addition to bone metabolism [4-7]. Vitamin D insufficiency / deficiency has been associated with hypertension worldwide [8-12] and the same happen with cardiovascular complications of hypertension [13-16]. As depicted in a recent review [17], the vitamin D may be involved in regulation of gene expression through the presence of vitamin D receptors in various cells. Regarding the regulation of blood pressure, there are evidences that 25(OH)D₃ acts through renin-angiotensin system, as well modulation of cell growth and proliferation including vascular smooth muscle cells and cardiomyocytes.

Moreover, several studies observed vitamin D insufficiency / deficiency even in sunny countries, specially, in adults and elderly [18-21]. Factors, which reduce it synthesis, such as high levels of air pollution and reduction of outdoor activities, may be the cause of this frame. Nevertheless, the effects of vitamin D insufficiency / deficiency in blood pressure has not been evaluated in Brazilian population.

Thus, the aim of the present study was to evaluated whether vitamin D status are related to blood pressure in adults.

METHODS

Study Design

This cross-sectional study was performed with free living adults who attend a center for primary health care at the School of Public Health, University of Sao Paulo.

Individuals under 18 years of age, pregnant and lactant women, as well as individuals who had previous cardiovascular events or reported diseases that could modify the vitamin D metabolism (chronic kidney disease, neoplasia, diabetes, osteoporosis) and black individuals were excluded. Individuals with serum levels of creatinine, calcium and phosphorus outside the reference values, and those that use vitamin D and/or calcium supplementation or multivitamin were also excluded.

A total of 460 subjects were evaluated. One hundred and twenty-eight subjects were excluded, among them fifty-seven were diabetics, nineteen presented alterations on serum levels of creatinine, calcium and phosphorus, six were outliers of PTH values, one were outlier of vitamin D and forty-five with others criteria of exclusion. Therefore, 332 individuals who accepted to participate were included in this analysis. Ethics committees of University of Sao Paulo approved the study protocol, and each participant signed an informed consent statement.

Measurements

Height was measured using a fixed stadiometer with a vertical backboard and movable headboard, with subjects standing on the floor. Weight was taken by asking each subject to stand on the center of the platform of a Tanita™ digital scale (Tanita Corporation of America Inc, Illinois, USA). Then, was calculated their body mass index ($BMI = \text{weight}/\text{height}^2$). Waist circumference was measured in standing subjects, with a tape placed at the midpoint level between the lower intercostals border and the anterior superior iliac supine while the subject was gently exhaling. Percent body fat mass and percent body fat-free mass were assessed by tetrapolar bioelectrical impedance analysis (Quantum BIA 101Q, RJL, Detroit, MI).

Blood pressure was obtained by automatic blood pressure monitor (Omron model HEM-712C, Omron Health Care, Inc, USA). Three measures were taken, at rest, with interval of five minutes, and average from last two measurements was considered to analysis. Participants were divided in three categories: 1) normal blood pressure (NBP): those with blood pressure $\leq 140/90$ mmHg; 2) high blood pressure (HBP): considered when subjects have $\geq 140/90$ mmHg; and 3) normal blood pressure by medication (NBPM): when subjects have normal blood pressure by pharmacological treatment.

After a 12-h fast, a blood sample were collected, frozen and stored at -80°C until analysis. Data were collected from August 2007 to January 2010. Serum triacylglycerol, total cholesterol, LDL-cholesterol, HDL-cholesterol and glucose were determined by enzymatic colorimetric assay (CelmTM, Barueri, SP, Brazil), and creatinine was determined by colorimetric assay (CelmTM, Barueri, SP, Brazil). Calcium and phosphorus were determined by colorimetric assay (BioclinTM, Belo Horizonte, MG, Brazil). Serum concentrations of 25-hydroxyvitamin D [25(OH)D₃] were measured by HPLC (Immundiagnostik AG, Bensheim, Germany) and intact PTH levels by electrochemiluminescence assay (Roche DiagnosticsTM, São Paulo, SP, Brazil) with a reference range within 15 and 65 pg/mL. High PTH was defined by values above 65 pg/mL and 25(OH)D₃ below 75 nmol/L was considered as insufficiency [22].

Statistical Analysis

Results were expressed as means and standard deviations. Statistical analysis were performed using SPSS (Statistical Package for the Social Sciences), version 17.0 (SPSS Inc, Chicago, United States). The significance level taken was 5%.

Logarithmic transformation was performed to obtain normal distribution for skewed variables (PTH, BMI, triacylglycerol, HDL-cholesterol). Analysis of variance (ANOVA) was used to evaluate differences between categories of blood pressure. The association of 25(OH)D₃ and PTH with covariates were examined using Pearson's correlation.

In secondary analysis, we also investigated whether the association among vitamin D, iPTH and blood pressure was confounded by other clinical conditions, i.e. dyslipidemia (total cholesterol, LDL-cholesterol and triacylglycerol) and obesity (BMI and waist circumference). For this, two models were used: model 1, adjusted for blood lipids (total cholesterol, LDL-cholesterol and triacylglycerol) and BMI; model 2, adjusted for blood lipids and waist circumference. To exclude the interference of pharmacological treatment in these analyses, we performed the same correlations without participants that use medication for hypertension. Finally, to better display of associations between PTH and blood pressure, we performed the correlation analysis without individuals who had high PTH in the whole sample.

Multiple linear regression models were used for testing 25(OH)D₃ and iPTH as a continuous variables for the prediction of systolic and diastolic blood pressure with whole sample and then without individuals under hypertension treatment.

RESULTS

The high blood pressure was present in 34% of whole sample and another 16% were taking medication for hypertension. Overweight and obesity was present in 75% of individuals. General characteristics of the whole sample and in the different subsamples are present in Table 1. As expected, BMI, waist circumference, glucose and triacylglycerol were significantly higher in individuals with high blood pressure and in those who were under hypertension treatment. No differences were observed on prevalence of vitamin D insufficiency and high PTH among blood pressure groups.

Table 2 shows the serum concentrations of vitamin D and other calcemic hormones in whole sample and in each blood pressure group. Levels of PTH were significantly higher in individuals with high blood pressure when compared with individuals with normal blood pressure.

Table 1. General characteristics of whole sample

Variable	Whole sample	Normal Blood Pressure	High Blood Pressure	Normal Blood Pressure by Medication	p
Subjects (n)	332	166	112	54	
Age (years)	50(15)	42(13)	57(14)*	59(11)*	0.000
BMI (kg/m ²)	29(6)	27(5)	30(6)*	31(6)*	0.000
%FFM	68(10)	69(10)	67(9)	65(9)*	0.015
%FM	32(10)	31(10)	33(9)	35(9)*	0.015
Waist circumference (cm)	97(13)	92(13)	101(14)*	100(12)*	0.000
Gender (%)					
Male	38	38	45	24	
Female	62	62	55	76	
Systolic BP (mmHg)	129(18)	118(11)	148(14)*	125(9)*#	0.000
Diastolic BP (mmHg)	80(11)	74(8)	89(11)*	77(7)*#	0.000
Total cholesterol (mg/dL)	190(41)	184(41)	198(42)*	193(39)	0.022
LDL-cholesterol (mg/dL)	120(37)	117(37)	125(37)	118(36)	NS
HDL-cholesterol (mg/dL)	43(12)	43(11)	43(12)	44(11)	NS
Triacylglycerol (mg/dL)	134(76)	122(77)	146(79)*	148(57)*	0.009
Glucose (mg/dL)	93(11)	91(11)	94(13)	97(12)*	0.006
Vitamin D insufficiency (%)	86	88	84	87	NS
High PTH (%)	12	10	14	13	NS

Data are mean(SD) for continuous variables and % for categorical variables. % FFM: Percent of fat-free mass; % FM: Percent of fat mass; High PTH was defined as > 65pg/mL; vitamin D insufficiency was defined as < 75nmol/L. (*) significant when compared with normal blood pressure group ($p<0.05$); (#) significant when compared with high blood pressure group ($p<0.05$).

Table 2. Mean serum concentration of calcemic hormones of whole sample and of each blood pressure group

Variable	Whole sample n=332	Normal Blood Pressure n=166	High Blood Pressure n=112	Normal Blood Pressure by Medication n=54	p
25(OH)D ₃ (nmol/L)	55.8(17.1)	55.6(17.7)	56.4(17.4)	55.3(15.0)	NS
PTH (pg/mL)	40.8(18.7)	37.7(17.7)	44.3(18.6)*	43.0(20.4)	0.006
Serum calcium (mg/dL)	9.3(0.5)	9.2(0.5)	9.4(0.4)*	9.4(0.5)	0.004
Serum phosphorus (mg/dL)	3.7(0.7)	3.7(0.7)	3.6(0.8)	3.7(0.9)	NS

Data are mean(SD). (*) significant when compared with normal blood pressure group ($p<0.05$); (#) significant when compared with high blood pressure group ($p<0.05$).

No significant association was observed between the concentration of 25(OH)D₃ and BP. However, vitamin D was positively associated with total cholesterol ($r=0.479$; $p=0.000$), LDL-cholesterol ($r=0.360$; $p=0.000$), log triacylglycerol ($r=0.491$; $p=0.000$). By analyzing the association between

25(OH)D₃ and BP with adjustments, none correlation was observed in model 1 as such as model 2.

By the other hand, a positive correlation was observed between iPTH and systolic BP ($r=0.168$; $p=0.002$), diastolic BP ($r=0.168$; $p=0.002$), BMI ($r=0.125$; $p=0.023$), waist circumference ($r=0.172$; $p=0.002$) and %FM ($r=0.158$; $p=0.004$) and negatively correlated with %FFM ($r=-0.158$; $p=0.004$). Furthermore, when data were adjusted, in model 1, the association between PTH and systolic BP ($r=0.160$; $p=0.004$) and diastolic BP ($r=0.137$; $p=0.014$) was maintained and a negative association was observed between PTH and vitamin D ($r=-0.148$; $p=0.008$). After adjustment, in model 2, the same associations occurs, positive with systolic BP ($r=0.146$; $p=0.009$) and diastolic BP ($r=0.124$; $p=0.027$) and negative with vitamin D ($r=-0.143$; $p=0.011$).

In multiple linear regression (Table 3), the iPTH and BMI only can account for 5% of the variation in systolic BP and 10% in diastolic BP.

Table 3. Regression coefficients(B) for parathyroid hormone (pg/dL), 25(OH)D₃ (nmol/L) and body mass index (kg/m²) regressed against blood pressure in whole sample (n=332).

	Systolic Blood Pressure			Diastolic Blood Pressure		
	B(SE)	B	R ²	B(SE)	B	R ²
Step 1						
Constant	55.39(18.10)			11.53(10.50)		
Vitamin D	0.06(0.06)	0.06		0.05(0.03)	0.08	
iPTH	13.70(4.76)	0.16*		7.36(2.76)	0.14*	
BMI	33.62(11.97)	0.15*	0.06	36.88(6.94)	0.28*	0.11
Step 2						
Constant	57.71(18.00)			13.48(10.45)		
iPTH	13.11(4.73)	0.15*		6.87(2.75)	0.13**	
BMI	35.07(11.90)	0.16*	0.05	38.10(6.92)	0.29*	0.10

* $p<0.01$; ** $p<0.05$

When, individuals taking medication for hypertension (n=54) were excluded in the analysis, a significant correlation was found between iPTH and systolic BP ($r=0.194$; $p=0.001$), diastolic BP ($r=0.211$; $p=0.001$) and waist circumference ($r=0.147$; $p=0.015$). The secondary analysis, including adjustment for waist circumference, model 2, vitamin D was negatively correlated, but not significantly, with systolic blood pressure ($r=-0.111$; $p=0.073$), whereas iPTH remained essentially unaltered with a positive

correlation with systolic BP ($r=0.178$; $p=0.004$) and diastolic BP ($r=0.161$; $p=0.009$).

Then, in multiple linear regression (Table 4), the model can explain approximately 6% of the variation in systolic BP and 13% of variation in diastolic BP of the individuals who not under hypertension treatment.

Table 4. Regression coefficients(B) for parathyroid hormone (pg/dL), 25(OH)D₃ (nmol/L) and body mass index (kg/m²) regressed against blood pressure in those individuals without medication for hypertension (n=278).

	Systolic Blood Pressure			Diastolic Blood Pressure		
	B(SE)	β	R ²	B(SE)	B	R ²
Step 1						
Constant	40.06(20.92)			0.06(11.95)		
Vitamin D	0.06(0.07)	0.05		0.04(0.40)	0.07	
iPTH	16.92(5.58)	0.18*		10.07(3.19)	0.18*	
BMI	41.75(14.01)	0.18*	0.06	42.35(8.00)	0.30*	0.11
Step 2						
Constant	40.85(20.89)			1.21(11.94)		
iPTH	16.54(5.56)	0.18*		9.77(3.18)	0.17	
BMI	43.78(13.80)	0.18*	0.06	43.93(7.89)	0.32	0.13

* $p<0.01$; ** $p<0.05$

In last analysis, individuals with high PTH (n=40) were excluded and the same correlations between iPTH and systolic BP ($r=0.175$; $p=0.003$) and diastolic BP ($r=0.151$; $p=0.009$) were observed.

DISCUSSION

In the present study, the PTH was associated with elevated blood pressure, whereas vitamin D was not correlated with BP even after the exclusion of individuals under hypertension treatment and either adjustment for blood lipids and waist circumference. The association between elevated levels of PTH and hypertension was primarily described by Hellstrom e cols. (1958) [23], in individuals with hyperparathyroidism. Over the years, the contribution of PTH in raised blood pressure was observed even in individuals with PTH in normal range [24-26]. Hagström e cols. (2009) [27] described that individuals with higher PTH had also higher risk for cardiovascular mortality, even when data were adjusted for established cardiovascular risk factors, besides this association remained in individuals with PTH in normal range. Similar to these studies, in our study, the PTH

were positively correlated with blood pressure and this association were also observed among individuals with PTH within normal values. In addition, all individuals had serum calcium within normal range.

Despite several studies suggest that low concentrations of vitamin D are associated with raised blood pressure [8, 28], and, in our sample 86% has been considered with vitamin D insufficiency, no association was observed between vitamin D and blood pressure. Scragg *et al.* [10], investigating 12,644 participants of NHANES III, found values for systolic and diastolic blood pressures respectively 3.0 and 1.6 mmHg lower in highest quintile of 25(OH)D₃ (≥ 85.7 nmol/L), when compared with the lowest quintile of vitamin D (25(OH)D₃ ≤ 40 nmol/L). This negative relationship between serum concentration of vitamin D and hypertension was also demonstrated in the Nurses Health Study and the Healthy Professional Follow-up Study [29]. In the present analysis, a tendency for negative association between vitamin D and systolic blood pressure was only present in individuals that not use medication for hypertension and with adjustment for blood lipids and waist circumference. This could indicate some kind of interaction between these medications with mechanism of vitamin D metabolism confounding the association between it and blood pressure. While, our sample was composed of many obese, adjustment for blood lipids and waist circumference, removed the effect of obesity (factor well established for the development of hypertension).

There are three main mechanisms that may explain the role of PTH on regulation of blood pressure. The PTH could act on cardiomyocyte promoting left ventricular hypertrophy [30, 31] and chronotropic effects on pacemaker cells [32], further, producing immediate and sustained rise in heart beats [33]. It seems to be due to involvement of intracellular calcium in this mechanism but the evidences are conflicting [32, 34]. Furthermore, the high levels of PTH seems to lead the exposed cardiac cells to prematurely death [33]. Another mechanism by which PTH can interfere on blood pressure is the structural and functional modifications in the vascular wall, promoted by PTH through alterations in endothelium and vascular smooth muscle cells, once

the PTH-receptor was described them [35]. Finally, some evidences shown that PTH could be involved in inflammatory response mediated production of interleukin-6 (IL-6) by osteoblasts and, possibly, by adipocytes [36]. Apart from, PTH has been observed positively correlated with fibrinogen, C-reactive protein [36] and leptin [37].

The role of PTH in regulation of mineral metabolism is well recognized. Its rise could be indicate of other disturbances such as vitamin D deficiency, hyperphosphatemia, hypercalcemia or chronic kidney disease (condition known to leads cardiovascular complications and higher risk of mortality). In our study, we seek to remove the subjects that were in these conditions, except by vitamin D insufficiency. For instance, 10% of our sample presented both high PTH and low vitamin D status.

It is important to emphasize the prevalence found of overweight and obesity among participants was very high (75%). Similar occurs with high indexes of waist circumference (measure linked to abdominal obesity) and levels of total cholesterol, LDL-cholesterol and triacylglycerol. These conditions as well as hypertension are strong risk factors for cardiovascular disease, and together reflect a serious problem in our environment that deserve attention.

The present study has some limitations. Since it has a cross-sectional design, these results do not allow us indicate causality and temporal associations. Furthermore, low concentrations of vitamin D in all groups may have been cause of null interactions between them. These concentrations of vitamin D and no association with blood pressure lead us to ask the real cut-point that must be considered to classify the individuals with vitamin D insufficiency when the aim is establish a predictor to cardiovascular disease in our population. From this point of view, the number and conditions of participants, as high percentage of overweight and obese, as well the vitamin D classification based in a bone metabolism effect may not have been sufficient to establish the association between vitamin D and blood pressure. Additionally, the role of inflammation through increased PTH should be further investigated.

In summary, the association between PTH and blood pressure observed in this study adds a new piece of information in literature regarding the involvement of vitamin D metabolism with blood pressure. The metabolic pathway, which causes this disorder, must be more investigated in clinical and prospective studies to better characterize and elucidate the relationship between them.

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6. CONSIDERAÇÕES FINAIS

Atualmente, as doenças cardiovasculares estão entre as principais causas de morte no Brasil e no mundo. Além disso, aumentam as taxas de hospitalização e acarretam altos custos para o tratamento, piorando os problemas de saúde pública. Infelizmente, esse paradigma tende a agravar-se durante as próximas décadas conforme o estimado pela Organização Mundial de Saúde. Em 2030, cerca de 23,6 milhões de pessoas morrerão desta doença em todo o mundo. São muitos os fatores que contribuem para o desenvolvimento dessas doenças e vários estudos que apontam a hipovitaminose D como integrante deste processo.

Em nosso estudo, a população apresentou alta prevalência de insuficiência de vitamina D (86%) e a concentração média de 25(OH)D₃ foi condizente com as concentrações observadas em todo o mundo. Contribuem para este cenário as poucas fontes alimentares de vitamina D, a baixa disponibilidade de alimentos fortificados em nosso país e a redução da síntese cutânea causada por fatores como: envelhecimento, utilização de protetor solar, altas taxas de poluição no ar, entre outros. No entanto, não foram observadas associações entre a vitamina D e a pressão arterial nos indivíduos avaliados. Possivelmente as características e o número dos indivíduos estudados afetaram esse resultado.

Contudo, associações entre PTH e pressão arterial observadas em nossa população são, também, descritas na literatura, proporcionando novos conhecimentos com relação ao envolvimento de hormônios calcêmicos na regulação da pressão arterial.

Ressalta-se, ainda, a prevalência de sobrepeso e obesidade observada nessa população, reforçando a necessidade de intervir nos hábitos alimentares e incentivar a prática da atividade física como fonte de prevenção de doenças crônicas, dentre elas, a hipertensão.

Enfim, esclarecer os mecanismos exatos pelos quais a vitamina D participa de processos implicados no desenvolvimento de doenças

cardiovasculares, como a regulação do sistema renina-angiotensina, a modulação da proliferação e crescimento de células, incluindo a célula muscular lisa vascular, os cardiomiócitos e as células endoteliais, é fundamental para contribuir na prevenção dessas doenças.

Além disso, a via metabólica pela qual o PTH provoca a elevação da pressão arterial, deve ser mais investigada em estudos clínicos prospectivos para melhor caracterizar e elucidar essa relação.

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