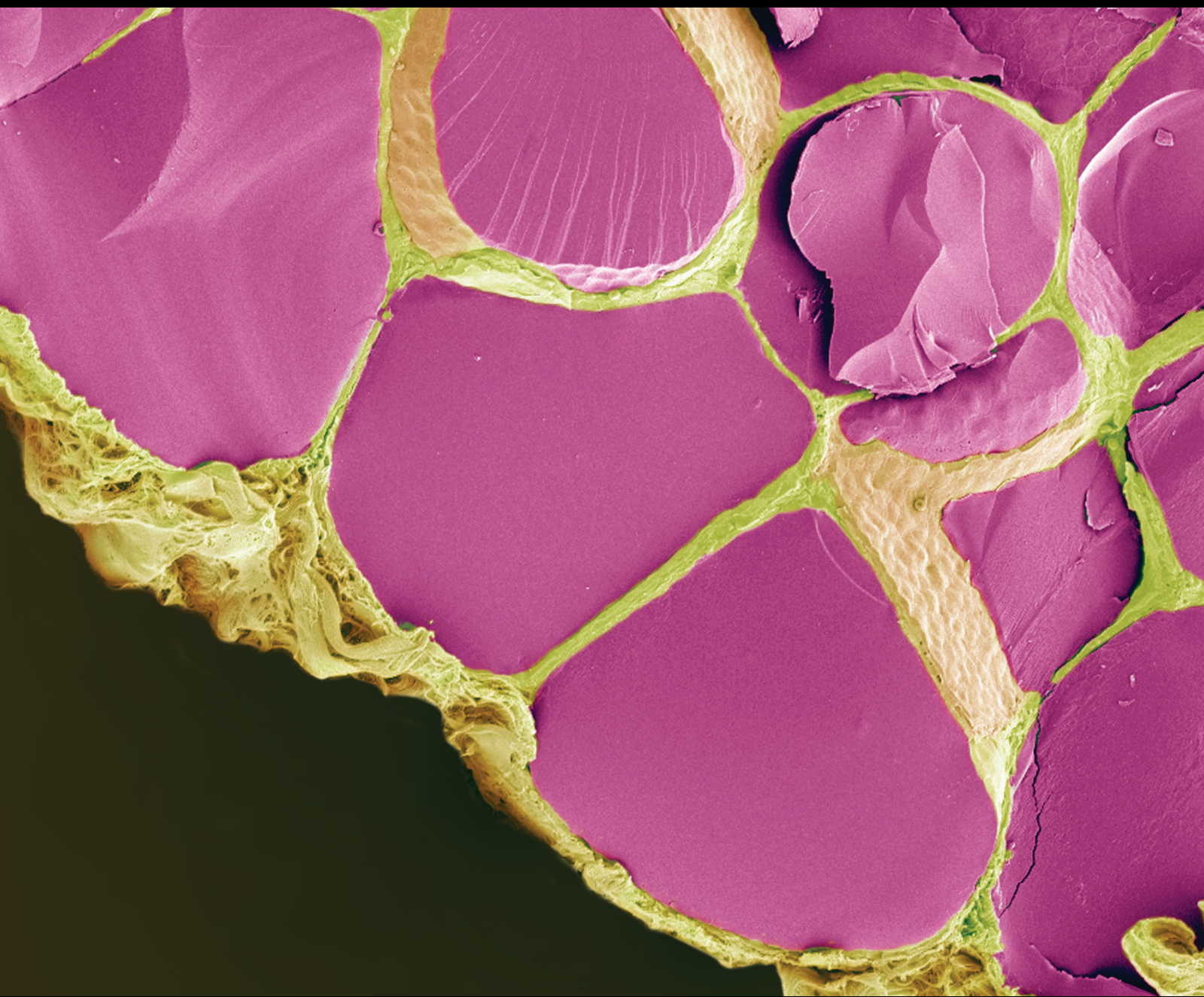
International Journal of Endocrinology

The Bone-Cardiovascular Axis: Mechanisms and Clinical Relevance

Lead Guest Editor: Nicolas Verheyen
Guest Editors: Cristiana Catena, Adriana J. van Ballegooijen, Martin R. Grübler, and Astrid Fahrleitner-Pammer



The Bone-Cardiovascular Axis: Mechanisms and Clinical Relevance

International Journal of Endocrinology

The Bone-Cardiovascular Axis: Mechanisms and Clinical Relevance

Lead Guest Editor: Nicolas Verheyen
Guest Editors: Cristiana Catena, Adriana J. van Ballegooijen, Martin R. Grübler, and Astrid Fahrleitner-Pammer

Editorial Board

Contents

Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 9689106, 2 pages
https://doi.org/10.1155/2018/9689106

Editorial

The Bone-Cardiovascular Axis: Mechanisms and Clinical Relevance

Nicolas Verheyen,[1] **Martin R. Grübler**,[2,3] **Cristiana Catena**,[4] **Astrid Fahrleitner-Pammer**,[5] **and Adriana J. van Ballegooijen**[6]

[1]*Department of Cardiology, Medical University of Graz, Graz, Austria*
[2]*Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Graz, Austria*
[3]*Department of Cardiology, Swiss Cardiovascular Center Bern, Bern University Hospital, University of Bern, Bern, Switzerland*
[4]*Hypertension Unit, Internal Medicine, Department of Experimental and Clinical Medical Sciences, University of Udine, Udine, Italy*
[5]*Department of Internal Medicine, Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria*
[6]*Department of Health Sciences, Vrije Universiteit Amsterdam and the Amsterdam Public Health Research Institute, Amsterdam, Netherlands*

Correspondence should be addressed to Nicolas Verheyen; nicolas.verheyen@medunigraz.at

Received 9 October 2017; Accepted 10 October 2017; Published 21 February 2018

Both bone and cardiovascular disease (CV) are leading causes of morbidity and mortality worldwide and particularly in ageing Western societies. Their common coincidence has been largely attributed to shared risk factors; however, increasing evidence also points towards direct mechanistic interweavement between bone metabolism, the vasculature, and the heart [1–4]. Direct and indirect crosslinks appear to become pathophysiologically relevant in the presence of specific comorbidities associated with imbalances in mineral and bone metabolism or the renin-angiotensin-aldosterone system (RAS) [5–7]. Under the umbrella of this special issue, experts in the field provide a broad and up-to-date overview and novel insights into hormonal interactions underlying the bone-cardiovascular axis.

A. Zittermann comprehensively reviews the role of vitamin D in bone and CV disease particularly stressing the great need for studies investigating the effects of vitamin D on CV health in patients with vitamin D deficiency.

Enriching the vitamin D discussion, A. J. van Ballegooijen et al. review the novel and increasingly important chapter on the mutual relationship between vitamins D and K and the impact of this interaction for both bone and CV health stating that optimal concentrations of both vitamins are needed to function properly.

In addition to these classical mineral hormones, the impact of hormones of the RAS, such as angiotensin II or aldosterone, on bone and CV health has recently attracted attention. C. Catena et al. provide an overview on the existing literature. They conclude that high aldosterone appears to be harmful particularly in concomitance with high salt intake and high parathyroid hormone (PTH).

In fact, there is a broad basis in the literature suggesting that interaction between the CV risk modifier PTH and the RAS is crucial for the development of bone and CV disease [8, 9]. S. Zaheer et al. extend this existing knowledge by showing that ACE inhibition leads to a reduction of PTH levels in patients with but not in patients without primary hyperparathyroidism.

Chronic kidney disease (CKD) is another specific condition where bone and cardiovascular disease are closely interweaved as a consequence of CKD-related mineral and bone disorders (CKD-MBD). In a novel murine model introduced by B. Frauscher et al., brown non-Agouti mice fed with high-phosphate diet developed media calcification,

secondary hyperparathyroidism, and low-turnover bone disease. This novel noninvasive model will provide the opportunity to investigate the bone-cardiovascular axis related with CKD-MBD.

Finally, the important aspect of gender differences in the clinical relevance of the bone-cardiovascular axis finds further substrate in epidemiological analyses of two German cohort studies ($n = 5680$) reported by V. Lange et al. The authors found significant associations between presence of carotid plaques and quantitative ultrasound parameters of the heel only in males and stress the importance of screening for cardiovascular disease in males with osteoporosis.

Conclusively, basic and clinical evidence on the bone-cardiovascular axis is growing, while the clinical relevance is only at the beginning to become unveiled and much remains to be done. This issue shall provide insights into the exciting and complex mechanisms underlying the bone-cardiovascular axis and open the reader's mind towards novel and innovative hypotheses that will contribute to the future shape of this research field. It is also intended to motivate researchers to further investigate the clinical relevance of the bone-cardiovascular axis in order to improve guidance for the prevention and treatment of the still unacceptably high burden of bone and CV disease.

We, the Editorial Team, have been delighted to lead this special issue and hope that the readership will enjoy reading it.

Nicolas Verheyen
Martin R. Grübler
Cristiana Catena
Astrid Fahrleitner-Pammer
Adriana J. van Ballegooijen

References

[1] L. Carbone, P. Buzková, H. A. Fink et al., "Hip fractures and heart failure: findings from the Cardiovascular Health Study," *European Heart Journal*, vol. 31, no. 1, pp. 77–84, 2010.

[2] R. Pfister, G. Michels, S. J. Sharp, R. Luben, N. J. Wareham, and K. T. Khaw, "Low bone mineral density predicts incident heart failure in men and women: the EPIC (European Prospective Investigation into Cancer and Nutrition)–Norfolk prospective study," *JACC: Heart Failure*, vol. 2, no. 4, pp. 380–389, 2014.

[3] N. Verheyen, A. Fahrleitner-Pammer, E. Belyavskiy et al., "Relationship between bone turnover and left ventricular function in primary hyperparathyroidism: the EPATH trial," *PLoS One*, vol. 12, no. 4, article e0173799, 2017.

[4] N. Veronese, B. Stubbs, G. Crepaldi et al., "Relationship between low bone mineral density and fractures with incident cardiovascular disease: a systematic review and meta-analysis," *Journal of Bone and Mineral Research*, vol. 32, no. 5, pp. 1126–1135, 2017.

[5] A. Vidal, Y. Sun, S. K. Bhattacharya, R. A. Ahokas, I. C. Gerling, and K. T. Weber, "Calcium paradox of aldosteronism and the role of the parathyroid glands," *American Journal of Physiology Heart and Circulatory Physiology*, vol. 290, no. 1, pp. H286–H294, 2006.

[6] D. M. Leistner, F. H. Seeger, A. Fischer et al., "Elevated levels of the mediator of catabolic bone remodeling RANKL in the bone marrow environment link chronic heart failure with osteoporosis," *Circulation: Heart Failure*, vol. 5, no. 6, pp. 769–777, 2012.

[7] A. Fahrleitner-Pammer, J. Herberth, S. R. Browning et al., "Bone markers predict cardiovascular events in chronic kidney disease," *Journal of Bone and Mineral Research*, vol. 23, no. 11, pp. 1850–1858, 2008.

[8] A. Tomaschitz, E. Ritz, B. Pieske et al., "Aldosterone and parathyroid hormone interactions as mediators of metabolic and cardiovascular disease," *Metabolism: Clinical and Experimental*, vol. 63, no. 1, pp. 20–31, 2014.

[9] J. M. Brown and A. Vaidya, "Interactions between adrenal-regulatory and calcium-regulatory hormones in human health," *Current Opinion in Endocrinology, Diabetes, and Obesity*, vol. 21, no. 3, pp. 193–201, 2014.

Hindawi
International Journal of Endocrinology
Volume 2017, Article ID 1659071, 8 pages
https://doi.org/10.1155/2017/1659071

Research Article

A New Murine Model of Chronic Kidney Disease-Mineral and Bone Disorder

Bianca Frauscher,[1] Katharina Artinger,[1] Alexander H. Kirsch,[1] Ida Aringer,[1] Foteini Moschovaki-Filippidou,[1] Máté Kétszeri,[1] Corinna Schabhüttl,[1] Peter P. Rainer,[2] Albrecht Schmidt,[2] Tatjana Stojakovic,[3] Astrid Fahrleitner-Pammer,[4] Alexander R. Rosenkranz,[1] Philipp Eller,[5] and Kathrin Eller[1]

[1]*Clinical Division of Nephrology, Medical University of Graz, Graz, Austria*
[2]*Clinical Division of Cardiology, Medical University of Graz, Graz, Austria*
[3]*Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria*
[4]*Clinical Division of Endocrinology and Diabetes, Medical University of Graz, Graz, Austria*
[5]*Intensive Care Unit, Department of Internal Medicine, Medical University of Graz, Graz, Austria*

Correspondence should be addressed to Philipp Eller; philipp.eller@medunigraz.at

Received 5 June 2017; Revised 31 August 2017; Accepted 11 September 2017; Published 14 December 2017

Academic Editor: Rosaria Meccariello

Chronic kidney disease (CKD) is associated with mineral and bone disorder (MBD), which is the main cause of the extensively increased cardiovascular mortality in the CKD population. We now aimed to establish a new murine experimental CKD-MBD model. Dilute brown non-Agouti (DBA/2) mice were fed with high-phosphate diet for 4 (HPD4) or 7 (HPD7) days, then with standard chow diet (SCD) and subsequently followed until day 84. They were compared to DBA/2 mice maintained on SCD during the whole study period. Both 4 and 7 days HPD-fed mice developed phosphate nephropathy with tubular atrophy, interstitial fibrosis, decreased glomerular filtration rate, and increased serum urea levels. The abdominal aorta of HPD-treated mice showed signs of media calcification. Histomorphometric analysis of HPD-treated mice showed decreased bone volume/tissue volume, low mineral apposition rate, and low bone formation rate as compared to SCD-fed mice, despite increased parathyroid hormone levels. Overall, the observed phenotype was more pronounced in the HPD7 group. In summary, we established a new, noninvasive, and therefore easy to perform reproducible CKD-MBD model, which showed media calcification, secondary hyperparathyroidism, and low-turnover bone disease.

1. Introduction

Chronic kidney disease (CKD) is a major health burden as per the 2011 US Renal Data System Annual Data Report; 15.1% of the US adult population has CKD [1]. CKD per se, but especially end-stage renal disease (ESRD), is associated with high morbidity and mortality [2]. Cardiovascular disease is the single greatest cause of mortality in CKD/ESRD and to a large extent is attributable to abnormal mineral metabolism leading to extensive arterial calcifications, a reduced vascular compliance, left ventricular hypertrophy, and sudden cardiac death [3]. When starting dialysis, 50% of the patients have fractures and the survival rate following a hip fracture is 0% [4, 5].

As opposed to nonuraemic subjects where arterial calcification typically affects intimal atherosclerotic plaques, patients with CKD predominantly develop calcification of the tunica media [6, 7]. It is current knowledge that deregulations in mineral and bone metabolism accompanying CKD are the major driving force for the occurrence of media calcification, which led to the term CKD-mineral and bone disorder (MBD) [8, 9]. It additionally includes renal osteodystrophy, which develops in the majority of CKD patients. The spectrum of renal osteodystrophy ranges

from low-turnover adynamic bone disease to high-turnover osteitis fibrosa and more than one type can coexist in the same patient [9]. Bone disease develops due to deregulations in PTH, FGF23, sclerostin, and Vitamin D levels, which are deregulated with declining kidney function [10]. All forms of renal osteodystrophy are accompanied by bone loss finally leading to a significantly increased risk of bone fractures including hip fractures in the CKD population [11, 12]. Especially, patients with adynamic bone disease are prone to develop extensive arterial calcification [4, 5]. Unfortunately, currently available biomarkers such as PTH, FGF23, and alkaline phosphatase can only poorly predict the respective form of renal osteodystrophy and are of limited use in guiding therapy. Thus, according to KDIGO guidelines, bone biopsy and bone histomorphometry remain the gold standard in diagnosing renal osteodystrophy. Yet, it is not recommended to routinely perform bone biopsy in ESRD patients [8, 9]. Nevertheless, few randomized controlled trials have evaluated different treatment strategies depending on the type of renal osteodystrophy. Murine CKD-MBD models are clearly needed to test putative therapeutic strategies for the treatment of vascular calcification and/or renal osteodystrophy. Recently, a subtotal nephrectomy/CKD model with uremic osteodystrophy and abnormalities in bone volume and mineralization has been published [13]. Here, we describe a new, noninvasive, and therefore easy to perform reproducible CKD-MBD model with secondary hyperparathyroidism and media calcification. Contrary to the subtotal nephrectomy/CKD model, we found low-turnover bone disease in our mice.

2. Material and Methods

2.1. Study Design. Female 8-week-old dilute brown non-agouti 2 (DBA/2NCrl, hereafter referred to as DBA/2) mice were obtained from Charles River (Sulzfeld, Germany) and housed in a virus/antibody-free environment in the laboratory animal facility of the Medical University of Graz. These mice are susceptible to ectopic renal calcification and media calcification when exposed to increased oral phosphate loads [14–16]. In order to cause renal damage, these mice were fed standard chow (SCD; $n = 8$) or high-phosphate diet for 4 (HPD4; $n = 4$) or 7 (HPD7; $n = 4$) days with subsequent return to SCD until day 84 after starting HPD diet. The high-phosphate diet (Altromin, Lage, Germany) contained 20.2 g of phosphorus, 9.4 g of calcium, 0.7 g of magnesium, and 500 IU/kg of vitamin D3. The standard chow contained 7.0 g of phosphorous, 10.0 g of calcium, 2.2 g of magnesium, and 1000 IU/kg of vitamin D3.

All animal experiments were approved by the Committee of the Ethics of Animal Experiments of the Austrian Ministry (BMWFW-66.010/0061-WF/V/3b/2016). All experiments were conducted under strict adherence of the law of Austria.

2.2. Metabolic Studies. For metabolic studies, blood was obtained at the end of the experiment from anaesthetized mice by retro-orbital bleeding. Serum urea levels were measured with standard laboratory techniques. Serum fibroblast growth factor 23 (FGF23) levels (Immutopics International, San Clemente, CA, USA) and serum parathyroid hormone (Pth) levels (Immutopics International) were quantified using commercially available enzyme-linked immunosorbent assay kits.

2.3. Evaluation of Histopathology, Histomorphometry, and Immunopathology. Formalin-fixed renal tissue and aortas were embedded in paraffin and cut into 4 μm sections prior to staining. The extent of media as well as renal calcification was determined histologically using alizarin red technique. Alizarin red staining was performed by incubating rehydrated paraffin sections in 2% Alizarin Red S solution (Sigma-Aldrich, St. Louis, MO, USA). Additionally, picrosirius red staining was performed by incubating rehydrated renal paraffin section in 0.1% Picrosirius Red solution (Sigma-Aldrich).

For the calcein-labelling, the mice were intraperitoneally injected with 20 mg/kg of calcein (Sigma-Aldrich) 7 and 2 days prior to sacrifice. Thereafter, the tibia was fixed in 70% ethanol and embedded in methyl methacrylate and sectioned. For further analysis, toluidine blue staining was used. Dewaxed and hydrated bone sections were immersed in toluidine blue working solution (1% Toluidine Blue O, Sigma-Aldrich; 2.5% sodium carbonate, 70% ethanol) for 5 minutes. Thereafter, sections were washed in dH2O, dehydrated in n-Butyl acetate and cover slipped. Bone histomorphometric parameters were obtained through tissue sections analysed by OsteoMeasure™ Software (OsteoMetrics, Decatur, GA, USA).

OCT-embedded (Sakura Finetek, St. Torrance, CA, USA) frozen tissue sections (4 μm) were cut for immunohistochemical stainings. The three-layer immunoperoxidase technique was used for the detection of infiltrating macrophages and T cells in the renal tissue. Macrophages were stained using a rat anti-mouse mAb (anti-CD68, clone FA-11; Serotec, Oxford, UK). A semiquantitative scoring system for kidney-infiltrating macrophages was performed as follows: 0 = 0–4 cells stained positive, 1 = 5–10 cells, 2 = 10–50 cells, 3 = 50–200 cells, and 4 = > 200 cells stained positive per low-power field.

For the detection of $CD4^+$ and $CD8^+$ T cells, rat anti-mouse CD4 (clone YTS191.1; Serotec) and CD8-α (clone KT15; Serotec) mAb were used. Biotin-conjugated goat anti-rat IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was used as a secondary antibody, followed by incubation with a streptavidin-biotin complex (Vector Laboratories, Burlingame, CA, USA) and subsequent development with 0.4% 3-amino-9-ethylcarbazole for 5 minutes and counterstaining with Gill's Haematoxylin. T cell quantitation was performed by counting the number of positive cells in six adjacent high power fields of renal cortex and medulla.

2.4. Reverse Transcription Real-Time Polymerase Chain Reaction. Murine tissue was stored at −80°C. Total RNA was isolated from the kidneys using TRI Reagent (Sigma-Aldrich) according to a standard protocol. Thereafter, 2 μg of total RNA was reverse transcribed using SuperScript III Transcription Kit (Invitrogen, Carlsbad, CA, USA) and random primers (Invitrogen). *Hprt* gene was served

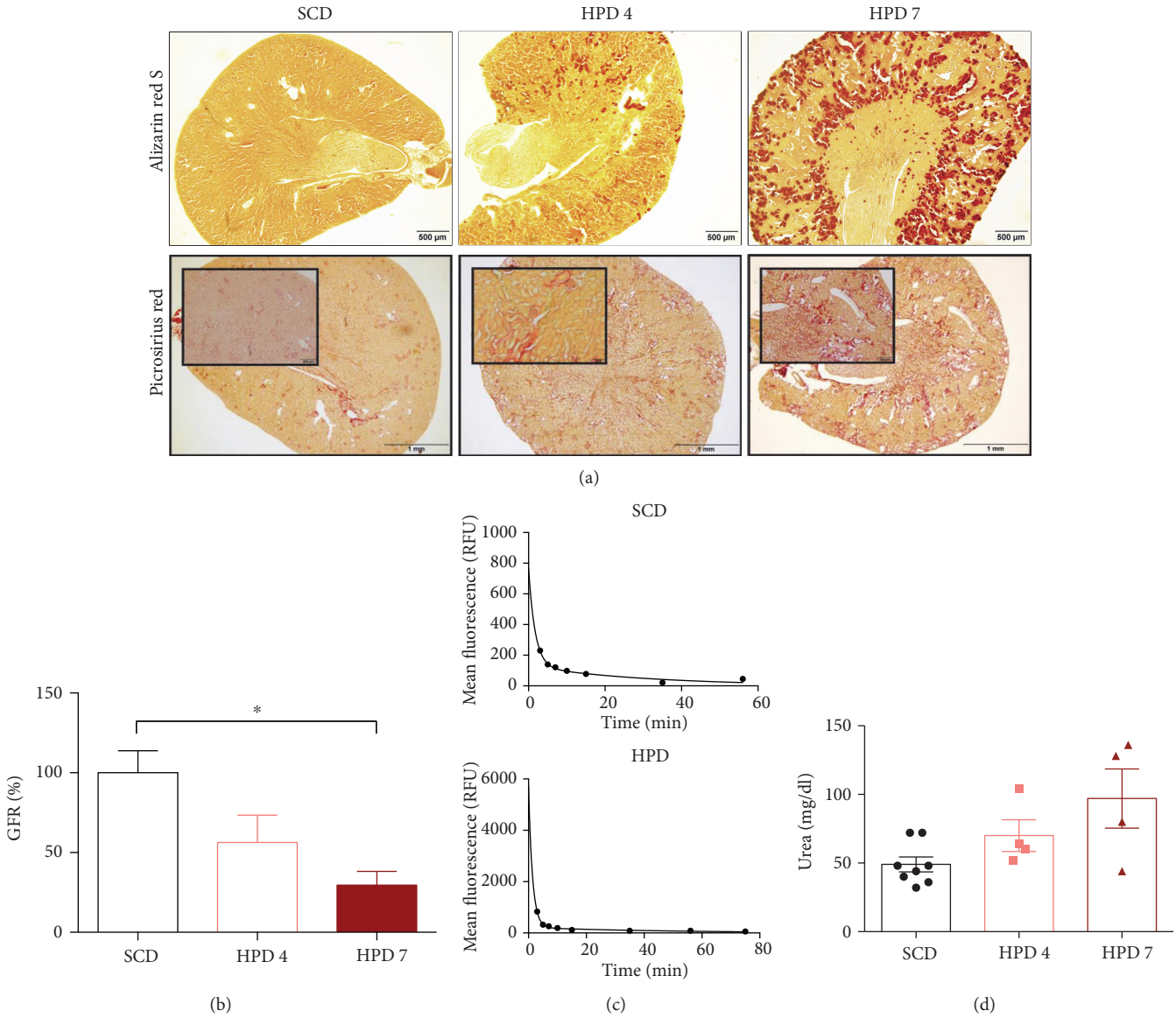


Figure 1: Kidney phenotype of the CKD model. Mice were either fed with HPD for 4 (HPD4, $n = 4$) or 7 days (HPD7, $n = 4$) and then followed on SCD until day 84. They were compared with mice on SCD for the complete study period (SCD, $n = 8$). (a) Kidneys were evaluated for calcium hydroxyapatite crystals and fibrosis by performing alizarin red and sirius red stain, respectively. Representative pictures are provided. (b) On day 84, FITC-inulin clearance to evaluate the glomerular filtration rate (GFR) was performed. The GFR in percentage compared to SCD is provided. (c) Representative GFR curves of SCD and 7-day HPD 7 mice are provided. (d) Serum urea was evaluated in mice on day 84. $^*p < 0.05$.

as the housekeeping reference and was assessed using SYBR Green Master Mix (Invitrogen) and by the following primers: forward 5′ GCT TCC TCA GAC CGG TTT TTG C 3′ and reverse 5′ ATC GCT AAT CAC GAC GCT GGG ACT G 3′. For quantification of *Foxp3*, *Gata3*, *Rorc*, *Tnfa*, *Tbx21*, *Ccr2*, and *CCr5*, the gene expression assays Mm00475162_m1, Mm00484683_m1, Mm01261022, Mm00443258_m1, Mm00450960, Mm00438270, and Mm01216171 (Applied Biosystems, Foster City, DA, USA) were used, respectively.

Real-Time PCR was performed in duplicates on a CF96 real-time detection system (Bio-Rad, Vienna, Austria). The data was evaluated using the 2-$^{\Delta\Delta}$CT method.

2.5. Measurement of the Glomerular Filtration Rate (GFR). GFR was measured by FITC-inulin clearance. FITC-inulin (Sigma-Aldrich; 5% in 0.85% NaCl) was dialyzed for 24 h against 0.85% NaCl. The dialyzed FITC-inulin solution was sterile filtered and injected intravenously (2 μl/g body weight). Three, 5, 7, 10, 15, 56, and 75 minutes after injection, blood was collected from the tail vein. After centrifugation, plasma was diluted 1 : 10 in 0.5 mol/L HEPES and fluorescence was measured. GFR was calculated using a two-compartment model of two-phase exponential decay.

2.6. Mouse Echocardiography. 2D-guided M-mode echoes (30 MHz) were obtained from short- and long-axis views at

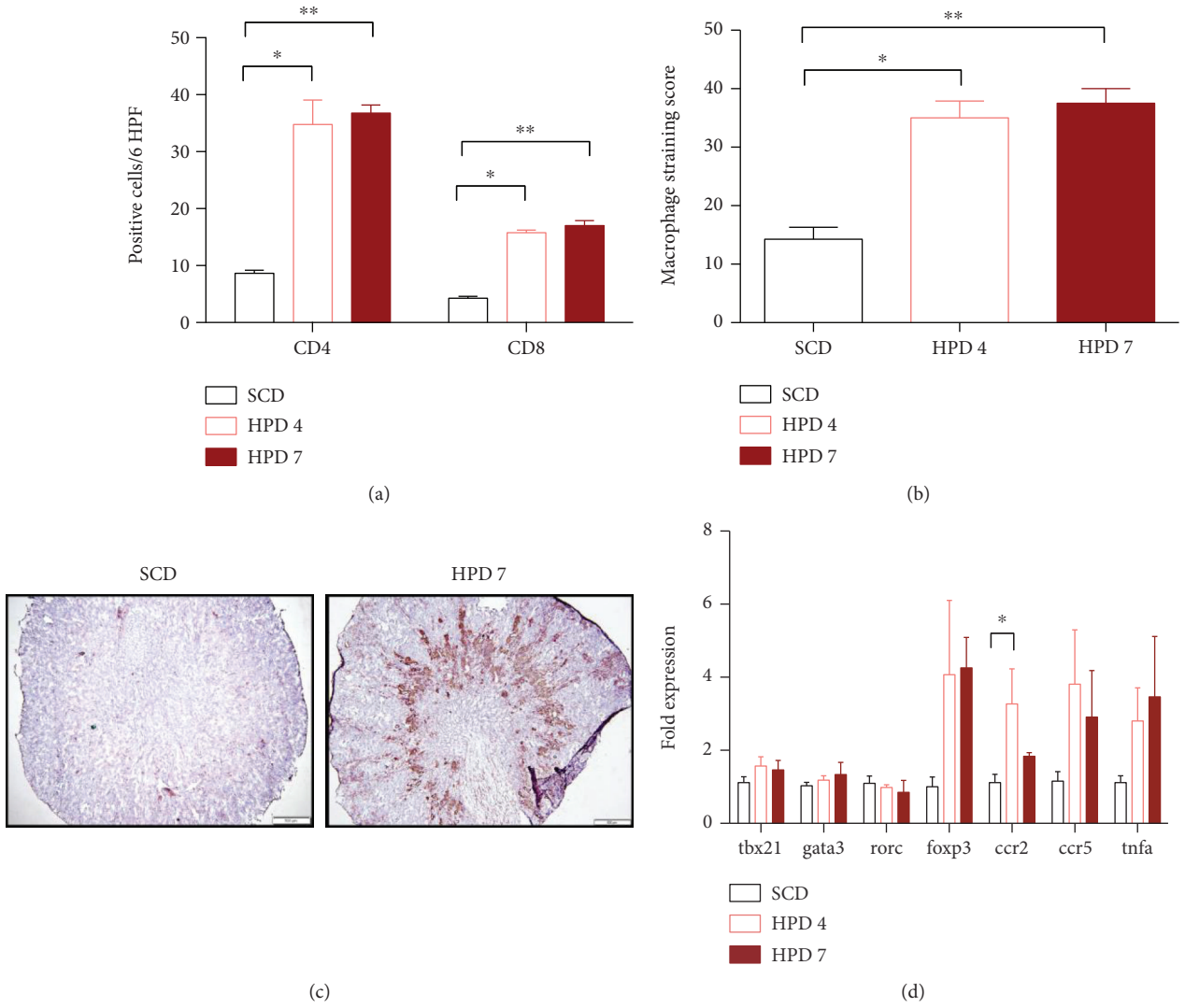


Figure 2: Immune cell infiltration into CKD kidneys. Kidneys of mice (SCD: $n = 8$; HPD4: $n = 4$; and HPD7: $n = 4$) were stained for (a) $CD4^+$ and $CD8^+$ T cells or (b) $CD68^+$ macrophages. (c) Representative pictures of CD68 stained kidney tissue from SCD and HPD7 mice are shown. (d) Quantitative PCR of respective genes was performed in kidney tissue. The fold expression compared to the mean mRNA expression of SCD mice is provided. $^*p < 0.05$ and $^{**}p < 0.01$.

the level of the largest LV-diameter using a VS-VEVO 770 High-Resolution Imaging System (Visualsonics, Toronto, Canada) equipped with a 30 MHz RMV (Real-time micro-visualization) scan head. Mice were lightly anesthetized with 2% isoflurane and were allowed to breathe spontaneously. The chest was shaved, acoustic coupling gel was applied, and a warming pad was used to maintain normothermia. Mice were imaged in a shallow left lateral decubitus position. LV end-diastolic (EDD) and end-systolic (ESD) dimensions were measured from original tracings by using the leading edge convention of the American Society of Echocardiography. LV percent fractional shortening (LVFS), LV mass (LVM), and end-diastolic wall-thickness/cavity ratio were calculated as previously described [17].

2.7. Statistical Analysis. Results from experiments are shown as means ± SEM. Testing for normal distribution was done using the Kolmogorov-Smirnov test with Dallal-Wilkinson-Lilliefors correction. When comparing the two groups, according to the distribution nonparametric Mann–Whitney *U* test or unpaired Student's *t*-test was used. When comparing the three groups, ANOVA or Kruskal-Wallis test was performed with subsequent Dunn's test with adjustment for multiple comparisons. A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. High-Phosphate Diet for 4 and 7 Days Induces Chronic Kidney Disease (CKD) after 84 Days Follow-Up. Female DBA/2 mice were fed for either 4 (HPD4) or 7 (HPD7) days with high-phosphate diet and then followed until day 84 on standard chow (SCD). They were compared to DBA/2 mice on SCD. At day 84, all mice subjected to the HPD developed

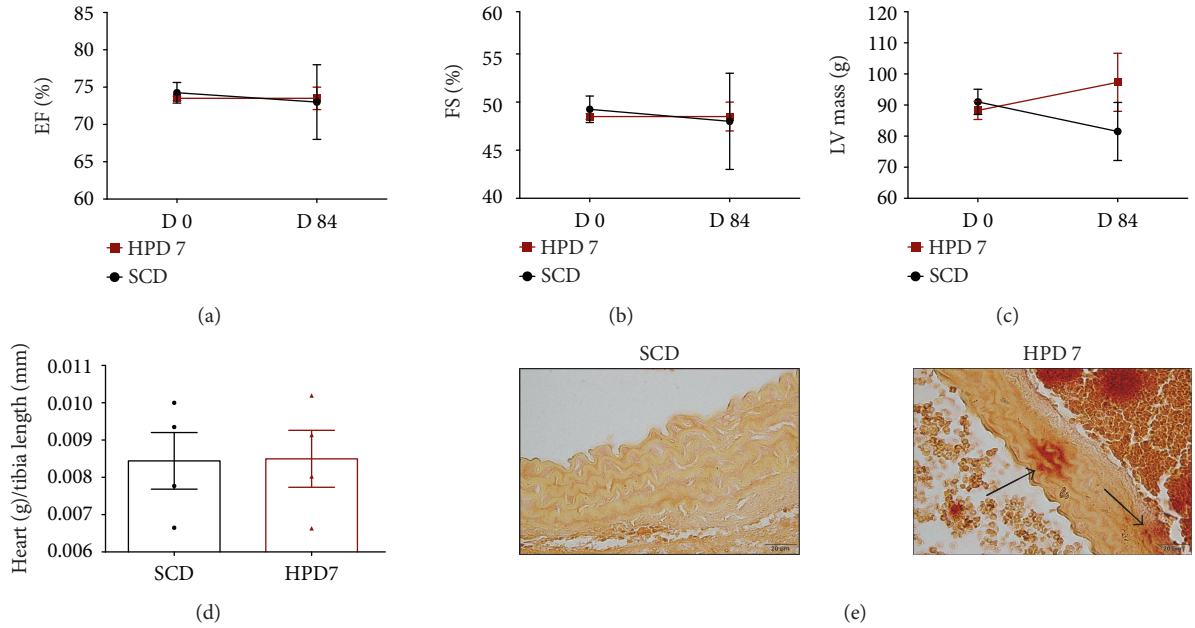


Figure 3: Cardiovascular phenotype of the CKD model. Echocardiography was performed before starting the CKD model ($n = 4$ per group). One group of mice was fed with HPD for 7 days (HPD7) and followed until day 84. This group was compared to mice fed with SCD throughout the study period. Both groups were evaluated by echocardiography on day 84 (SCD: $n = 4$; HPD7: $n = 2$). Evaluation included (a) EF, (b) FS, and (c) LV mass. (d) Abdominal aortas of the 7-day HPD group and the SCD group were stained with alizarin red. Calcifications were detected in the media of aortas (arrow). Representative pictures are shown.

phosphate nephropathy with tubular calcium hydroxyapatite crystals, which was stained positive with alizarin red. Only small and significantly less alizarin red positive deposits were found in DBA/2 mice on standard chow (SCD) (Figure 1(a), upper panel). To evaluate fibrosis, a sirius red stain was performed. All HPD mice displayed significantly increased fibrotic areas especially in the peritubular region without affecting the glomeruli (Figure 1(a), lower panel). The histological phenotype was increased in the HPD7 mice as compared to the HPD4 mice.

To quantify kidney function, we performed FITC-inulin clearance, which showed decreased GFR in both HPD groups as compared to the control SCD group, but significance was only reached in the HPD7 group compared to SCD mice (Figures 1(b) and 1(c)). In line, serum urea was increased in both HPD groups compared to SCD (Figure 1(d)).

The tubular calcium hydroxyapatite precipitations were associated with a prominent renal infiltration of leukocytes (Figure 2). $CD4^+$ and $CD8^+$ T cells were found to infiltrate the kidneys of mice treated with HPD throughout the interstitial area, whereas significantly decreased numbers were detected in the SCD group (Figure 2(a)). The infiltrate mainly consisted of $CD68^+$ macrophages, which were found in significantly increased numbers in HPD-treated mice (Figures 2(b) and 2(c)). In line, we detected an increase in macrophage mRNA markers such as *C-C chemokine receptor* (*Ccr*) *2* and *Ccr 5* as well as *Tnfa* in the kidneys of HPD mice (Figure 2(d)). The TH1, TH2, and TH17 markers *Tbx21*, *Gata3*, and *Rorc*, respectively, were not regulated on the transcriptional level (Figure 2(d)). Only the regulatory T cell marker *FoxP3* was increased in HDP mice compared to SCD-treated mice (Figure 2(d)).

3.2. Cardiovascular Changes in Mice Treated with High-Phosphate Diet for 7 Days Followed by 84 Days Standard Chow. HPD7 mice or SCD mice were evaluated by echocardiography before starting the diet and at day 84 of follow-up. A major limitation of the echocardiographic observations is the fact that (n) numbers on day 84 in HPD7 mice were small ($n = 2$). No difference in left ventricular (LV) systolic function (EF and FS) or mass was found between the mice at baseline. At day 84, preliminary observations in HPD7 mice showed no difference in EF and SF compared to SCD mice, while LV mass tended to increase in HPD7 mice (Figures 3(a), 3(b), and 3(c)). Heart weights did not differ between HPD7 and SCD mice (Figure 3(d)). Of note, no significant myocardial calcifications were detected in our mice (data not shown).

The abdominal aortas of mice were evaluated for calcification by performing alizarin stains. Calcified areas were detected in the media of the aortas of HPD7 mice, whereas no calcifications were found in SCD mice (Figure 3(e)).

3.3. A Model of CKD-Associated Low-Turnover Bone Disease. The surrogate parameters for bone disease in CKD parathyroid hormone (Pth) and fibroblast growth factor 23 (FGF23) were evaluated. Whereas we detected a significant increase in Pth in mice on day 84 in the HPD7 group compared to SCD mice, no difference was found in FGF23 levels between the three groups (Figures 4(a) and 4(b)).

Mice were calcein-labelled and after sacrifice, tibias were evaluated on day 84 by bone histomorphometry. Overall, the bone structure was more deteriorated in the HPD7 group. In detail, we detected a decrease in the bone volume/tissue volume (BV/TV), mineral apposition rate

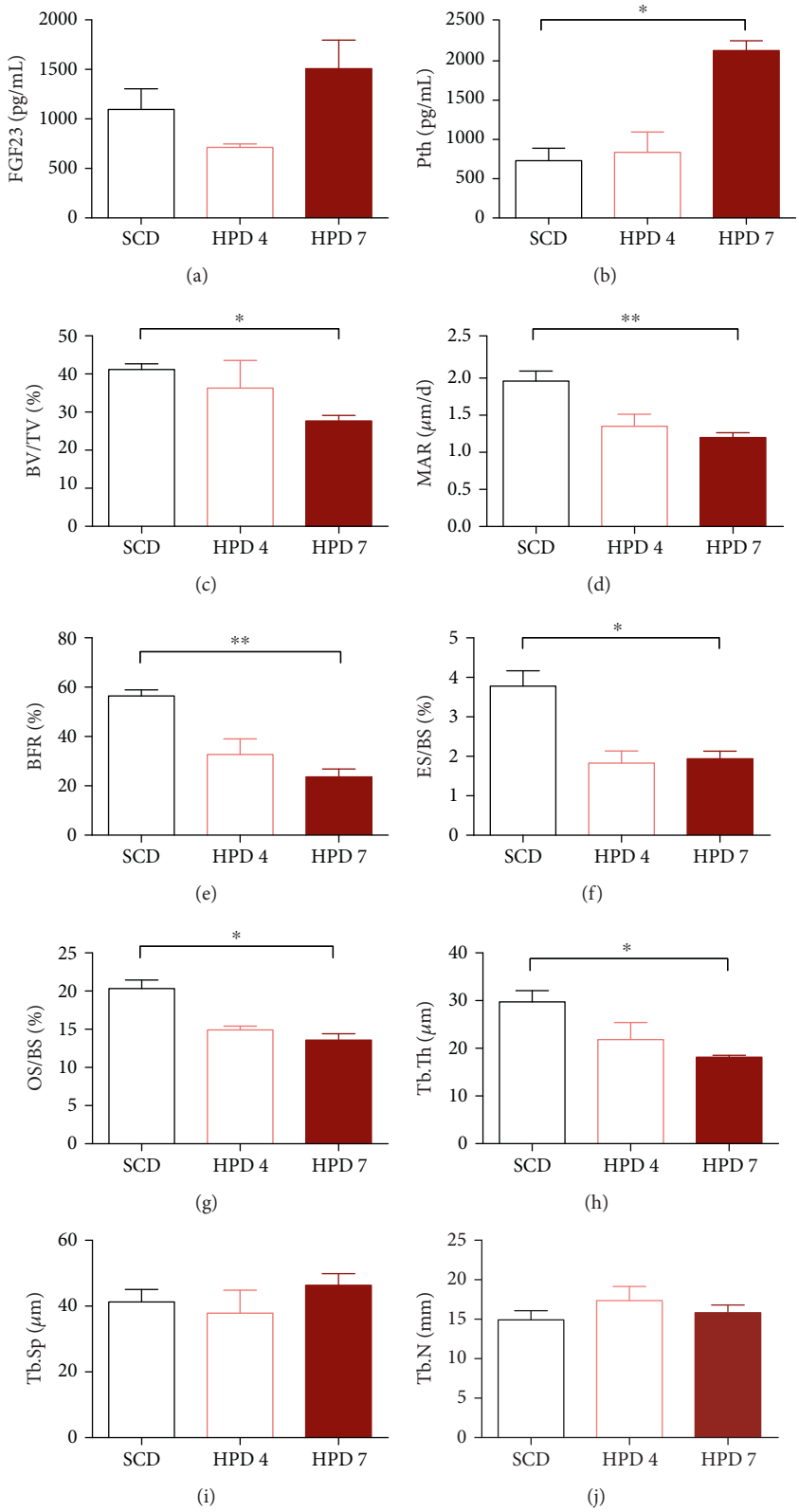


Figure 4: Bone histomorphometry and histology of CKD mice. Mice were either fed with HPD for 4 (HPD4, $n = 4$) or 7 days (HPD7, $n = 4$) and then followed on SCD until day 84. They were compared with mice on SCD for the complete study period (SCD, $n = 8$). On day 84, (a) serum Pth levels and (b) FGF23 levels were evaluated. Furthermore, mice were calcein-labelled and the tibia was analysed by using bone histomorphometry. (c) Bone volume/tissue volume (BV/TV), (d) mineral apposition rate (MAR), (e) bone formation rate (BFR), (f) eroded surface/bone surface (ES/BS), (g) osteoid surface/bone surface (OS/BS), (h) trabecular thickness (Tb.Th), (i) trabecular separation (Tb. Sp.), and (j) trabecular number (Tb.N) are provided. $^{*}p < 0.05$ and $^{**}p < 0.01$.

(MAR), bone formation rate (BFR), eroded surface/bone surface (ES/BS), osteoid surface/bone surface (OS/BS), and trabecular thickness (Tb.Th) in the HPD4 and HPD7 group as compared to SCD mice, but significance was only reached in the HPD7 group (Figures 4(c), 4(d), 4(e), 4(f), 4(g), and 4(h)). The trabecular separation (Tb.Sp) and trabecular number (Tb.N) did not differ between the groups (Figures 4(i) and 4(j)).

4. Discussion

In this manuscript, we present a new model of CKD, which has features of MBD as shown by arterial media calcification, secondary hyperparathyroidism, and low-turnover bone disease.

The classical CKD model is the 5/6 nephrectomy model, which is difficult to perform consistently in mice, since robust CKD is in most cases not induced. Thus, nowadays, most groups use the CKD model established by Gagnon et al., where the majority of the renal surface of one kidney is coagulated followed by nephrectomy of the other kidney after some weeks of recovery [18]. Nevertheless, mortality during and after this procedure is high and surgical procedures are necessary to induce CKD [13], which is an inherent source of bias. In our model, we do not need surgical interventions and the mortality of these mice is very low (5 to 10%; data not shown) since we stop HPD on day 4 or 7, respectively. As shown previously, mortality increases rapidly in our mice when fed with HPD for more than 10 days [19]. Of note, choosing the DBA/2 strain is of critical importance to induce CKD, since C57BL/6 mice do not develop critical calcification neither in the kidney nor in the cardiovascular system [20]. This is explained by the fact that DBA/2 mice have an alternative splice variant of the *Abcc6* gene resulting in an increased susceptibility to develop tissue calcification [14–16].

In our CKD model, mice suffer from CKD reflecting CKD stage 3 in humans since GFR declined by 50% compared to SCD mice, which differs to the surgically induced CKD stage 5 model [13, 21, 22]. Thus, our model provides the opportunity to study early CKD-MBD changes in different organs, which is of particular interest since only early interventions seem to improve mortality and morbidity in our CKD patients.

Our CKD mice develop cardiovascular changes such as media sclerosis in the abdominal aorta. From our data, we cannot clearly tell, whether the mice also develop concentric left ventricular hypertrophy since we have low (n) numbers in echocardiography and heart weights did not differ between the groups. In HPD-induced acute kidney injury model due to phosphate nephropathy, we found the picture of dystrophic cardiac calcinosis resulting in the significantly increased mortality in the mice [19]. In the presented CKD model, the cardiac picture looked differently since we did not detect relevant calcifications in the myocardium (data not shown), but preliminary observations by echocardiography showed that the mice developed some extent of cardiac hypertrophy probably due to hypertension which more closely resembles the human CKD-MBD phenotype. Nevertheless, increasing (n) numbers in echocardiography and further evaluations such as blood pressure measurement are necessary to characterize the cardiac phenotype in detail. In the presented CKD model, we detected media sclerosis predominantly in the abdominal aorta, which is in line with our previous data published in an HPD-induced acute kidney injury mouse model [23]. Both in CKD patients and in our CKD mouse model, there is a different susceptibility of the ascending aorta and the abdominal aorta to vascular media calcification [23]. The mechanisms for this observational finding remain elusive so far. It was speculated that due to their different embryonic origin, vascular smooth muscle cells in different parts of the aorta have a different susceptibility to calcification [23, 24].

In our CKD mice, we detected a low-turnover bone disease by performing bone histomorphometry even though Pth levels were significantly increased in HPD mice. This highlights the fact that Pth is an imperfect marker for evaluating bone disease in CKD, and bone histomorphometry should be considered as gold standard for diagnosing bone disease in CKD [8, 9]. Interestingly, we detected FGF23 levels to be increased only after 14 days (data not shown), whereas no difference was detectable on day 84. Obviously, the early FGF23 increase is a physiological reaction to the HPD in order to clear the excessive oral phosphate intake but normalizes after the long observation interval of 11 weeks on standard chow diet. Nevertheless, it would be interesting to also study FGF23 expression in the bone of our mice since increased expression levels have been described recently in the bone of CKD patients with renal osteodystrophy [25]. To our knowledge, this is the first experimental CKD model describing a low-turnover bone disease. Others published a model of renal osteodystrophy in a surgically induced subtotal nephrectomy/CKD model [13, 21, 22]. Contrary to our model, histomorphometry was evaluated in the lumbar vertebrae rather than in the tibiae [13]. They performed microCT in the tibia-trabecular region and found increased bone volume and decreased mineral density in the metaphysis of their mice [13], whereas we found decreased bone volume and mineralization in the tibia of our CKD model. Nevertheless, we need to further analyse the bone disease in our model by methods of histology and confocal microscopy to gain additional insights into our newly described mouse model.

In summary, our mouse model offers a new and easy to perform reproducible tool to study the pathogenesis and treatment options of CKD-MBD and especially of low-turnover bone disease in CKD.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank Annemarie Ferstl-Rohrbacher for helping them performed bone histomorphometry. PhD student Bianca Frauscher is enrolled in the PhD program of the Medical University of Graz in Molecular Medicine. This work was further supported by the Austrian Science Fund (FWF) (P-27537 to Philipp Eller). The PhD students Ida

Aringer and Foteini Moschovaki-Filippidou received funding from the Austrian Science Fund (FWF) (W1241) and the Medical University of Graz through the PhD Program Molecular Fundamentals of Inflammation (DK-MOLIN).

References

[1] A. J. Collins, R. N. Foley, B. Chavers et al., "United States Renal Data System 2011 Annual Data Report: Atlas of chronic kidney disease & end-stage renal disease in the United States," *American Journal of Kidney Diseases*, vol. 59, no. 1, Supplement 1, p. A7, e1–420, 2012.

[2] A. S. Go, G. M. Chertow, D. J. Fan, C. E. McCulloch, and C. Y. Hsu, "Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization," *The New England Journal of Medicine*, vol. 351, no. 13, pp. 1296–1305, 2004.

[3] G. M. London, "Cardiovascular calcifications in uremic patients: clinical impact on cardiovascular function," *Journal of the American Society of Nephrology*, vol. 14, no. 9, Supplement 4, pp. S305–S309, 2003.

[4] S. A. Jamal, O. Ljunggren, C. Stehman-Breen et al., "Effects of denosumab on fracture and bone mineral density by level of kidney function," *Journal of Bone and Mineral Research*, vol. 26, no. 8, pp. 1829–1835, 2011.

[5] T. L. Nickolas, M. B. Leonard, and E. Shane, "Chronic kidney disease and bone fracture: a growing concern," *Kidney International*, vol. 74, no. 6, pp. 721–731, 2008.

[6] M. Vervloet and M. Cozzolino, "Vascular calcification in chronic kidney disease: different bricks in the wall?," *Kidney International*, vol. 91, no. 4, pp. 808–817, 2017.

[7] G. M. London, A. P. Guerin, S. J. Marchais, F. Metivier, B. Pannier, and H. Adda, "Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality," *Nephrology Dialysis Transplantation*, vol. 18, no. 9, pp. 1731–1740, 2003.

[8] Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group, "KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD)," *Kidney International Supplement*, vol. 76, no. 113, pp. S1–130, 2009.

[9] S. Moe, T. Drüeke, J. Cunningham et al., "Definition, evaluation, and classification of renal osteodystrophy: a position statement from kidney disease: improving global outcomes (KDIGO)," *Kidney International*, vol. 69, no. 11, pp. 1945–1953, 2006.

[10] S. Yamada and C. M. Giachelli, "Vascular calcification in CKD-MBD: roles for phosphate, FGF23, and klotho," *Bone*, vol. 100, pp. 87–93, 2016.

[11] A. M. Alem, D. J. Sherrard, D. L. Gillen et al., "Increased risk of hip fracture among patients with end-stage renal disease," *Kidney International*, vol. 58, no. 1, pp. 396–399, 2000.

[12] S. M. Kim, J. Long, M. Montez-Rath, M. Leonard, and G. M. Chertow, "Hip fracture in patients with non-dialysis-requiring chronic kidney disease," *Journal of Bone and Mineral Research*, vol. 31, no. 10, pp. 1803–1809, 2016.

[13] D. Cejka, D. Parada-Rodriguez, S. Pichler et al., "Only minor differences in renal osteodystrophy features between wild-type and sclerostin knockout mice with chronic kidney disease," *Kidney International*, vol. 90, no. 4, pp. 828–834, 2016.

[14] R. S. Jansen, S. Duijst, S. Mahakena et al., "ABCC6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation-brief report," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 34, no. 9, pp. 1985–1989, 2014.

[15] H. Meng, I. Vera, N. Che et al., "Identification of Abcc6 as the major causal gene for dystrophic cardiac calcification in mice through integrative genomics," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 11, pp. 4530–4535, 2007.

[16] T. G. M. F. Gorgels, X. Hu, G. L. Scheffer et al., "Disruption of Abcc6 in the mouse: novel insight in the pathogenesis of pseudoxanthoma elasticum," *Human Molecular Genetics*, vol. 14, no. 13, pp. 1763–1773, 2005.

[17] S. Sedej, A. Schmidt, M. Denegri et al., "Subclinical abnormalities in sarcoplasmic reticulum Ca^{2+} release promote eccentric myocardial remodeling and pump failure death in response to pressure overload," *Journal of the American College of Cardiology*, vol. 63, no. 15, pp. 1569–1579, 2014.

[18] R. F. Gagnon and B. Gallimore, "Characterization of a mouse model of chronic uremia," *Urological Research*, vol. 16, no. 2, pp. 119–126, 1988.

[19] A. H. Kirsch, N. Smaczny, V. Riegelbauer et al., "Regulatory T cells improve nephrocalcinosis but not dystrophic cardiac calcinosis in DBA/2 mice," *The American Journal of Pathology*, vol. 183, no. 2, pp. 382–390, 2013.

[20] P. Eller, K. Eller, A. H. Kirsch et al., "A murine model of phosphate nephropathy," *The American Journal of Pathology*, vol. 178, no. 5, pp. 1999–2006, 2011.

[21] I. G. Nikolov, N. Joki, T. Nguyen-Khoa et al., "Chronic kidney disease bone and mineral disorder (CKD-MBD) in apolipoprotein E-deficient mice with chronic renal failure," *Bone*, vol. 47, no. 1, pp. 156–163, 2010.

[22] E. A. González, R. J. Lund, K. J. Martin et al., "Treatment of a murine model of high-turnover renal osteodystrophy by exogenous BMP-7," *Kidney International*, vol. 61, no. 4, pp. 1322–1331, 2002.

[23] A. H. Kirsch, A. Kirsch, K. Artinger et al., "Heterogeneous susceptibility for uraemic media calcification and concomitant inflammation within the arterial tree," *Nephrology Dialysis Transplantation*, vol. 30, no. 12, pp. 1995–2005, 2015.

[24] M. Leroux-Berger, I. Queguiner, T. T. Maciel, A. Ho, F. Relaix, and H. Kempf, "Pathologic calcification of adult vascular smooth muscle cells differs on their crest or mesodermal embryonic origin," *Journal of Bone and Mineral Research*, vol. 26, no. 7, pp. 1543–1553, 2011.

[25] F. G. Graciolli, K. R. Neves, F. Barreto et al., "The complexity of chronic kidney disease-mineral and bone disorder across stages of chronic kidney disease," *Kidney International*, vol. 91, no. 6, pp. 1436–1446, 2017.

Hindawi
International Journal of Endocrinology
Volume 2017, Article ID 4397028, 8 pages
https://doi.org/10.1155/2017/4397028

Review Article

Salt, Aldosterone, and Parathyroid Hormone: What Is the Relevance for Organ Damage?

Cristiana Catena, Gian Luca Colussi, Gabriele Brosolo, Nicole Bertin, Marileda Novello, Andrea Palomba, and Leonardo A. Sechi

Internal Medicine, Department of Medicine, University of Udine, Udine, Italy

Correspondence should be addressed to Leonardo A. Sechi; sechi@uniud.it

Received 25 May 2017; Revised 6 August 2017; Accepted 27 August 2017; Published 19 September 2017

Academic Editor: Małgorzata Kotula-Balak

Structured interventions on lifestyle have been suggested as a cost-effective strategy for prevention of cardiovascular disease. Epidemiologic studies demonstrate that dietary salt restriction effectively decreases blood pressure, but its influence on cardiovascular morbidity and mortality is still under debate. Evidence gathered from studies conducted in patients with primary aldosteronism, essential hypertension, or heart failure demonstrates that long-term exposure to elevated aldosterone results in cardiac structural and functional changes that are independent of blood pressure. Animal experiments and initial clinical studies indicate that aldosterone damages the heart only in the context of an inappropriately elevated salt status. Recent evidence suggests that aldosterone might functionally interact with the parathyroid hormone and thereby affect calcium homeostasis with important sequelae for bone mineral density and strength. The interaction between aldosterone and parathyroid hormone might have implications also for the heart. Elevated dietary salt is associated on the one hand with increased urinary calcium excretion and, on the other hand, could facilitate the interaction between aldosterone and parathyroid hormone at the cellular level. This review summarizes the evidence supporting the contribution of salt and aldosterone to cardiovascular disease and the possible cardiac and skeletal consequences of the mutual interplay between aldosterone, parathyroid hormone, and salt.

1. Introduction

Arterial hypertension is the most frequent modifiable cardiovascular risk factor. The NHANES (National Health and Nutrition Examination Survey) has estimated a prevalence of hypertension of 30% among the adult population, and that approximately 85% of people between 55 and 65 years will develop hypertension within their lifetime [1]. Because blood pressure control in the population is a difficult task, prevention and treatment of hypertension through interventions on patients' lifestyle have been suggested as a cost-effective strategy [2–4]. Among these interventions, a reduction of dietary salt intake could be beneficial for blood pressure control and prevention of heart failure [5].

Evidence gathered in the last decades indicates that, in addition to the well-known renal tubular actions, aldosterone regulates many cellular functions. These cellular effects of aldosterone result in the regulation of specific responses including tissue remodeling, hypertrophy, and fibrosis [6]. In fact, chronic exposure to aldosterone levels that are inappropriately elevated for the salt status causes cardiovascular damage independent of blood pressure [7]. Past animal experiments reported that chronic exposure to elevated aldosterone causes myocardial fibrosis in rats that are maintained on a high-salt diet [8] and that these changes are prevented by either administration of aldosterone antagonists or adrenalectomy [9]. In addition to these animal data, studies conducted in patients with primary aldosteronism [10] or essential hypertension [11] provided evidence that long-term exposure to inappropriately elevated aldosterone leads to a variety of organ sequelae occurring beyond what could be expected from the increase in blood pressure. Also, indirect evidence of the untoward effects of aldosterone on the cardiovascular

system was obtained in clinical studies that investigated the effects of either aldosterone antagonists or adrenalectomy on patients with primary aldosteronism [10, 12].

Increasing evidence indicates that, beyond its cardiovascular effects, aldosterone excess might affect also mineral metabolism and have specific relevance for calcium homeostasis [13–15]. Because primary hyperparathyroidism is associated with poor cardiovascular outcome, a role in cardiovascular disease has been attributed also to the parathyroid hormone (PTH) [13–15]. Recent studies have demonstrated a reciprocal interaction between aldosterone and PTH, and there is growing evidence that the salt status might have a role within this interaction. This interaction between aldosterone and PTH could have clinical relevance because it could lead, on the one hand, to cardiac structural and functional changes that facilitate development and progression of heart failure and, on the other hand, to decreased bone mineral density and strength. In this narrative review, we outline the evidence supporting the contribution of salt and aldosterone to cardiovascular disease and the possible consequences of the mutual interplay between salt, aldosterone, and PTH on cardiac and skeletal damage.

2. The Role of Salt

The association between dietary salt consumption, hypertension, and cardiovascular disease has long been the subject of important epidemiological studies. Because of significant discrepancies among the findings of these studies, this association remains under debate [16].

2.1. Dietary Salt and Blood Pressure. Dietary salt consumption has long been associated with blood pressure regulation. In fact, hypertensive patients have been classified as "salt-resistant" or "salt-sensitive" depending upon their blood pressure response to an oral or intravenous salt load. Salt is distributed in the extracellular fluid and, as such, participates in blood pressure regulation [17]. The effects of salt on blood pressure, however, can be attributed to changes in extracellular volume only in part, and additional mechanisms might be involved, including changes in vascular responses to vasoactive substances and interaction with a variety of hormonal systems [18].

The relationship between dietary salt intake and blood pressure was initially investigated in the International Study of Salt and Blood Pressure (INTERSALT) population study [19]. This study demonstrated that populations with high salt intake had higher blood pressure and a greater age-related blood pressure increase than populations with low salt intake. Although prevalence of hypertension in populations with low salt consumption was unremarkable, this increased significantly after migration of these populations to geographical areas where salt intake was high. Later on, the same INTERSALT group reported a direct relationship of daily urinary sodium excretion with blood pressure in a cross-sectional investigation conducted in over 30 countries [20]. A Cochrane systematic review and meta-analysis of randomised trials concluded that decreasing daily salt intake by 4.4 grams corresponding to 1.76 grams of sodium leads to a small but statistically significant fall in blood pressure in both hypertensive and normotensive subjects [21]. In the Dietary Approaches to Stop Hypertension (DASH) study, a typical western diet was compared to a diet enriched in fruits, vegetables, and low-fat dairy products, and within each dietary group, the study subjects were assigned to eat food with different salt contents for a month [22]. In this study, a dose-response relationship between dietary salt and blood pressure levels was observed in subjects eating both the DASH diet and the typical western diet. More recently, the Prospective Urban Rural Epidemiology (PURE) study examined 102,216 adults from 667 communities all over the world to investigate the relationship between salt intake and blood pressure and to clarify whether this relationship varies across different geographical areas of the world [23]. The study countries were categorized into four different income levels, and urinary sodium excretion was estimated from a morning specimen and divided into three levels. Sodium excretion was higher in men than in women, in rural than in urban areas, and was inversely related to gross national income. This study indicated that for each 1-gram increment in urinary sodium excretion corresponding to 2.5 grams of salt, systolic and diastolic blood pressure increased by 2.1 and 0.8 mmHg, respectively. The association between urinary sodium and blood pressure had a slope that was steeper in patients with sodium excretion > 5 grams/day (corresponding to >12.5 grams of salt), in patients with hypertension, and in elderly subjects. Overall, this study indicated that the proportion of populations eating a low-sodium diet worldwide is rather small and indicated that salt intake is only weakly related to blood pressure. Thus, epidemiologic evidence indicates that dietary salt affects blood pressure, but its influence seems to be small and restricted to subjects with high salt consumption.

2.2. Dietary Salt and Cardiac Damage. Despite the evidence supporting the contribution of salt to blood pressure regulation, the potential benefits of dietary salt restriction on cardiovascular morbidity and mortality are uncertain. The association of daily salt intake, as assessed by urinary sodium excretion, with a composite outcome of cardiovascular events and mortality was prospectively investigated in a 3-year follow-up study [24]. An increased risk of the composite outcome was associated with dietary sodium excretion of more than 7 grams/day (corresponding to 17.5 grams of salt). Also, the risk of death and cardiovascular events considered separately was increased, and the association between dietary salt and cardiovascular outcomes was strongest among hypertensive patients. To notice, in this study, increased risk of cardiovascular events was also associated with daily sodium excretion below 3 grams/day (corresponding to 7.5 grams of salt), suggesting a J-shaped relationship. In another study by the NutriCode group, the impact of dietary salt on cardiovascular outcomes was examined by the use of a complex analysis technique [25]. Salt intake was quantified from data obtained in surveys conducted in more than 60 different countries, and the effects of salt on blood pressure and

cardiovascular events were calculated with a meta-analysis. This study reported a significant dose-response relationship between salt intake and cardiovascular events and estimated that 1.65 million deaths for cardiovascular causes could be ascribed to dietary salt worldwide. However, a recent meta-analysis of seven prospective studies that compared cardiovascular mortality in patients undergoing dietary interventions to decrease salt consumption and patients on a liberal diet did not report any benefits of dietary interventions [26]. This held true also when the study subjects with normal blood pressure, hypertension, or heart failure were considered separately. In summary, although some important cumulative analyses suggest possible benefits of dietary salt restriction on cardiovascular morbidity and mortality, definitive conclusions cannot be drawn.

3. Aldosterone and the Heart

Landmark studies have tested the effects of aldosterone antagonists on patients with systolic cardiac failure reporting a highly significant decrease in mortality as compared to placebo [27] and supporting the view that elevated aldosterone could be harmful to the heart. Later on, studies conducted in patients with primary aldosteronism, essential hypertension, and diastolic heart failure have yielded further evidence that elevated plasma aldosterone might have untoward cardiac effects [28] and might foreshadow the onset of heart failure.

3.1. Primary Aldosteronism. Primary aldosteronism is associated with cardiac changes that might reflect the ability of inappropriately elevated circulating aldosterone to cause myocardial damage beyond that induced by high blood pressure itself. Longitudinal retrospective studies have shown that patients with primary aldosteronism have a greater risk of atrial fibrillation and coronary artery and cerebrovascular disease than matched patients with essential hypertension [29–32]. Also, and of greatest relevance, both surgical and medical treatments of primary aldosteronism decrease cardiovascular risk to the level of patients with essential hypertension [10, 12, 30]. Cardiac ultrasound evaluations have reported a greater increase in left ventricular mass in primary aldosteronism than in other forms of hypertensive disease [33, 34] suggesting inappropriate left ventricular hypertrophy for the hemodynamic load. In primary aldosteronism, excess ventricular hypertrophy occurs in conjunction with an abnormal pattern of ventricular filling indicating diastolic dysfunction [35]. The cardiac findings obtained in patients with primary aldosteronism were corroborated by the demonstration that also patients with familial hyperaldosteronism type 1 who have normal blood pressure and elevated aldosterone have increased left ventricular wall thickness and diastolic dysfunction in comparison to matched normotensive patients [36]. Long-term observation after treatment of primary aldosteronism showed that both adrenalectomy and spironolactone caused a significant decrease in ventricular mass [34, 37] and that the extent of this decrease was directly related to pretreatment plasma aldosterone levels [38].

3.2. Essential Hypertension and Left Ventricular Diastolic Dysfunction. Because of the relevance of left ventricular hypertrophy and diastolic dysfunction in patients with essential hypertension, the possible contribution of circulating aldosterone to these cardiac changes has been extensively investigated. Initial observations indicated that aldosterone antagonists decrease left ventricular mass in patients with essential hypertension and left ventricular hypertrophy [37, 39] and improve myocardial function in hypertensive patients with diastolic heart failure [40]. However, cross-sectional evidence subsequently obtained in treatment-naïve essential hypertensive patients indicated that plasma aldosterone has no independent relationship with left ventricular diastolic properties [41]. Consistently, a recent study of hypertensive patients with diastolic dysfunction reported no change in the ventricular filling pattern after addition of spironolactone to previous antihypertensive treatment, despite a significant reduction in ventricular mass [42]. It has to be considered that lack of association between left ventricular diastolic dysfunction and plasma aldosterone levels might be related to the limitation of plasma aldosterone as a measure of the overall mineralocorticoid activity.

In 44 elderly patients with cardiac failure and preserved ejection fraction, eplerenone improved left ventricular diastolic function more than conventional treatment [43]. In the Chronic Renal Impairment in Birmingham (CRIB II) study, spironolactone improved markers of left ventricular relaxation suggesting that aldosterone blockers might be beneficial in the management of patients with diastolic heart failure [44], a hypothesis that was subsequently tested in two important trials. In the Aldo-DHF trial, spironolactone improved left ventricular diastolic function, but had no effects on maximal exercise capacity in patients with heart failure and preserved ejection fraction [45]. Similarly, in a subgroup of patients with heart failure and preserved systolic function included in the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) trial, spironolactone significantly reduced a composite cardiovascular endpoint [46]. In summary, plasma aldosterone levels seem to be marginally relevant for left ventricular diastolic dysfunction in hypertensive subjects, but the use of aldosterone antagonists in the treatment of heart failure with preserved systolic function has so far provided encouraging results.

4. The Contribution of Salt to Aldosterone-Related Cardiac Damage

The hypothesis of an interplay between dietary salt and aldosterone in causing cardiac damage was extensively supported by the findings of animal studies [8, 9]. Some of the untoward effects of salt loading might depend on mineralocorticoid receptor activation resulting from changes in the intracellular redox state [47, 48]. Aldosterone affects the redox potential of diverse cell types increasing the generation of reactive oxygen species, and this effect is potentiated by exposure to high concentration of salt [49]. Therefore, an inappropriately high salt status might sensitize mineralocorticoid receptors and

explain why salt interacts with aldosterone in the induction of cardiac damage.

In the clinical setting, observations on the contribution of salt to aldosterone-mediated cardiac damage are restricted to a few studies [50]. In a population study, a significant and independent correlation of the left ventricular mass index with both 24-hour urinary sodium and aldosterone excretion was reported by Jin et al. [51]. In 182 hypertensive patients who were treated for 3 years with either angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, du Cailar et al. observed a direct relationship of the percentage change in ventricular mass with the absolute changes in urinary sodium and plasma aldosterone levels [52]. In 90 essential hypertensive patients free of clinically relevant cardiovascular complications, aldosterone levels measured after intravenous saline load were found to be independently related to left ventricular mass, suggesting that limited ability of salt to modulate aldosterone production could contribute to ventricular hypertrophy [53]. In 21 patients with primary aldosteronism, Pimenta et al. found that urinary sodium independently predicts left ventricular mass [54]. More recently, we have shown that an increased left ventricular mass is associated with daily urinary sodium excretion and plasma aldosterone levels in 65 patients with primary aldosteronism [55]. Also, and most important, we found that the extent of a reduction in left ventricular mass obtained after either surgical or medical treatment of primary aldosteronism was independently correlated with the decrease in urinary sodium observed during treatment. Thus, the hypothesis of an interaction between salt and circulating aldosterone in causing damage to the heart is currently supported by robust animal data and initial clinical evidence.

5. Interplay of Salt and Aldosterone with Calcium Metabolism

5.1. Relevance to the Bone. An interaction between components of the renin-angiotensin-aldosterone system and hormones involved in calcium homeostasis was initially suggested in patients with salt-sensitive hypertension by Resnick et al. [56] and has been reviewed in previous articles [13–15]. These authors reported for the first time a significant increase in PTH levels in patients with primary aldosteronism. Later on, a similar increase in serum PTH was demonstrated in association with increased urinary calcium excretion [57], lower serum calcium concentrations [58], and comparable vitamin D levels [59] in patients with primary aldosteronism in comparison to patients with essential hypertension. Consistent with these findings, increased prevalence of osteoporosis and increased risk of bone fracture have been reported in patients with primary aldosteronism who were recruited in different geographical areas and were compared to matched patients with essential hypertension [60, 61]. Also, and most important, normalization of serum calcium and PTH levels was reported to follow surgical treatment of patients with aldosterone-producing adenomas [59] as well as treatment with spironolactone of patients with bilateral adrenal hyperplasia [58]. These findings are in agreement with those of studies that demonstrated that administration of spironolactone to aldosterone-salt-treated rats improves cortical bone strength [62]. Consistently, treatment of primary aldosteronism was associated with significant recovery of the bone mineral density at different skeletal sites from decreased density that was detected at baseline [57]. These observations support the hypothesis that an increased fracture risk in patients with primary aldosteronism might result from secondary hyper-PTH due to aldosterone-induced hypercalciuria and subsequent hypocalcemia (Figure 1).

In support of the close interplay existing between PTH and aldosterone, recent evidence indicates that type-1 PTH receptors are expressed in aldosterone-producing adenomas [63] and explains why PTH elevation might increase aldosterone secretion. On the other hand, mineralocorticoid receptors have been detected in the nuclei of parathyroid cells indicating the possibility that aldosterone directly regulates PTH production [63]. In this context, dietary salt consumption could play an important role in as much as an inappropriate salt status causes activation of mineralocorticoid receptors leading to an increased oxidative stress and promoting tissue damage [64]. It was also suggested that salt retention and extracellular fluid expansion caused by elevated circulating aldosterone could decrease sodium reabsorption in the distal tubule leading to an increased urinary calcium excretion [65].

5.2. Relevance to the Heart. The interplay between salt, aldosterone, and PTH has received robust support from experimental animal studies. Treatment of rats with aldosterone and 1% dietary salt increases urinary and intestinal calcium excretion causing hypocalcemia and increased PTH secretion [66]. In these rats, blockade of mineralocorticoid receptors with the use of spironolactone decreases urinary and fecal calcium losses restoring normal calcium homeostasis [67]. The same effect of spironolactone was reported in patients with chronic heart failure [68]. When healthy subjects are exposed to a dietary salt excess, urinary calcium excretion increases significantly [69], an effect that is significantly more pronounced in patients with primary aldosteronism than in patients with essential hypertension [70]. On the other hand, many studies have shown that aldosterone causes renal calcium wasting in healthy subjects in the presence of dietary salt excess [71] and data of the Styrian Hypertension Study indicate that even in patients with essential hypertension, the interaction between aldosterone, calcium, and PTH varies depending upon the dietary salt intake [13].

In the setting of heart failure, aldosterone secretion is increased as a result of renin-angiotensin axis activation and causes salt and fluid retention. The inappropriate elevation of aldosterone for the salt status increases urinary and intestinal calcium losses with subsequent activation of PTH production [72]. This explains why elevated PTH is frequently associated with increased circulating aldosterone in patients with heart failure [73] and supports the hypothesis that PTH might concur with aldosterone in causing worsening of cardiac function in these patients [72]. Elevation of PTH levels facilitates calcium uptake in many cell types including cardiomyocytes, leading to mitochondrial calcium

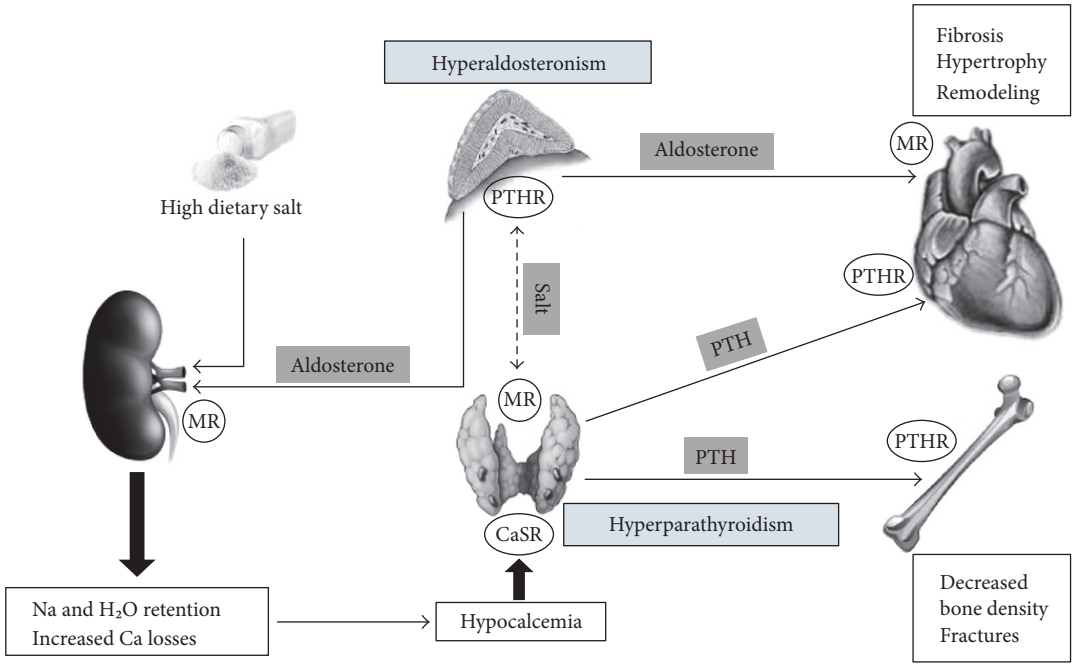


Figure 1: Overview of the mechanisms resulting from the interaction between aldosterone and PTH with the potential role of salt and the related impact on the heart and bone. MR: mineralocorticoid receptor; PTHR: parathyroid hormone receptor; CaSR: calcium-sensing receptor.

overload. This, in turn, decreases the ability of the cell to efficiently generate ATP thereby leading to cell death and myocardial tissue damage [70] and inducing further worsening of cardiac function [74]. In summary, both animal and human studies support the hypothesis of a functional interaction between aldosterone and PTH that could vary according to the salt status. This interaction might have an impact on bone structure and cardiac function in different disease conditions. Specifically designed studies with aldosterone blockers or other types of interventions would be needed to reach conclusive views on the pathophysiologic relevance of these mechanisms.

6. Conclusions

Robust scientific evidence demonstrates a relationship between salt intake and blood pressure and supports the benefits of salt restriction in hypertension. Conversely, the possible benefits of dietary salt restriction on cardiovascular outcomes are still debated because intervention studies and cumulative analyses have not been able to provide thoroughly convincing results. Current evidence unquestionably supports the view that elevated aldosterone causes cardiovascular damage well beyond what could be expected just from blood pressure elevation. Animal studies clearly indicate that aldosterone-dependent cardiac damage is strictly dependent on the salt status and this view is corroborated by the results of some human studies. A close interaction between aldosterone, calcium, and PTH has been demonstrated that is dependent on the salt status both in healthy subjects and in disease states. This complex interplay of salt with aldosterone and PTH might contribute to the development and progression of organ damage in patients with primary aldosteronism or primary hyper-PTH, in patients with heart failure, and in subjects with dietary salt excess. These observations might have important therapeutic implications inasmuch as dietary salt restriction, aldosterone blockers, calcimimetic drugs, and PTH receptor blockers might prove beneficial for cardiovascular and bone protection in these conditions. The effects of all these interventions will have to be tested in appropriately designed studies.

Conflicts of Interest

The authors have no conflict of interest to declare.

Acknowledgments

Cristiana Catena received support from the European Cooperation in the field of Scientific and Technical Research (COST-BM1301) grant. This work was supported by a generous contribution of the PierSilverio Nassimbeni Foundation to Cristiana Catena and Leonardo A. Sechi.

References

[1] R. S. Vasan, A. Beiser, S. Seshadri et al., "Residual lifetime risk for developing hypertension in middle-age women and men: the Framingham heart study," *The Journal of American Medical Association*, vol. 287, no. 8, pp. 1003–1010, 2002.

[2] N. M. Kaplan and L. H. Opie, "Controversies in hypertension," *Lancet*, vol. 367, no. 9505, pp. 168–176, 2006.

[3] G. L. Colussi, C. Catena, S. Baroselli et al., "Omega-3 fatty acids: from biochemistry to their clinical use in the prevention

of cardiovascular disease," *Recent Patents on Cardiovascular Drug Discovery*, vol. 2, no. 1, pp. 13–21, 2007.

[4] C. Catena, M. Novello, L. Dotto, S. De Marchi, and L. A. Sechi, "Serum lipoprotein(a) concentrations and alcohol consumption in hypertension: possible relevance for cardiovascular damage," *Journal of Hypertension*, vol. 21, no. 2, pp. 281–288, 2003.

[5] C. Catena, G. L. Colussi, G. Brosolo et al., "Salt, hypertension and cardiovascular disease," *Journal of Clinical and Laboratory Investigation Updates*, vol. 2, no. 2, pp. 46–49, 2014.

[6] C. Catena, G. L. Colussi, F. Nait, F. Martinis, F. Pezzutto, and L. A. Sechi, "Aldosterone and the heart: still an unresolved issue?," *Frontiers in Endocrinology*, vol. 5, p. 168, 2014.

[7] G. P. Rossi, L. A. Sechi, G. Giacchetti, V. Ronconi, P. Strazzullo, and J. W. Funder, "Primary aldosteronism: cardiovascular, renal and metabolic implications," *Trends in Endocrinolology & Metabolism*, vol. 19, no. 3, pp. 88–90, 2008.

[8] D. V. Martinez, R. Rocha, M. Matsumura et al., "Cardiac damage prevention by eplerenone: comparison with low sodium diet or potassium loading," *Hypertension*, vol. 39, no. 2, pp. 614–618, 2002.

[9] R. Rocha, A. E. Rudolph, G. E. Frierdich et al., "Aldosterone induces a vascular inflammatory phenotype in the rat heart," *American Journal Physiology: Heart and Circulatory Physiology*, vol. 283, no. 5, pp. H1802–H1810, 2002.

[10] L. A. Sechi, G. L. Colussi, A. Di Fabio, and C. Catena, "Cardiovascular and renal damage in primary aldosteronism: which outcomes after treatment," *American Journal of Hypertension*, vol. 23, no. 12, pp. 1253–1260, 2010.

[11] C. Catena, G. L. Colussi, M. Valeri, and L. A. Sechi, "Association of aldosterone with left ventricular mass in hypertension: interaction with plasma fibrinogen levels," *American Journal of Hypertension*, vol. 26, no. 1, pp. 111–117, 2013.

[12] C. Catena, G. L. Colussi, A. Di Fabio et al., "Mineralocorticoid antagonists treatment versus surgery in primary aldosteronism," *Hormone and Metabolic Research*, vol. 42, no. 6, pp. 440–445, 2010.

[13] A. Tomaschitz, E. Ritz, B. Pieske et al., "Aldosterone and parathyroid hormone interactions as mediators of metabolic and cardiovascular disease," *Metabolism*, vol. 63, no. 1, pp. 20–31, 2013.

[14] A. Vaidya, J. M. Brown, and J. S. Williams, "The renin-angiotensin-aldosterone system and calcium-regulatory hormones," *Journal of Human Hypertension*, vol. 29, no. 9, pp. 515–521, 2015.

[15] E. Asbach, M. Bekeran, and M. Reincke, "Parathyroid gland function in primary aldosteronism," *Hormone and Metabolic Research*, vol. 47, no. 13, pp. 994–999, 2015.

[16] K. J. Aaron and P. W. Sanders, "Role of dietary salt and potassium intake in cardiovascular health and disease: a review of the evidence," *Mayo Clinic Proceedings*, vol. 88, no. 9, pp. 987–995, 2013.

[17] S. Mohan and N. R. C. Campbell, "Salt and high blood pressure," *Clinical Science*, vol. 117, no. 1, pp. 1–11, 2009.

[18] I. Drenjancevic-Peric, B. Jelakovic, J. H. Lombard, M. P. Kunert, A. Kibel, and M. Gros, "High-salt diet and hypertension: focus on the renin-angiotensin system," *Kidney and Blood Pressure Research*, vol. 34, no. 1, pp. 1–11, 2011.

[19] J. J. Carvalho, R. G. Baruzzi, P. F. Howard et al., "Blood pressure in four remote populations in the INTERSALT study," *Hypertension*, vol. 14, no. 3, pp. 238–246, 1989.

[20] INTERSALT Cooperative Research Group, "Intersalt: an international study of electrolyte excretion and blood pressure: results for 24 hour urinary sodium and potassium excretion," *British Medical Journal*, vol. 297, no. 6644, pp. 319–328, 1988.

[21] F. He and G. MacGregor, "Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials," *British Medical Journal*, vol. 346, article f1325, 2013.

[22] S. M. Sacks, L. P. Svetkey, W. M. Vollmer et al., "Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet," *New England Journal of Medicine*, vol. 344, no. 1, pp. 3–10, 2001.

[23] A. Mente, M. J. O'Donnel, S. Rangarajan et al., "Association of urinary sodium and potassium excretion with blood pressure," *New England Journal of Medicine*, vol. 371, no. 7, pp. 601–611, 2014.

[24] M. O'Donnell, A. Mente, S. Rangarajan et al., "Urinary sodium and potassium excretion, mortality, and cardiovascular events," *New England Journal of Medicine*, vol. 371, no. 7, pp. 612–623, 2014.

[25] D. Mozaffarian, S. Fahimi, G. M. Singh et al., "Global sodium consumption and death from cardiovascular causes," *New England Journal of Medicine*, vol. 371, no. 7, pp. 624–634, 2014.

[26] R. S. Taylor, K. E. Ashton, T. Moxham, L. Hooper, and S. Ebrhaim, "Reduced dietary salt for the prevention of cardiovascular disease: a meta-analysis of randomized controlled trials (Cochrane review)," *American Journal of Hypertension*, vol. 24, no. 8, pp. 843–853, 2011.

[27] C. Catena, G. L. Colussi, L. Marzano, and L. A. Sechi, "Aldosterone and the heart: from basic research to clinical evidence," *Hormone and Metabolic Research*, vol. 44, no. 3, pp. 181–187, 2012.

[28] C. Catena, G. L. Colussi, G. Brosolo, M. Novello, and L. A. Sechi, "Aldosterone and left ventricular remodeling," *Hormone and Metabolic Research*, vol. 47, no. 13, pp. 981–986, 2015.

[29] P. Milliez, X. Girerd, P. F. Plouin, J. Blacher, M. E. Safar, and J. J. Mourad, "Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism," *Journal of the American College of Cardiolology*, vol. 45, pp. 1243–1248, 2005.

[30] C. Catena, G. Colussi, E. Nadalini et al., "Cardiovascular outcomes in patients with primary aldosteronism after treatment," *Archives of Internal Medicine*, vol. 168, no. 1, pp. 80–85, 2008.

[31] E. Born-Frontsberg, M. Reincke, L. C. Rump et al., "Cardiovascular and cerebrovascular comorbidities of hypokalemic and normokalemic primary aldosteronism: results of the German Conn's Registry," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 4, pp. 1125–1130, 2009.

[32] S. Savard, L. Amar, P. F. Plouin, and O. Steichen, "Complications in patients with primary hyperaldosteronism: a controlled cross-sectional study," *Hypertension*, vol. 62, no. 2, pp. 331–336, 2013.

[33] M. L. Muiesan, M. Salvetti, A. Paini et al., "Inappropriate left ventricular mass in patients with primary aldosteronism," *Hypertension*, vol. 52, no. 3, pp. 529–534, 2008.

[34] C. Catena, G. L. Colussi, R. Lapenna et al., "Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism," *Hypertension*, vol. 50, no. 5, pp. 911–918, 2007.

[35] G. P. Rossi, V. Di Bello, C. Ganzaroli et al., "Excess aldosterone is associated with alterations of myocardial texture in primary aldosteronism," *Hypertension*, vol. 40, no. 1, pp. 23–27, 2002.

[36] M. Stowasser, J. Sharman, R. Leano et al., "Evidence of abnormal left ventricular structure and function in normotensive individuals with familial hyperaldosteronism type I," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 9, pp. 5070–5076, 2005.

[37] G. L. Colussi, C. Catena, and L. A. Sechi, "Spironolactone, eplerenone and the new aldosterone blockers in endocrine and primary hypertension," *Journal of Hypertension*, vol. 31, no. 1, pp. 3–15, 2013.

[38] C. Catena, G. L. Colussi, L. Marzano, and L. A. Sechi, "Predictive factors of left ventricular mass changes after treatment of primary aldosteronism," *Hormone and Metabolic Research*, vol. 44, no. 3, pp. 188–193, 2012.

[39] B. Pitt, N. Reichek, R. Willenbrock et al., "Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-left ventricular hypertrophy study," *Circulation*, vol. 108, no. 15, pp. 1831–1838, 2003.

[40] P. M. Mottram, B. Haluska, R. Leano, D. Cowley, M. Stowasser, and T. H. Marwick, "Effect of aldosterone antagonism on myocardial dysfunction in hypertensive patients with diastolic heart failure," *Circulation*, vol. 110, no. 5, pp. 558–565, 2004.

[41] C. Catena, N. Verheyen, S. Pilz et al., "Plasma aldosterone and left-ventricular diastolic dysfunction in treatment-naïve patients with hypertension. Tissue-Doppler imaging study," *Hypertension*, vol. 65, no. 6, pp. 1231–1237, 2015.

[42] A. Gupta, C. G. Schiros, K. K. Gaddam et al., "Effect of spironolactone on diastolic function in hypertensive left ventricular hypertrophy," *Journal of Human Hypertension*, vol. 29, no. 4, pp. 241–246, 2015.

[43] G. J. Mak, M. T. Ledwidge, C. J. Watson et al., "Natural history of markers of collagen turnover in patients with early diastolic dysfunction and impact of eplerenone," *Journal of the American College of Cardiology*, vol. 54, no. 18, pp. 1674–1682, 2009.

[44] N. C. Edwards, C. J. Ferro, H. Kirkwood et al., "Effect of spironolactone on left ventricular systolic and diastolic function in patients with early stage chronic kidney disease," *American Journal of Cardiology*, vol. 106, no. 10, pp. 1505–1511, 2010.

[45] F. Edelmann, R. Wachter, A. G. Schmidt et al., "Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserved ejection fraction: the Aldo-DHF randomized controlled trial," *The Journal of American Medical Association*, vol. 309, no. 8, pp. 781–791, 2013.

[46] M. A. Pfeffer, B. Clagget, S. F. Assmann et al., "Regional variation in patients and outcomes in the treatment of preserved cardiac function heart failure with an aldosterone antagonist (TOPCAT) trial," *Circulation*, vol. 131, no. 1, pp. 34–42, 2015.

[47] J. W. Funder, "Mineralocorticoid receptor activation and oxidative stress," *Hypertension*, vol. 50, no. 5, pp. 840-841, 2007.

[48] L. A. Sechi, M. Novello, G. L. Colussi et al., "Relationship of plasma renin with a prothrombotic state in hypertension: relevance for organ damage," *American Journal of Hypertension*, vol. 21, no. 12, pp. 1347–1353, 2008.

[49] C. Fan, Y. Kawai, S. Inaba et al., "Synergy of aldosterone and high salt induces vascular smooth muscle hypertrophy through up-regulation of NOX1," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 111, no. 1-2, pp. 29–36, 2008.

[50] C. Catena, G. L. Colussi, and L. A. Sechi, "Aldosterone, organ damage and dietary salt," *Clinical and Experimental Pharmacology and Physiology*, vol. 40, no. 12, pp. 922–928, 2013.

[51] Y. Jin, T. Kuznetsova, M. Maillard et al., "Independent relations of left ventricular structure with the 24-hour urinary excretion of sodium and aldosterone," *Hypertension*, vol. 54, no. 3, pp. 489–495, 2009.

[52] G. du Cailar, P. Fesler, J. Ribstein, and A. Mimran, "Dietary sodium, aldosterone, and left ventricular mass changes during long-term inhibition of the renin-angiotensin system," *Hypertension*, vol. 56, no. 5, pp. 865–870, 2010.

[53] C. Catena, N. D. Verheyen, M. Url-Michitsch et al., "Association of post-saline load plasma aldosterone levels with left ventricular hypertrophy in primary hypertension," *American Journal of Hypertension*, vol. 29, no. 3, pp. 303–310, 2016.

[54] E. Pimenta, R. D. Gordon, A. H. Ahmed et al., "Cardiac dimensions are largely determined by dietary salt in patients with primary aldosteronism: results of a case-control study," *Journal of Clinical Endocrinology & Metabolism*, vol. 96, no. 9, pp. 2813–2820, 2011.

[55] C. Catena, G. L. Colussi, M. Novello et al., "Dietary salt intake is a determinant of cardiac changes after treatment of primary aldosteronism: a prospective study," *Hypertension*, vol. 68, no. 1, pp. 204–212, 2016.

[56] L. M. Resnick, F. B. Muller, and J. H. Laragh, "Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism," *Annals of Internal Medicine*, vol. 105, no. 5, pp. 649–654, 1986.

[57] L. Ceccoli, V. Ronconi, L. Giovannini et al., "Bone health and aldosterone excess," *Osteoporosis International*, vol. 24, no. 11, pp. 2801–2807, 2013.

[58] E. Rossi, C. Sani, F. Perazzoli, M. C. Casoli, A. Negro, and C. Dotti, "Alterations of calcium metabolism and of parathyroid function in primary aldosteronism, and their reversal by spironolactone or by surgical removal of aldosterone-producing adenomas," *American Journal of Hypertension*, vol. 8, no. 9, pp. 884–893, 1995.

[59] C. Maniero, A. Fassina, T. M. Seccia et al., "Mild hyperparathyroidism: a novel surgically correctable feature of primary aldosteronism," *Journal of Hypertension*, vol. 30, no. 2, pp. 390–395, 2012.

[60] L. Petramala, M. Zinnamosca, A. Settevendemmie et al., "Bone and mineral metabolism in patients with primary aldosteronism," *International Journal of Endocrinology*, vol. 2014, Article ID 836529, 6 pages, 2014.

[61] V. C. Wu, C. H. Chang, C. Y. Wang et al., "Risk of fracture in primary aldosteronism: a population-based cohort study," *Journal of Bone and Mineral Research*, vol. 32, no. 4, pp. 743–752, 2017.

[62] A. Vidal, Y. Sun, S. K. Bhattacharya, R. A. Ahokas, I. C. Gerling, and K. T. Weber, "Calcium paradox of aldosteronism and the role of the parathyroid glands," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 290, no. 1, pp. H286–H294, 2006.

[63] C. Maniero, A. Fassina, V. Guzzardo et al., "Primary hyperparathyroidism with concurrent primary aldosteronism," *Hypertension*, vol. 58, no. 3, pp. 341–346, 2011.

[64] O. Skott, T. R. Uhrenholt, J. Schjerning, P. B. Hansen, L. E. Rasmussen, and B. L. Jensen, "Rapid actions of aldosterone in vascular health and disease—friend or foe?," *Pharmacology & Therapeutics*, vol. 111, no. 2, pp. 495–507, 2006.

[65] V. S. Chhokar, Y. Sun, S. K. Bhattacharya et al., "Hyperparathyroidism and the calcium paradox of aldosteronism," *Circulation*, vol. 111, no. 7, pp. 871–878, 2005.

[66] A. L. Runyan, V. S. Chhokar, Y. Sun, S. K. Bhattacharya, J. W. Runyan, and K. T. Weber, "Bone loss in rats with aldosteronism," *American Journal of Medical Sciences*, vol. 330, no. 1, pp. 1–7, 2005.

[67] V. S. Chhokar, Y. Sun, S. K. Bhattacharya et al., "Loss of bone minerals and strength in rats with aldosteronism," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 287, no. 5, pp. H2023–H2026, 2004.

[68] L. D. Carbone, J. D. Cross, S. H. Raza et al., "Fracture risk in men with congestive heart failure risk reduction with spironolactone," *Journal of the American College of Cardiology*, vol. 52, no. 2, pp. 135–138, 2008.

[69] D. A. McCarron, L. I. Rankin, W. M. Bennett, S. Krutzik, M. R. McClung, and F. C. Luft, "Urinary calcium excretion at extremes of sodium intake in normal man," *American Journal of Nephrology*, vol. 1, no. 2, pp. 84–90, 1981.

[70] E. Rossi, F. Perazzoli, A. Negro et al., "Acute effects of intravenous sodium chloride load on calcium metabolism and on parathyroid function in patients with primary aldosteronism compared with subjects with essential hypertension," *American Journal of Hypertension*, vol. 11, no. 1, pp. 8–13, 1998.

[71] F. P. Cappuccio, N. D. Markandu, and G. A. MacGregor, "Renal handling of calcium and phosphate during mineralocorticoid administration in normal subjects," *Nephron*, vol. 48, no. 4, pp. 280–283, 1988.

[72] M. R. Rutledge, V. Farah, A. A. Adeboye, M. R. Seawell, S. K. Bhattacharya, and K. T. Weber, "Parathyroid hormone, a crucial mediator of pathologic cardiac remodeling in aldosteronism," *Cardiovascular Drugs and Therapy*, vol. 27, no. 2, pp. 161–170, 2013.

[73] R. N. Khouzam, D. A. Dishmon, V. Farah, S. D. Flax, L. D. Carbone, and K. T. Weber, "Secondary hyperparathyroidism in patients with untreated and treated congestive heart failure," *American Journal of the Medical Sciences*, vol. 331, no. 1, pp. 30–34, 2006.

[74] Y. Cheema, J. N. Sherrod, W. Zhao et al., "Mitochondriocentric pathway to cardiomyocyte necrosis in aldosteronism: cardioprotective responses to carvedilol and nebivolol," *Journal of Cardiovascular Pharmacology*, vol. 58, no. 1, pp. 80–86, 2011.

Hindawi
International Journal of Endocrinology
Volume 2017, Article ID 7454376, 12 pages
https://doi.org/10.1155/2017/7454376

Review Article

The Synergistic Interplay between Vitamins D and K for Bone and Cardiovascular Health: A Narrative Review

Adriana J. van Ballegooijen,[1] Stefan Pilz,[2,3] Andreas Tomaschitz,[4] Martin R. Grübler,[2,5] and Nicolas Verheyen[6]

[1]*Department of Health Sciences, Vrije Universiteit Amsterdam and the Amsterdam Public Health Research Institute, Amsterdam, Netherlands*
[2]*Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Graz, Austria*
[3]*Department of Epidemiology and Biostatistics, VU University Medical Center and the Amsterdam Public Health Research Institute, Amsterdam, Netherlands*
[4]*Bad Gleichenberg Clinic, Bad Gleichenberg, Austria*
[5]*Department of Cardiology, Swiss Cardiovascular Center Bern, Bern University Hospital, University of Bern, Bern, Switzerland*
[6]*Department of Cardiology, Medical University of Graz, Graz, Austria*

Correspondence should be addressed to Adriana J. van Ballegooijen; hanne.van.ballegooijen@vu.nl

Received 2 June 2017; Accepted 17 August 2017; Published 12 September 2017

Academic Editor: Constantinos Pantos

Vitamins D and K are both fat-soluble vitamins and play a central role in calcium metabolism. Vitamin D promotes the production of vitamin K-dependent proteins, which require vitamin K for carboxylation in order to function properly. The purpose of this review is to summarize available evidence of the synergistic interplay between vitamins D and K on bone and cardiovascular health. Animal and human studies suggest that optimal concentrations of both vitamin D and vitamin K are beneficial for bone and cardiovascular health as supported by genetic, molecular, cellular, and human studies. Most clinical trials studied vitamin D and K supplementation with bone health in postmenopausal women. Few intervention trials studied vitamin D and K supplementation with cardiovascular-related outcomes. These limited studies indicate that joint supplementation might be beneficial for cardiovascular health. Current evidence supports the notion that joint supplementation of vitamins D and K might be more effective than the consumption of either alone for bone and cardiovascular health. As more is discovered about the powerful combination of vitamins D and K, it gives a renewed reason to eat a healthy diet including a variety of foods such as vegetables and fermented dairy for bone and cardiovascular health.

1. Introduction

Worldwide, a large group of people is prescribed to a supplemental regime of both vitamin D and calcium. In Europe, depending on a country and sex, between 1 and 66% of the adult population use vitamin D supplements [1, 2]. Over the last decade, large vitamin D supplementation is promoted to restore 25-hydroxyvitamin D (25(OH)D) concentrations and is considered to be safe with doses up to 4000 international units (IU) per day [3]. However, little is known about potential long-term high-dose vitamin D supplementation [2, 4].

Vitamin D is a fat-soluble vitamin that can be ingested by foods such as fatty fish, dairy products, and eggs, but is mainly synthesized by the human skin when exposed to sunlight. In the liver, vitamin D is hydroxylated to 25(OH)D, the main circulating vitamin D metabolite that is measured to assess and classify vitamin D status. Circulating 25(OH)D is further metabolized by the kidney for full biological activity into its most active form 1,25-dihydroxyvitamin D (1,25(OH)D) also known as calcitriol. Calcitriol is also produced endogenously by extrarenal production through peripheral 1-α-hydroxylase and has positive effects on immune function and anticancer activity [5–7]. Vitamin D

plays a main role in regulating calcium metabolism by increasing intestinal calcium absorption [8]. Ample evidence recommends vitamin D supplementation for the prevention of falls and fractures [9, 10]; however, evidence suggests calcium precipitation in the vasculature and other potential side effects [4, 11–14].

Vitamin K is another fat-soluble vitamin that exists in two forms of vitamin K: vitamin K_1 (phylloquinone, mainly found in green leafy vegetables) and vitamin K_2 (menaquinone, mainly found in fermented dairy and produced by lactic acid bacteria in the intestine) [15]. Vitamin K stores are limited, but they can be recycled to a certain extent [16]. Vitamin K_1 is principally transported to the liver, regulating the production of coagulation factors, while vitamin K_2 is transported to extrahepatic tissues, such as bone and the vascular wall, regulating the activity of matrix Gla protein (MGP) and osteocalcin (bone Gla protein)—the main vitamin K-dependent proteins. They require vitamin K for carboxylation in order to function properly. When circulating concentrations of vitamin K are insufficient, a greater proportion of MGP and osteocalcin remain uncarboxylated, which is associated with unfavorable outcomes such as cardiovascular disease, lower BMD, and osteoporosis [17]. The current recommendation for vitamin K_1 intake is 70 μg/day for all adults defined by an adequate intake [18]. This amount is solely based on maintaining coagulation function and might not be enough for optimal carboxylation of other vitamin K-dependent proteins, which require higher amounts of vitamin K [19].

The role of vitamin K in cardiovascular health has mainly been studied in isolation [20]; however, a growing body of evidence suggests a synergistic effect of vitamin K combined with vitamin D [21–26]. Vitamin D promotes the production of vitamin K-dependent proteins, as shown in rats by Karl et al. already in 1985 [27]. These findings cannot be explained by our current understanding of the biochemical role of vitamin K, but suggest that vitamin D may influence vitamin K-dependent proteins [28].

The purpose of this narrative review is to summarize available evidence in the field of the synergistic interplay between vitamins D and K on bone and cardiovascular health. The primary focus is on the general population and includes observational studies that investigated both vitamin D and vitamin K status with outcome measures and supplementation studies that administered both vitamins D and K.

2. Interaction of Vitamins D and K for Bone Health

2.1. Experimental Studies. In experimental models, the exploration of the interaction between vitamins D and K on bone health is ongoing for decades and a fair amount of literature is available. Recent understanding suggests that vitamin D enhances vitamin K-dependent bone protein concentrations and induces bone formation in vitro [29–31] with stimulation of osteoblast-specific gene expression [32]. Osteoblast-specific expression of osteocalcin is controlled at the transcriptional level by 1,25(OH)D through the 1,25(OH)D-responsive element within the promoter of the osteocalcin gene [32]. The underlying mechanism of mineralization induced by vitamin K in the presence of 1,25(OH)D was different from vitamin K alone [33]. In rats, 1,25(OH)D receptor binding can undergo gamma-carboxylation in the presence of vitamin K. This means that 1,25(OH)D receptor carboxylation can potentially modify the intrinsic biochemical properties of the nuclear receptors and modulates its binding to DNA [34].

The effect of 1,25(OH)D and warfarin—a vitamin K antagonist—on the vitamin K cycle was studied in cultured osteoblasts [26]. Epoxide reductase, one of the key enzymes in the vitamin K cycle, was strongly inhibited by warfarin, whereas it was not affected by 1,25(OH)D, meaning that the vitamin K metabolic cycle functions normally in human osteoblasts.

Human osteoblast cell cultures indicate that glycoxidation interferes with the maturation of osteoblasts; however, this process may be counterbalanced by adding vitamins D and K, which reverses the detrimental glycoxidation on several bone markers [35]. Therefore, the addition of vitamins D and K may induce important biochemical changes in bone, which may exert therapeutic effects on bone metabolic diseases such as osteoporosis [36].

2.2. Animal Models. A growing body of evidence is also documenting the interaction between vitamins D and K in animal models. The effect of vitamin K of bone mineralization is enhanced by plasma 25(OH)D concentration. Vitamin K was administered to prevent osteoporosis in ovariectomized rats, but bone loss was only prevented in rats fed with a diet containing vitamin D or vitamin D supplementation [37, 38]. These findings suggest that combined treatment with vitamins D and K is more effective than vitamin K alone particularly in the early phase of estrogen deficiency after menopause.

Vitamin K and vitamin D supplementation on calcium balance was investigated in young rats fed with a normal or low calcium diet, plus vitamin K and/or vitamin D [39]. Vitamin K supplementation promoted the reduction in urinary calcium excretion and stimulated intestinal calcium absorption in rats on a normal calcium diet. Vitamin D supplementation stimulated intestinal calcium absorption with prevention of the abnormal elevation of serum PTH concentrations, prevented hypocalcemia in rats fed with a low calcium diet, and stimulated intestinal calcium absorption in rats fed with a normal calcium diet. The stimulation of intestinal calcium absorption was associated with increased 1,25(OH)D concentrations. An additive effect of vitamin K and vitamin D on intestinal calcium absorption was only found in rats fed with a normal calcium diet. This study shows the differential effects of vitamin K and vitamin D supplementation on calcium balance in young rats fed with a normal or low calcium diet.

2.3. Observational Evidence. Human evidence for the role of 1,25(OH)D in stimulating vitamin K-dependent proteins is scarce. In hemodialysis patients, vitamin D analog users had much higher concentrations of bone Gla protein (BGP) than nonusers indicating that vitamin D administration

may play a role in stimulating vitamin K-dependent protein activity [40]. More research on the stimulating role of vitamin D on vitamin K-dependent proteins is urgently needed to study the underlying mechanisms.

Some observational studies support the hypothesis that optimal concentrations of both vitamins D and K support bone mineralization and lower fracture risk. In a cross-sectional study among Japanese older men, lower 25(OH)D and vitamin K_1 concentrations were concomitantly associated with BMD, indicating a nonestrogen-dependent pathway in men [41]. In a case-control study of 184 Norwegian older adults, the combination of low vitamin K_1 and low 25(OH)D was synergistically associated with hip fractures: odds ratio 7.6 (95% CI 2.3, 26.7) [42]. In the NOREPOS study, another Norwegian population study, similar results were observed among 1318 older adults [43]. During 8.2-year follow-up, the combination of both low vitamin D and K_1 concentrations was associated with a greater hip fracture risk, hazard ratio 1.41 (95% CI 1.09, 1.82), compared to the high vitamin D and vitamin K category. No increased risk was observed in the groups low in 1 vitamin only. These results indicate that the combination of low concentrations of vitamin K_1 and 25(OH)D is associated with increased risk of hip fractures.

2.4. Human Intervention Studies. A small study among 15 healthy women indicated that 3 weeks of supplementation of 20 ml extra virgin olive oil enriched with vitamins D, K, and B_6 resulted in lower concentrations of uncarboxylated osteocalcin [44]. This means that a vitaminized oil can influence vitamin K-dependent proteins within multiple weeks.

An increasing amount of randomized controlled trials have demonstrated the combined effects of vitamins D and K on postmenopausal osteoporosis mostly pursued in Japan with a study duration between 8 weeks and 3 years (Table 1). A randomized trial with 4 arms (diet, menaquinone-4, cholecalciferol, and menaquinone-4 + cholecalciferol) showed that only the vitamin K plus vitamin D arm increased BMD [45]. Similar results were found in another trial with postmenopausal women with osteoporosis ≥ 5 years after menopause [46]. After 2 years of follow-up, the longitudinal changes in BMD were significant compared with those in the calcium lactate-, vitamin D-, and vitamin K-only groups ($P < 0.001$). A modest synergistic effect of vitamins D and K was found after 2 years in healthy older women from nutritionally relevant intakes of vitamin K_1 together with supplements of calcium plus vitamin D_3 on bone mineral concentration compared to either vitamin D or K alone or placebo [47]. The complementary effect of vitamin K_1 (1 mg/day) and a mineral + vitamin D supplement (8 μg/day) was most effective in reducing bone loss at the femoral neck after 3 years among postmenopausal women versus vitamin D alone or placebo [48]. The addition of vitamin K to vitamin D and calcium supplements compared to vitamin D and calcium alone in postmenopausal Korean women increased BMD and reduced uncarboxylated osteocalcin concentrations after 6 months compared to vitamin D and calcium alone [49]. In postmenopausal women, 1 year of oral supplementation with extra virgin olive oil enriched with vitamins D_3, K_1, and B_6 or extra virgin olive oil reduced uncarboxylated osteocalcin concentrations and increased the T-score of BMD [50]. These findings indicate that combined administration of vitamin D and vitamin K appears to be useful in increasing BMD in postmenopausal women. It should be noted that these studies found beneficial effects at some but not all BMD sites measured. Furthermore, treatment with vitamins D and K with calcium increased BMD in older female patients with Alzheimer's disease and led to the prevention of nonvertebral fracture odds ratio: 7.5 (95% CI 5.6, 10.1); however, no placebo capsules were administered, hampering the interpretation of the results [51].

Not all studies observed synergistic effects of vitamin D and K supplementation. A small study among adults with Crohn's disease in Ireland showed generally no effect of combined vitamin D and K supplementation versus placebo on bone mass after 1 year, except a modest increase in bone mass of the total radius [52]. Among healthy women, 1 year of vitamin D and calcium + vitamin K supplementation either by phylloquinone or menaquinone-4 supplementation had no effect on BMD compared to calcium and vitamin D alone [53]. This study does not support a combined role for vitamin D + K supplementation in osteoporosis prevention; however, the relatively short study duration and the inclusion of healthy women could explain the null finding. It is however questionable if BMD can be improved in 12 months since changes in BMD usually require at least 1 year of follow-up time.

Among healthy older men and women, no difference was observed between multivitamin and calcium and vitamin D compared with the addition of vitamin K on BMD after 3 years [19]. An additive effect was noticeable for decreased percentage of uncarboxylated osteocalcin, which indicates an improved vitamin K status in the treatment group.

The ECKO trial among postmenopausal women with osteopenia showed no beneficial effect of vitamin D and calcium + vitamin K supplementation versus vitamin D and calcium alone after 2 years of follow-up in vitamin D-sufficient women [54]. However, the risk of fractures—a clinically more meaningful endpoint—was lower in the vitamin D and calcium + vitamin K groups: hazard ratio 0.41 (95 CI 0.1, 1.18) at 2 years and 0.45 (95% CI 0.20, 0.98) after 4 years of follow-up. This result on fracture risk indicates that bone quality rather than quantity is more important as not all trials showed synergistic effects of vitamin D and K supplementation on bone mineral density.

The protective effect of vitamin D with K on prednisolone-induced loss of BMD in patients with chronic glomerulonephritis after 8 weeks of treatment was similar in the vitamin D-only group [55], meaning that the addition of vitamin K had no synergistic effect. The elevation in serum calcium concentrations in the vitamin D group was, however, attenuated in the vitamin D + K group.

Taken together the evidence for combined vitamin D and K supplementation, the majority of the studies found beneficial effects for BMD among postmenopausal women.

Table 1: Summary of clinical trials of combined vitamin D and K supplementation on bone health.

Author, year	Country	Participants	Treatment	Study duration	Outcome	Results for the highest versus the lowest quartiles
Iwamoto et al., 2000 [46]	Japan	$N = 92$ osteoporotic women ≥ 5 years after menopause, mean age 64 years	(i) Calcium (calcium lactate, 2 g/day) (ii) Vitamin D_3 0.75 μg/day (iii) Vitamin K_2 45 mg/day (iv) Vitamin D_3 plus vitamin K_2	2 years	Bone mineral density % change	Combined vitamins D and K increased BMD
Ushiroyama et al., 2002 [45]	Japan	$N = 126$ postmenopausal women with osteopenia and osteoporosis, mean age 53 years	(i) Diet (ii) Vitamin K_2 45 mg/day MK-4 (iii) 1-α hydroxylcholecalciferol 1 μg/day (iv) Vitamin K_2 + 1-α hydroxylcholecalciferol	2 years	Bone mineral density % change	K + D group increased BMD % change at 2 years $P < 0.001$
Braam et al., 2003 [48]	Netherlands	$N = 155$ postmenopausal women between 50 and 60 years	(i) Placebo (ii) Mineral + vitamin D (8 μg/day) (iii) Mineral + vitamin D + vitamin K_1 1 mg	3 years	Bone loss	Mineral + vitamin D + vitamin K showed reduced bone loss of the femoral neck
Yonemura et al., 2004 [55]	Japan	$N = 60$ patients with chronic glomerulonephritis, mean age 32 years, 53% female	(i) Control (ii) Vitamin D (alfacalcidol 0.5 mg) (iii) Vitamin K_2 MK-4 45 mg/d (iv) Vitamins D plus K	8 weeks	Bone mineral density	The preventive effect in groups K and D + K was similar to D
Sato et al., 2005 [51]	Japan	$N = 200$ older women with Alzheimer's disease, mean age 78 years	(i) Placebo (ii) 45 mg menatetrenone, 1000 IU ergocalciferol, and 600 mg calcium	2 years	Bone mineral density and fractures	BMD increased in vitamin D + K group Odds ratio nonvertebral fractures 7.5 (95% CI 5.6, 10.1)
Bolton-Smith et al., 2007 [47]	UK	$N = 244$ healthy women, mean age 68 years	(i) Placebo (ii) 200 mg/d vitamin K_1 (iii) 400 IU vitamin D_3 + 1000 mg calcium (iv) Vitamins K_1 and D_3 plus calcium	2 years	Bone mineral content	Combined vitamin K with vitamin D plus calcium associated with an increase in bone mineral content at the ultradistal radius
Booth et al. 2008 [19]	US	$N = 401$ healthy men and women, mean age 69, 59% female	(i) Multivitamin + 10 μg vitamin D and 600 mg calcium (ii) Multivitamin + vitamin D + calcium + 500 μg vitamin K_1	3 years	Bone mineral density	No differences in change in BMD Vitamin D + K group lower uncarboxylated osteocalcin concentrations
Cheung et al., 2008 [54]	Canada	$N = 440$ postmenopausal women with osteopenia, mean age 59 years	(i) 1500 mg calcium + 800 IU vitamin D (ii) 5 mg of vitamin K_1 + calcium and vitamin D	2–4 years	Bone mineral density	No effect on BMD
Binkley et al., 2009 [53]	US	$N = 381$ postmenopausal women, mean age 62 years	(i) Calcium 315 mg + vitamin D_3 200 IU (ii) Phylloquinone 1 mg + calcium and vitamin D_3 (iii) MK-4 (45 mg day) + calcium and vitamin D_3	1 year	Bone mineral density	No effect on BMD
Je et al., 2011 [49]	South Korea	$N = 78$ Korean postmenopausal women, mean age 68 years	(i) Vitamin D 400 IU + calcium (630 mg) (ii) Vitamin D + calcium +45 mg of vitamin K_2	6 months	Bone mineral density	BMD increased significantly in the vitamin D + K group

Table 1: Continued.

Author, year	Country	Participants	Treatment	Study duration	Outcome	Results for the highest versus the lowest quartiles
O'Connor et al., 2014 [52]	Ireland	$N = 46$ adults with Crohn's disease, mean age 45 years	(i) Placebo (ii) Phylloquinone 1 mg, vitamin D 10 μg, and calcium 500 mg/d	1 year	Bone mineral density	Small effect on BMD of the total radius for vitamin D + K group
Mazzanti et al., 2015 [50]	Italy	60 healthy postmenopausal women, mean age 55 years	(i) Extra virgin olive oil (ii) Extra virgin olive oil enriched with vitamins D_3, K_1, and B_6	1 year	Bone mineral density	Vitaminized oil D, K, and B_6 increased the T-score of BMD

BMD: bone mineral density; MK-4: menaquinone-4.

3. Interaction between Vitamins D and K for Cardiovascular Health

Besides bone health, also, the interaction between vitamins D and K with regard to cardiovascular health receives growing research interest. MGP—the vascular marker of vitamin K status—needs γ-glutamate carboxylation to inhibit vascular calcification [56]. In an experimental rat model, warfarin was administered to induce vitamin K deficiency and caused arterial calcification [57], which was accelerated when given toxic doses of vitamin D and resulted in premature death.

The Czech MONICA study cross-sectionally observed that subjects in the highest quartile of dephosphorylated-uncarboxylated MGP (dp-ucMGP) plus the lowest quartile of 25(OH)D concentrations had the highest pulse wave velocity in middle-aged healthy adults [58]. Further, potential interaction between vitamin K status and polymorphisms of the vitamin D receptors was investigated. Pulse wave velocity was higher with the number of G-allele polymorphisms and highest in the top quartile of dp-ucMGP for the GG vitamin D receptor genotype.

A Dutch prospective cohort indicates that the combination of low vitamin D < 50 mmol/L and low K status ≥ 323 mmol/L dp-ucMGP was associated with increased systolic and diastolic blood pressures and incident hypertension after 6 years of follow-up [59]. Up to now, no study investigated the combination of optimal vitamin D and K status in relation to coronary artery calcification and cardiovascular events after long-term follow-up. This would give valuable insight if vitamins D and K are involved in developing cardiovascular disease.

So far, two human intervention studies in healthy populations have investigated the combined effect of vitamins D and K on vascular function and calcification (Table 2) [60, 61]. In postmenopausal women, after 3 years of supplementation (1000 μg/d vitamin K_1 + 320 IU vitamin D), the vitamin D + K group maintained vessel wall characteristics of the carotid artery, whereas the control group and the vitamin D-only group significantly worsened over 3 years of follow-up [60]. However, vitamin K status was not measured as a marker of compliance to investigate what would have occurred following supplementation. Further, in a 3-year, double-blind, randomized controlled trial in older men and women free of clinical CVD, daily supplemental vitamin K in amounts achievable by high dietary intake of green, leafy vegetables (500 μg/day) combined with 600 mg calcium carbonate and 10 μg (400 IU) vitamin D did not result in lower coronary artery calcium progression as assessed by computerized tomography compared to the calcium + vitamin D group. In a subgroup analysis of participants who were ≥85% adherent to supplementation, there was less coronary artery calcium progression in the vitamin K + calcium and vitamin D groups than in the calcium and vitamin D group alone [61]; however, MGP carboxylation status was not determined. These data are hypothesis generating, and further studies are warranted to clarify the mechanism.

Among overweight type 2 diabetic patients with coronary heart disease, cosupplementation for 12 weeks of vitamins D (10 μg) and K (180 μg) and calcium (1000 mg) had beneficial effects on maximum levels of left carotid intima-media thickness and insulin metabolism markers [62]; however, no effect on right intima-media thickness was found and the results could be a chance finding. Unfortunately, circulating markers of vitamin K concentrations and vitamin K-dependent proteins were not taken into account to get a better mechanistic understanding.

Two trials studied the effect of vitamin D versus vitamin D + K in nondialyzed CKD patients on vascular calcification and cardiovascular risk factors for 9 months [63, 64]. In 42 CKD patients, the increase in carotid intima-media thickness (IMT) was significantly lower in the K (90 μg menaquinone-7) + D (10 μg vitamin D) group compared with the D-only group after 9 months [63]. Another small trial ($n = 38$) from the same research group did not show differences between the D versus D + K groups on cardiovascular risk markers [64]. These few studies show some potential for the combined effect of vitamins D + K versus D alone on subclinical CVD risk markers. It should be noted that very few clinical studies have been conducted in this field and that vitamin D + K supplements have been often combined with different micronutrients making it difficult to solely pinpoint the effect of vitamin D + K. These limited studies indicate that joint supplementation might benefit cardiovascular health.

4. Vitamins D and K with Glucose Metabolism and Inflammation

Another pathway that might affect CVD risk is via disturbances in glucose metabolism. Among Iranian vitamin D-deficient women with polycystic ovary syndrome—a dysmetabolic disorder—cosupplementation of calcium (1000 mg) and vitamins D (400 IU) and K (180 μg) for 8 weeks improved markers of insulin metabolism and lipid concentrations compared to placebo [65]. The joint supplementation of vitamins D and K might improve insulin metabolism through an effect on upregulation of the insulin receptor genes, the regulation of insulin secretion from the pancreatic beta-cell, the enhancement of β-cell proliferation, and suppression of parathyroid hormone [66–69].

Further, another feature in which both vitamins D and K overlap is on inflammation, which is strongly related to the development of CVD and osteoporosis [70]. In the same Iranian clinical trial among vitamin D-deficient women with polycystic ovary syndrome, the joint supplementation of calcium with vitamins D and K had beneficial effects on endocrine and oxidative stress markers, however no effect on inflammatory markers [71].

5. Effects of Long-Term Vitamin D Supplementation

A large group of people uses both vitamin D and calcium for the prevention of falls and fractures. Given the fact that 25(OH)D is converted to 1,25(OH)D, vitamin D supplementation stimulates the production of 1,25(OH)D [72]. This means that long-term vitamin D supplementation could promote the production of large amounts of vitamin K-

Table 2: Summary of clinical trials of combined vitamin D and K supplementation on cardiovascular health and disease.

Author, year	Country	Participants	Treatment	Study duration	Outcome	Results for the highest versus the lowest quartiles
Braam et al., 2004 [60]	Netherlands	$N = 181$ postmenopausal women, means age 55, 100% female	(i) Placebo (ii) Minerals + 8 μg vitamin D (iii) Minerals + 8 μg vitamin D + 1 mg vitamin K_1	3 years	Vessel wall characteristics	MDK group unchanged, placebo and minerals + vitamin D decreased elastic properties
Shea et al., 2009 [61]	US	$N = 388$ healthy men and postmenopausal women, mean age 66 y, 60% female	(i) Multivitamin + 10 μg vitamin D and 600 mg calcium (ii) Multivitamin + vitamin D + calcium + 500 μg vitamin K_1	3 years	Coronary artery calcification	No difference between vitamin K_1 group and control group
Asemi et al., 2016 [62]	Iran	$N = 66$ overweight diabetic patients with coronary heart disease, mean age 65 y, 47% female	(i) Placebo (ii) Vitamin D (10 μg), K (180 μg), and calcium (1000 mg)	12 weeks	Carotid IMT	Lower left carotid intima-media thickness and improved insulin metabolism markers
			Chronic kidney disease patients			
Kurnatowska et al., 2015 [63], 2016 [64]	Poland	$N = 42$ nondialyzed CKD patient stages, mean age 60 y, 3–5, 45% female	(i) 10 μg cholecalciferol (ii) 10 μg cholecalciferol + 90 μg MK-7	270 days	Carotid IMT	Reduced progression IMT, reduced dp-ucMGP and osteocalcin

IMT: intima-media thickness; dp-ucMGP: dephosphorylated-uncarboxylated matrix Gla protein; MK-7: menaquinone-7.

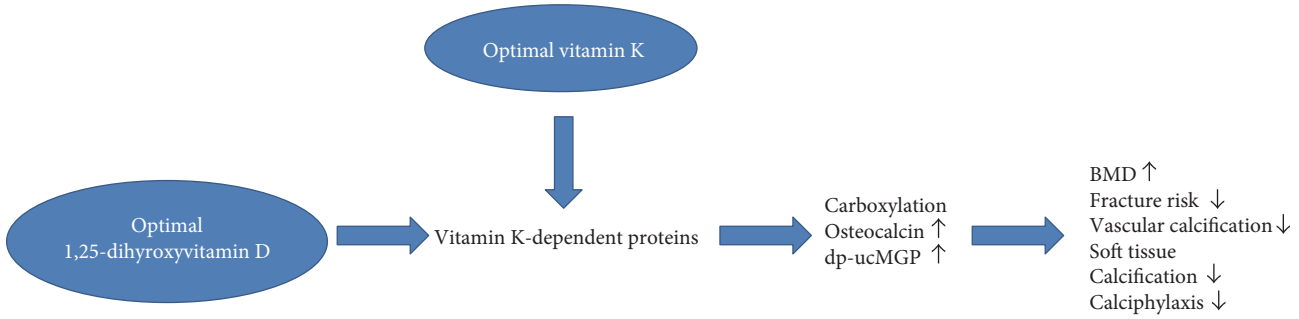


Figure 1: Simplified overview of potential synergy between vitamins D and K and bone and cardiovascular health. dp-ucMGP: dephosphorylated-uncarboxylated matrix Gla protein: BMD: bone mineral density. Genetic, molecular, cellular, and human evidence support that optimal concentrations of both vitamin D and vitamin K are beneficial for bone and cardiovascular health. Vitamin K is needed for the carboxylation of vitamin K-dependent proteins such as osteocalcin and matrix Gla protein, while vitamin D promotes the production of vitamin K-dependent protein concentrations. These vitamin K-dependent proteins are needed for extrahepatic organs such as the bone and the vascular system. This will result in bone mineralization and will inhibit soft tissue calcification, which will ultimately lead to lower risks of fractures and coronary heart disease.

dependent proteins, which remain inactive because there is not enough vitamin K to carboxylate (Figure 1). We propose a new hypothesis that if vitamin D concentrations are constantly high, there might not be enough vitamin K for activation of vitamin K-dependent proteins. Consequently, excess vitamin D diminishes the ability of vitamin K-dependent proteins to function properly, to stimulate bone mineralization, and to inhibit soft tissue calcification.

Further, increasing vitamin D intake through dietary or supplemental source increases intestinal calcium absorption, particularly when combined with calcium supplementation, and promotes hypercalcemia [73]. In this context, a human trial was performed in older women who received either 1200 mg calcium or 1200 mg calcium and 800 IU vitamin D per day over a 12-week period [74]. At the end of the 12 weeks, neither group observed a change in calcium concentrations, meaning that calcium was either excreted or stored somewhere. Increased calcium intake by itself may not be problematic as long as there is a steady state between optimal vitamin D and vitamin K concentrations. The disbalance between vitamin D and vitamin K promotes an environment in which excess calcium will be deposited into our vascular tissue instead of bone. The migration of calcification into the vascular tissue is described by the double burden of atherosclerosis and osteoporosis [75–77]. Additionally, as vitamin D increases calcium absorption, it might also promote hypercalcemia as seen in the Women's Health Initiative, which found a 24% higher risk of myocardial infarction in individuals taking calcium and vitamin D supplements and a greater risk for urinary tract stone occurrence: hazard ratio 1.17 (95% CI 1.02, 1.34) [11, 13, 14]. One prospective study found that higher 1,25(OH)D concentrations were strongly associated with the incidence of hypertension, while 25(OH)D was inversely associated with hypertension risk [78]. Higher 1,25(OH)D was associated with lower urinary calcium excretion, which could mean that the calcium meant for bone is stored somewhere else. Unfortunately, vitamin K status was not measured which would have given valuable insight into the association between vitamins D and K with calcium excretion.

6. Calciphylaxis and Vitamin K Antagonist Use

Calciphylaxis is a syndrome of calcification of the blood vessels, coagulopathy, and skin necrosis. It is seen mostly in patients with end-stage kidney disease, but can occur in the absence of kidney failure. Vitamin K antagonist use may contribute to its development [79]. The syndrome may cause a substantial morbidity and mortality. However, it should be acknowledged that the term calciphylaxis refers to a heterogeneous disorder that is characterized by soft tissue and vascular necrosis and has a clinical presentation from mild to severe. The underlying causes of calciphylaxis are not well understood; however, reported risk factors include female sex, obesity, elevated calcium*phosphate product, warfarin use, and vitamin D derivatives, for example, calcitriol, calcium-based binders, or systemic steroids, low blood albumin concentrations, and type 2 diabetes [80]. A recent study among patients with hemodialysis with calciphylaxis versus hemodialysis showed that cases had higher plasma uncarboxylated MGP concentrations than controls, which suggest a role of MGP in the pathophysiology of calciphylaxis. The fraction of total MGP that was carboxylated was also lower in cases than in controls. Vitamin K deficiency-mediated reduction in relative carboxylated MGP concentration may play a role in the pathogenesis of calciphylaxis [81]. This could be further mediated by the combined use of vitamin D derivatives and warfarin. Further, another study indicated that vitamin K antagonist use predisposes to the development of calciphylaxis in end-stage renal disease [82]. More evidence on the combined role of vitamin K antagonist use and vitamin D on bone and cardiovascular health is urgently needed.

7. Vitamin D and K Supplementation

Based on the current body of evidence, there is not enough evidence to recommend combined vitamin D and K supplementation for the prevention and treatment of osteoporosis. Most trials studied low-dose vitamin D in isolation (400–800 IU daily), which demonstrated only

modest or null effects on BMD and fracture prevention in mostly ≥65 years postmenopausal women [6–8]. Large clinical trials of moderate–high dose (≥800 IU daily) vitamin D supplementation (cholecalciferol) are currently in progress.

The most widely used vitamin K form for supplementation is vitamin K_2 and more specifically menaquinone-4 and menaquinone-7. Menaquinone-4 is more used in trials with bone outcomes, while menaquinone-7 is more in trials with cardiovascular outcomes with dosages between 90–360 μg. Menaquinone-7 has a higher bioavailability and may be of particular importance for extrahepatic tissue [83]. No cut-off value for vitamin K status nor vitamin K supplementation is available yet. Future studies are needed to determine whether vitamin D combined with vitamin K rich foods or vitamin K supplementation could improve bone and cardiovascular health.

8. Recommendations for Future Research

The recommendations for future research are as follows:

(i) Evaluate the role of vitamin D administration in vitamin K-dependent proteins in human populations

(ii) Question the possible long-term consequences of high-dose vitamin D supplementation

(iii) Assess the combined role of vitamin K antagonist use and vitamin D in bone and cardiovascular health

(iv) Investigate the joint supplementation of vitamins D and K on hard clinical endpoints

9. Conclusion

Taken together, animal and human studies suggest that optimal concentrations of both vitamin D and vitamin K are beneficial for bone and cardiovascular health as supported by genetic, molecular, cellular, and some human studies. However, vitamin D and calcium supplementation along with vitamin K deficiency might also induce long-term soft tissue calcification and CVD, particularly in vitamin K antagonist users and other high-risk populations. At this moment, we should be careful about supplementing high-dose vitamin D, unless indicated differently. More clinical data about the potential interplay between vitamin D and vitamin K metabolism is urgently needed before broader treatment recommendations can be given.

The consumption of a well-balanced diet is key for population-based primary prevention of chronic diseases. As more is discovered about the powerful combination of vitamins D and K, it gives a renewed reason to eat a healthy diet including a variety of foods such as vegetables and fermented dairy for bone and cardiovascular health.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] G. Skeie, T. Braaten, A. Hjartaker et al., "Use of dietary supplements in the European prospective investigation into cancer and nutrition calibration study," *European Journal of Clinical Nutrition*, vol. 63, Supplement 4, pp. S226–S238, 2009.

[2] A. Spiro and J. L. Buttriss, "Vitamin D: an overview of vitamin D status and intake in Europe," *Nutrition Bulletin*, vol. 39, pp. 322–350, 2014.

[3] EFSA Panel on Dietetic Products, NaA, "Scientific opinion on the tolerable upper intake level of vitamin D," *EFSA Journal*, vol. 10, p. 2813, 2012.

[4] A. Zittermann, J. B. Ernst, S. Prokop et al., "Effect of vitamin D on all-cause mortality in heart failure (EVITA): a 3-year randomized clinical trial with 4000 IU vitamin D daily," *European Heart Journal*, vol. 38, no. 29, pp. 2279–2286, 2017.

[5] J. S. Adams, B. Rafison, S. Witzel et al., "Regulation of the extrarenal CYP27B1-hydroxylase," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 144, Part A, pp. 22–27, 2014.

[6] A. Dusso, A. Brown, and E. Slatopolsky, "Extrarenal production of calcitriol," *Seminars in Nephrology*, vol. 14, pp. 144–155, 1994.

[7] H. S. Cross and E. Kallay, "Nutritional regulation of extrarenal vitamin D hydroxylase expression - potential application in tumor prevention and therapy," *Future Oncology*, vol. 1, pp. 415–424, 2005.

[8] J. C. Fleet, "The role of vitamin D in the endocrinology controlling calcium homeostasis," *Molecular and Cellular Endocrinology*, vol. 17, pp. 30221–30226, 2017.

[9] G. Bjelakovic, L. L. Gluud, D. Nikolova et al., "Vitamin D supplementation for prevention of mortality in adults," *Cochrane Database of Systematic Reviews*, article CD007470, 2014.

[10] A. Avenell, J. C. Mak, and D. O'Connell, "Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men," *Cochrane Database of Systematic Reviews*, article CD000227, 2014.

[11] M. J. Bolland, A. Grey, A. Avenell, G. D. Gamble, and I. R. Reid, "Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis," *British Medical Journal*, vol. 342, article d2040, 2011.

[12] I. Thiele, J. Linseisen, C. Meisinger et al., "Associations between calcium and vitamin D supplement use as well as their serum concentrations and subclinical cardiovascular disease phenotypes," *Atherosclerosis*, vol. 241, pp. 743–751, 2015.

[13] D. Challoumas, A. Stavrou, A. Pericleous, and G. Dimitrakakis, "Effects of combined vitamin D–calcium supplements on the cardiovascular system: should we be cautious?," *Atherosclerosis*, vol. 238, pp. 388–398, 2015.

[14] R. B. Wallace, J. Wactawski-Wende, M. J. O'Sullivan et al., "Urinary tract stone occurrence in the Women's Health Initiative (WHI) randomized clinical trial of calcium and vitamin D supplements," *The American Journal of Clinical Nutrition*, vol. 94, pp. 270–277, 2011.

[15] S. L. Booth and A. RajabiAl, "Determinants of vitamin K status in humans," *Vitamins and Hormones*, vol. 78, pp. 1–22, 2008.

[16] M. J. Shearer and P. Newman, "Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis," *Journal of Lipid Research*, vol. 55, pp. 345–362, 2014.

[17] S. A. Lanham-New, "Importance of calcium, vitamin D and vitamin K for osteoporosis prevention and treatment," *The Proceedings of the Nutrition Society*, vol. 67, pp. 163–176, 2008.

[18] D. Turck, J. L. Bresson, B. Burlingame et al., "EFSA panel on dietetic products, nutrition and allergies. Dietary reference values for vitamin K," *EFSA Journal*, vol. 15, p. 4780, 2017.

[19] S. L. Booth, G. Dallal, M. K. Shea, C. Gundberg, J. W. Peterson, and B. Dawson-Hughes, "Effect of vitamin K supplementation on bone loss in elderly men and women," *The Journal of Clinical Endocrinology and Metabolism*, vol. 93, pp. 1217–1223, 2008.

[20] A. J. Ballegooijenvan and J. W. Beulens, "The role of vitamin K status in cardiovascular health: evidence from observational and clinical studies," *Current Nutrition Reports*, pp. 1–9, 2017.

[21] X. Fu, X. D. Wang, H. Mernitz, R. Wallin, M. K. Shea, and S. L. Booth, "9-cis retinoic acid reduces 1alpha,25-dihydroxycholecalciferol-induced renal calcification by altering vitamin K-dependent gamma-carboxylation of matrix gamma-carboxyglutamic acid protein in A/J male mice," *The Journal of Nutrition*, vol. 138, pp. 2337–2341, 2008.

[22] N. C. Arbour, H. M. Darwish, and H. F. DeLuca, "Transcriptional control of the osteocalcin gene by 1,25-dihydroxyvitamin D-2 and its 24-epimer in rat osteosarcoma cells," *Biochimica et Biophysica Acta*, vol. 1263, pp. 147–153, 1995.

[23] Y. Seyama, M. Horiuch, M. Hayashi, and Y. Kanke, "Effect of vitamin K2 on experimental calcinosis induced by vitamin D2 in rat soft tissue," *International Journal for Vitamin and Nutrition Research*, vol. 66, pp. 36–38, 1996.

[24] E. C. Breen, A. J. Wijnenvan, J. B. Lian, G. S. Stein, and J. L. Stein, "In vivo occupancy of the vitamin D responsive element in the osteocalcin gene supports vitamin D-dependent transcriptional upregulation in intact cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, pp. 12902–12906, 1994.

[25] J. D. Fraser and P. A. Price, "Induction of matrix Gla protein synthesis during prolonged 1,25-dihydroxyvitamin D3 treatment of osteosarcoma cells," *Calcified Tissue International*, vol. 46, pp. 270–279, 1990.

[26] N. Miyake, K. Hoshi, Y. Sano, K. Kikuchi, K. Tadano, and Y. Koshihara, "1,25-Dihydroxyvitamin D3 promotes vitamin K2 metabolism in human osteoblasts," *Osteoporosis International*, vol. 12, pp. 680–687, 2001.

[27] P. I. Karl, D. L. Carnes, and P. A. Friedman, "Effects of 1,25-dihydroxycholecalciferol administration on the rat renal vitamin K-dependent carboxylating system," *FEBS Letters*, vol. 192, pp. 243–246, 1985.

[28] D. D. Bikle, "Vitamin D metabolism, mechanism of action, and clinical applications," *Chemistry & Biology*, vol. 21, pp. 319–329, 2014.

[29] Y. Koshihara and K. Hoshi, "Vitamin K2 enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts in vitro," *Journal of Bone and Mineral Research*, vol. 12, pp. 431–438, 1997.

[30] P. A. Price and S. A. Baukol, "1,25-Dihydroxyvitamin D3 increases synthesis of the vitamin K-dependent bone protein by osteosarcoma cells," *The Journal of Biological Chemistry*, vol. 255, pp. 11660–11663, 1980.

[31] P. A. Price and S. A. Baukol, "1,25-dihydroxyvitamin D3 increases serum levels of the vitamin K-dependent bone protein," *Biochemical and Biophysical Research Communications*, vol. 99, pp. 928–935, 1981.

[32] S. A. Kerner, R. A. Scott, and J. W. Pike, "Sequence elements in the human osteocalcin gene confer basal activation and inducible response to hormonal vitamin D3," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, pp. 4455–4459, 1989.

[33] Y. Koshihara, K. Hoshi, H. Ishibashi, and M. Shiraki, "Vitamin K2 promotes 1alpha,25(OH)2 vitamin D3-induced mineralization in human periosteal osteoblasts," *Calcified Tissue International*, vol. 59, pp. 466–473, 1996.

[34] I. N. Sergeev and A. W. Norman, "Vitamin K-dependent gamma-carboxylation of the 1,25-dihydroxyvitamin D3 receptor," *Biochemical and Biophysical Research Communications*, vol. 189, pp. 1543–1547, 1992.

[35] R. Sanguineti, F. Monacelli, A. Parodi et al., "Vitamins D3 and K2 may partially counterbalance the detrimental effects of pentosidine in ex vivo human osteoblasts," *Journal of Biological Regulators and Homeostatic Agents*, vol. 30, pp. 713–726, 2016.

[36] A. Gigante, M. Torcianti, E. Boldrini et al., "Vitamin K and D association stimulates in vitro osteoblast differentiation of fracture site derived human mesenchymal stem cells," *Journal of Biological Regulators and Homeostatic Agents*, vol. 22, pp. 35–44, 2008.

[37] K. Hara, Y. Akiyama, T. Tomiuga, M. Kobayashi, T. Nakamura, and T. Tajima, "Influence of vitamin D3 on inhibitory effect of vitamin K2 on bone loss in ovariectomized rats," *Nihon Yakurigaku Zasshi*, vol. 104, pp. 101–109, 1994.

[38] S. Matsunaga, H. Ito, and T. Sakou, "The effect of vitamin K and D supplementation on ovariectomy-induced bone loss," *Calcified Tissue International*, vol. 65, pp. 285–289, 1999.

[39] J. Iwamoto, A. Seki, Y. Sato, H. Matsumoto, T. Tadeda, and J. K. Yeh, "Vitamin K2 promotes bone healing in a rat femoral osteotomy model with or without glucocorticoid treatment," *Calcified Tissue International*, vol. 86, pp. 234–241, 2010.

[40] M. Fusaro, S. Giannini, M. Gallieni et al., "Calcimimetic and vitamin D analog use in hemodialyzed patients is associated with increased levels of vitamin K dependent proteins," *Endocrine*, vol. 51, pp. 333–341, 2016.

[41] M. Tamatani, S. Morimoto, M. Nakajima et al., "Decreased circulating levels of vitamin K and 25-hydroxyvitamin D in osteopenic elderly men," *Metabolism*, vol. 47, pp. 195–199, 1998.

[42] A. C. Torbergsen, L. O. Watne, T. B. Wyller et al., "Vitamin K1 and 25(OH)D are independently and synergistically associated with a risk for hip fracture in an elderly population: a case control study," *Clinical Nutrition*, vol. 34, pp. 101–106, 2015.

[43] T. E. Finnes, C. M. Lofthus, H. E. Meyer et al., "A combination of low serum concentrations of vitamins K1 and D is associated with increased risk of hip fractures in elderly Norwegians: a NOREPOS study," *Osteoporosis International*, vol. 27, pp. 1645–1652, 2016.

[44] A. Vignini, L. Nanetti, F. Raffaelli et al., "Effect of supplementation with fortified olive oil on biochemical markers of bone turnover in healthy women," *Mediterranean Journal of Nutrition and Metabolism*, vol. 1, pp. 117–120, 2008.

[45] T. Ushiroyama, A. Ikeda, and M. Ueki, "Effect of continuous combined therapy with vitamin K(2) and vitamin D(3) on bone mineral density and coagulofibrinolysis function in postmenopausal women," *Maturitas*, vol. 41, pp. 211–221, 2002.

[46] J. Iwamoto, T. Takeda, and S. Ichimura, "Effect of combined administration of vitamin D3 and vitamin K2 on bone mineral density of the lumbar spine in postmenopausal women with osteoporosis," *Journal of Orthopaedic Science*, vol. 5, pp. 546–551, 2000.

[47] C. Bolton-Smith, M. E. McMurdo, C. R. Paterson et al., "Two-year randomized controlled trial of vitamin K1 (phylloquinone) and vitamin D3 plus calcium on the bone health of older women," *Journal of Bone and Mineral Research*, vol. 22, pp. 509–519, 2007.

[48] L. A. Braam, M. H. Knapen, P. Geusens et al., "Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age," *Calcified Tissue International*, vol. 73, pp. 21–26, 2003.

[49] S. H. Je, N. S. Joo, B. H. Choi et al., "Vitamin K supplement along with vitamin D and calcium reduced serum concentration of undercarboxylated osteocalcin while increasing bone mineral density in Korean postmenopausal women over sixty-years-old," *Journal of Korean Medical Science*, vol. 26, pp. 1093–1098, 2011.

[50] L. Mazzanti, M. Battino, L. Nanetti et al., "Effect of 1-year dietary supplementation with vitaminized olive oil on markers of bone turnover and oxidative stress in healthy post-menopausal women," *Endocrine*, vol. 50, pp. 326–334, 2015.

[51] Y. Sato, T. Kanoko, K. Satoh, and J. Iwamoto, "Menatetrenone and vitamin D2 with calcium supplements prevent nonvertebral fracture in elderly women with Alzheimer's disease," *Bone*, vol. 36, pp. 61–68, 2005.

[52] E. M. O'Connor, G. Grealy, J. McCarthy et al., "Effect of phylloquinone (vitamin K1) supplementation for 12 months on the indices of vitamin K status and bone health in adult patients with Crohn's disease," *The British Journal of Nutrition*, vol. 112, pp. 1163–1174, 2014.

[53] N. Binkley, J. Harke, D. Krueger et al., "Vitamin K treatment reduces undercarboxylated osteocalcin but does not alter bone turnover, density, or geometry in healthy postmenopausal North American women," *Journal of Bone and Mineral Research*, vol. 24, pp. 983–991, 2009.

[54] A. M. Cheung, L. Tile, Y. Lee et al., "Vitamin K supplementation in postmenopausal women with osteopenia (ECKO trial): a randomized controlled trial," *PLoS Medicine*, vol. 5, article e196, 2008.

[55] K. Yonemura, H. Fukasawa, Y. Fujigaki, and A. Hishida, "Protective effect of vitamins K2 and D3 on prednisolone-induced loss of bone mineral density in the lumbar spine," *American Journal of Kidney Diseases*, vol. 43, pp. 53–60, 2004.

[56] L. J. Schurgers, E. C. Cranenburg, and C. Vermeer, "Matrix Gla-protein: the calcification inhibitor in need of vitamin K," *Thrombosis and Haemostasis*, vol. 100, pp. 593–603, 2008.

[57] P. A. Price, S. A. Faus, and M. K. Williamson, "Warfarin-induced artery calcification is accelerated by growth and vitamin D," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, pp. 317–327, 2000.

[58] O. Mayer Jr., J. Seidlerova, P. Wohlfahrt et al., "Synergistic effect of low K and D vitamin status on arterial stiffness in a general population," *The Journal of Nutritional Biochemistry*, vol. 46, pp. 83–89, 2017.

[59] A. J. Ballegooijenvan, A. Cepelis, M. Visser, I. A. Brouwer, N. M. Schoorvan, and J. W. Beulens, "Joint association of low vitamin D and vitamin K status with blood pressure and hypertension," *Hypertension*, vol. 69, pp. 1165–1172, 2017.

[60] L. A. Braam, A. P. Hoeks, F. Brouns, K. Hamulyák, M. J. Gerichhausen, and C. Vermeer, "Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study," *Thrombosis and Haemostasis*, vol. 91, pp. 373–380, 2004.

[61] M. K. Shea, C. J. O'Donnell, U. Hoffmann et al., "Vitamin K supplementation and progression of coronary artery calcium in older men and women," *The American Journal of Clinical Nutrition*, vol. 89, pp. 1799–1807, 2009.

[62] Z. Asemi, F. Raygan, F. Bahmani et al., "The effects of vitamin D, K and calcium co-supplementation on carotid intima-media thickness and metabolic status in overweight type 2 diabetic patients with CHD," *The British Journal of Nutrition*, vol. 116, pp. 286–293, 2016.

[63] I. Kurnatowska, P. Grzelak, A. Masajtis-Zagajewska et al., "Effect of vitamin K2 on progression of atherosclerosis and vascular calcification in nondialyzed patients with chronic kidney disease stages 3-5," *Polskie Archiwum Medycyny Wewnętrznej*, vol. 125, pp. 631–640, 2015.

[64] I. Kurnatowska, P. Grzelak, A. Masajtis-Zagajewska et al., "Plasma desphospho-uncarboxylated matrix Gla protein as a marker of kidney damage and cardiovascular risk in advanced stage of chronic kidney disease," *Kidney & Blood Pressure Research*, vol. 41, pp. 231–239, 2016.

[65] M. Karamali, M. Ashrafi, M. Razavi et al., "The effects of calcium, vitamins D and K co-supplementation on markers of insulin metabolism and lipid profiles in vitamin D-deficient women with polycystic ovary syndrome," *Experimental and Clinical Endocrinology & Diabetes*, vol. 125, pp. 316–321, 2017.

[66] B. Maestro, S. Molero, S. Bajo, N. Dávila, and C. Calle, "Transcriptional activation of the human insulin receptor gene by 1,25-dihydroxyvitamin D(3)," *Cell Biochemistry and Function*, vol. 20, pp. 227–232, 2002.

[67] I. N. Sergeev and W. B. Rhoten, "1,25-Dihydroxyvitamin D3 evokes oscillations of intracellular calcium in a pancreatic beta-cell line," *Endocrinology*, vol. 136, pp. 2852–2861, 1995.

[68] N. Sakamoto, I. Wakabayashi, and K. Sakamoto, "Low vitamin K intake effects on glucose tolerance in rats," *International Journal for Vitamin and Nutrition Research*, vol. 69, pp. 27–31, 1999.

[69] M. Yoshida, P. F. Jacques, J. B. Meigs et al., "Effect of vitamin K supplementation on insulin resistance in older men and women," *Diabetes Care*, vol. 31, pp. 2092–2096, 2008.

[70] G. Crepaldi and S. Maggi, "Epidemiologic link between osteoporosis and cardiovascular disease," *Journal of Endocrinological Investigation*, vol. 32, pp. 2–5, 2009.

[71] M. Razavi, M. Jamilian, M. Karamali, F. Bahmani, E. Aghadavod, and Z. Asemi, "The effects of vitamin D-K-calcium co-supplementation on endocrine, inflammation, and oxidative stress biomarkers in vitamin D-deficient women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial," *Hormone and Metabolic Research*, vol. 48, pp. 446–451, 2016.

[72] P. Lips, A. Wiersinga, F. C. Ginkelvan et al., "The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects," *The Journal of Clinical Endocrinology and Metabolism*, vol. 67, pp. 644–650, 1988.

[73] M. Peacock, "Calcium metabolism in health and disease," *Clinical Journal of the American Society of Nephrology*, vol. 5, Supplement 1, pp. S23–S30, 2010.

[74] H. A. Bischoff, H. B. Stahelin, W. Dick et al., "Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial," *Journal of Bone and Mineral Research*, vol. 18, pp. 343–351, 2003.

[75] D. Uylden, M. T. Nurmohamed, L. H. Tuylvan, H. G. Raterman, and W. F. Lems, "(Sub)clinical cardiovascular disease is associated with increased bone loss and fracture risk; a systematic review of the association between cardiovascular disease and osteoporosis," *Arthritis Research & Therapy*, vol. 13, article R5, 2011.

[76] S. K. Seo, B. H. Yun, E. B. Noe, J. W. Suh, Y. S. Choi, and B. S. Lee, "Decreased bone mineral density is associated with coronary atherosclerosis in healthy postmenopausal women," *Obstetrics & Gynecology Science*, vol. 58, pp. 144–149, 2015.

[77] R. Zhou, H. Zhou, M. Cui et al., "Association between aortic calcification and the risk of osteoporosis in a Chinese cohort: the Chongqing osteoporosis study," *Calcified Tissue International*, vol. 93, pp. 419–425, 2013.

[78] A. J. Ballegooijenvan, R. T. Gansevoort, H. J. Lambers-Heerspink et al., "Plasma 1,25-dihydroxyvitamin D and the risk of developing hypertension: the prevention of renal and vascular end-stage disease study," *Hypertension*, vol. 66, pp. 563–570, 2015.

[79] T. Coates, G. S. Kirkland, R. B. Dymock et al., "Cutaneous necrosis from calcific uremic arteriolopathy," *American Journal of Kidney Diseases*, vol. 32, pp. 384–391, 1998.

[80] G. Arseculeratne, A. T. Evans, and S. M. Morley, "Calciphylaxis–a topical overview," *Journal of the European Academy of Dermatology and Venereology*, vol. 20, pp. 493–502, 2006.

[81] S. U. Nigwekar, D. B. Bloch, R. M. Nazarian et al., "Vitamin K-dependent carboxylation of matrix Gla protein influences the risk of calciphylaxis," *Journal of the American Society of Nephrology*, vol. 28, no. 6, pp. 1717–1722, 2017.

[82] P. A. Galloway, R. El-Damanawi, V. Bardsley et al., "Vitamin K antagonists predispose to calciphylaxis in patients with end-stage renal disease," *Nephron*, vol. 129, pp. 197–201, 2015.

[83] T. Sato, L. J. Schurgers, and K. Uenishi, "Comparison of menaquinone-4 and menaquinone-7 bioavailability in healthy women," *Nutrition Journal*, vol. 11, p. 93, 2012.

Hindawi
International Journal of Endocrinology
Volume 2017, Article ID 3206240, 11 pages
https://doi.org/10.1155/2017/3206240

Review Article

The Biphasic Effect of Vitamin D on the Musculoskeletal and Cardiovascular System

Armin Zittermann

Clinic for Thoracic and Cardiovascular Surgery, Heart and Diabetes Center North Rhine-Westphalia, Ruhr University Bochum, Bad Oeynhausen, Germany

Correspondence should be addressed to Armin Zittermann; azittermann@hdz-nrw.de

Received 25 April 2017; Accepted 10 July 2017; Published 23 August 2017

Academic Editor: Cristiana Catena

This narrative review summarizes beneficial and harmful vitamin D effects on the musculoskeletal and cardiovascular system. Special attention is paid to the dose-response relationship of vitamin D with clinical outcomes. In infants and adults, the risk of musculoskeletal diseases is highest at circulating 25-hydroxyvitamin D (25OHD) concentrations below 25 nmol/L and is low if 40–60 nmol/L are achieved. However, evidence is also accumulating that in elderly people the risk of falls and fractures increases again at circulating 25OHD levels > 100 nmol/L. Cohort studies report a progressive increase in cardiovascular disease (CVD) events at 25OHD levels < 50 nmol/L. Nevertheless, meta-analyses of randomized controlled trials suggest only small beneficial effects of vitamin D supplements on surrogate parameters of CVD risk and no reduction in CVD events. Evidence is accumulating for adverse vitamin D effects on CVD outcomes at 25OHD levels > 100 nmol/L, but the threshold may be influenced by the level of physical activity. In conclusion, dose-response relationships indicate deleterious effects on the musculoskeletal system and probably on the cardiovascular system at circulating 25OHD levels < 40–60 nmol/L and >100 nmol/L. Future studies should focus on populations with 25OHD levels < 40 nmol/L and should avoid vitamin D doses achieving 25OHD levels > 100 nmol/L.

1. Introduction

During the last two decades, the scientific interest in vitamin D has increased exponentially, as indicated by the fact that 65% of the 71,000 vitamin D articles available in the US National Library of Medicine by February 2017 have been published since 1997 [1]. However, the importance of vitamin D for bone health has already been known for almost 100 years. In the early 1920s, vitamin D was found to cure rickets, a bone disease that occurred endemically in infants and toddlers in many European countries and North America during the industrialization in the 19th and early 20th century [2, 3]. In some cities, up to 80% of children were afflicted by rickets [3]. Rickets prophylaxis was first performed by the administration of UV-irradiated ergosterol using doses of up to 5 mg ergosterol [4]. As early as in the 1920s, it was also recognized that administration of these doses was associated with soft tissue calcification in some children [4], indicating that beneficial vitamin D effects on bone health may lead to adverse effects on the cardiovascular system. Nowadays, rickets prophylaxis is performed with a daily dose of 400 IU vitamin D. This dose can be regarded as effective and safe [5, 6]. Although the importance and safety of vitamin D in infants are well understood, the relevance of vitamin D for the musculoskeletal and the cardiovascular system still remains a topic of scientific interest that has been extensively investigated both in experimental animals and in humans during recent years. However, the focus has moved from infancy to geriatrics, since low vitamin D status, bone diseases, and cardiovascular diseases are all prevalent in this age group [7–9].

The present narrative review gives an overview of the effects of vitamin D on the musculoskeletal and cardiovascular system. Results of experimental studies, cohort studies, Mendelian randomization studies, and randomized controlled trials (RCTs) are used to discuss both beneficial and potentially harmful vitamin D effects. Particular emphasis is paid to those studies that achieve a high level of scientific

Table 1: Daily vitamin D recommendations and daily upper tolerable intake levels by different organizations [6, 10–12].

Life Stage Group	Recommendations			Upper tolerable intake level		
	D-A-CH[1,2]	IOM[3]	ES[4,5]	EFSA[6]	IOM	ES
Infants						
0–6 months	400	400	400–1000	1000	1000	2000
6 to 12 months	400	400	400–1000	1000	1500	2000
Children						
1–3 yr	800	600	600–1000	2000	2500	4000
4–8 yr	800	600	600–1000	2000	3000	4000
Males						
9–13 yr	800	600	600–1000	2000–4000	4000	4000
14–18 yr	800	600	600–1000	4000	4000	4000
19–30 yr	800	600	1500–2000	4000	4000	10,000
31–50 yr	800	600	1500–2000	4000	4000	10,000
51–70 yr	800	600	1500–2000	4000	4000	10,000
70+ yr	800	800	1500–2000	4000	4000	10,000
Females						
9.13 yr	800	600	600–1000	4000	4000	4000
14–18 yr	800	600	600–1000	4000	4000	4000
19–30 yr	800	600	1500–2000	4000	4000	10,000
31–50 yr	800	600	1500–2000	4000	4000	10,000
51–70 yr	800	600	1500–2000	4000	4000	10,000
70+ yr	800	800	1500–2000	4000	4000	10,000
Pregnancy						
14–18 yr	800	600	600–1000	4000	4000	4000
19–30 yr	800	600	1500–2000	4000	4000	10,000
31–50 yr	800	600	1500–2000	4000	4000	10,000
Lactation						
14–18 yr	800	600	600–1000	4000	4000	4000
19–30 yr	800	600	1500–2000	4000	4000	10,000
31–50 yr	800	600	1500–2000	4000	4000	10,000

[1]German, Austrian, Swiss Nutrition Societies. [2]In the absence of skin synthesis of vitamin D. [3]Institute of Medicine. [4]Endocrine Society. [5]For patients at risk for 25-hydroxyvitamin D levels < 50 nmol/L. [6]European Food Safety Authority. Vitamin D data are presented as international units.

evidence such as Mendelian randomization studies and meta-analyses of RCTs. Special attention is also paid to the dose-response relationship of vitamin D with clinical outcomes.

2. Research Strategy

A systematic literature search in PubMed was performed without language restrictions for relevant publications released until the end of February 2017. The following search terms were used: "vitamin D" or "vitamin D supplementation" or "cholecalciferol" or "25-hydroxyvitamin D" or "VDR knockout" or "1α-hydroxylase deletion" or "CYP27B1 deletion" or "CYP2R1 deletion" and "bone" or "rickets" or "osteomalacia" or "osteoporosis" or "fracture" or "falls" or "cardiovascular disease" or "heart failure" or "hypertension" or "cardiovascular mortality" or "myocardial infarction" or "stroke." Personal collections of articles on this topic as well as references from selected articles were also used to extend the search. Some articles were not cited due to space limitations.

3. Vitamin D Metabolism and Actions

Adequate vitamin D supply can be achieved through dietary vitamin D intake, vitamin D supplement use, and/or skin exposure to solar ultraviolet (UV) B radiation. In the absence of skin synthesis of vitamin D, a daily oral dose of 400 IU and 800 IU is regarded to be adequate for infants and the general population beyond infancy, respectively [10]. The upper tolerable intake level is age dependently considered to be 1000 to 4000 IU [6, 11]. For adult patients who are at risk of inadequate vitamin D status, the Endocrine Society recommends a daily vitamin D dose of 1500 to 2000 IU and considers daily doses of up to 10,000 IU as safe (Table 1).

Vitamin D is activated by a hepatic 25-hydroxylation (principle hydroxylase: CYP2R1-hydroxylase; additional hydroxylase: CYP27R1-hydroxylase) and a renal a 1α-hydroxylation (CYP27B1-hxdroxylase) into its active hormonal form 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) (Figure 1). The best indicator for defining human vitamin D status is the circulating 25-hydroxyvitamin D (25OHD) concentration.

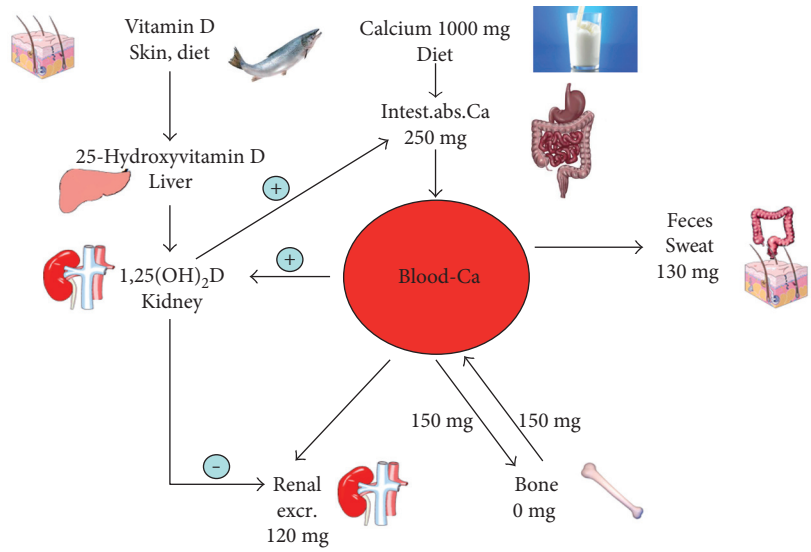


Figure 1: Calcium and vitamin D metabolism at the example of a young adult.

The usefulness of this parameter for assessing vitamin D-dependent biochemical actions can be explained by the fact that various local tissues including enterocytes also possess 1α-hydroxylase activity [12] and that $1,25(OH)_2D$ is reduced in case of deficient 25OHD levels [13]. However, classifications of circulating 25OHD concentrations are inconsistent: the North American Institute of Medicine (IOM) [6] has classified values < 30 nmol/L as deficient, 30–49.99 nmol/L as insufficient, 50–125 nmol/L as adequate, and >125 nmol/L as potentially harmful. The Endocrine Society considers 25OHD levels < 50 nmol/L as deficient and levels between 50 and 74.99 nmol/L as insufficient [14]. Moreover, from their clinical practise guideline [14], it can indirectly be assumed that the Endocrine Society considers 25OHD levels of 75 up to 250 nmol/L as adequate and >250 nmol/L as potentially harmful.

Vitamin D plays a pivotal role in the regulation of calcium and phosphate metabolism and the maintenance of adequate blood levels of these minerals. In case of low serum ionized calcium concentrations (e.g., during low dietary calcium intake), renal $1,25(OH)_2D$ synthesis is activated by parathyroid hormone (PTH), whereas PTH and renal 1α-hydroxylation of 25OHD are suppressed by high plasma calcium levels [15]. Renal 1α-hydroxylase is also suppressed by fibroblast growth factor- (FGF-) 23, a phosphaturic hormone which is secreted by bone cells. FGF-23 is stimulated by high serum phosphate levels and promotes phosphaturia to maintain serum phosphate levels within the normal range [15].

With respect to beneficial and potential harmful vitamin D effects, it is noteworthy that no evidence of a threshold in calcium absorption rate was found with a serum 25OHD level ranging from deficient concentrations up to 150 nmol/L [16–18]. Since both an increase in oral calcium intake and higher serum 25OHD levels are associated with a rise of intestinal absorbed calcium, calcium and vitamin D can replace each other relative to their effects on calcium supply (Figure 1). In line with this assumption, circulating 25OHD levels > 45 nmol/L can ensure low PTH levels even when the calcium intake level is less than 800 mg/day, while a calcium intake above 1200 mg/day is not sufficient to maintain adequate serum PTH, as long as vitamin D status is below 45 nmol/L [19]. Highest PTH levels have been reported in individuals with 25OHD levels < 25 nmol/L and calcium intakes < 800 mg/day [19].

Besides its role in maintaining mineral homeostasis, $1,25(OH)_2D$ has been shown to play an important role in the musculoskeletal and the cardiovascular system. Briefly, in skeletal muscle cells, vitamin D affects cell proliferation and differentiation and the transport of calcium and phosphate across skeletal muscle cell membranes, suppresses the expression of myostatin, a negative regulator of muscle mass, upregulates the expression of follistatin and insulin-like growth factor 2, induces the expression of a number of myogenic transcription factors, regulates muscle cell differentiation by inducing cell cycle arrest, prevents muscular degeneration, and reverses myalgia [20]. In the cardiovascular system, vitamin D downregulates proinflammatory cytokines, metalloproteinases, and natriuretic peptides [21, 22] and upregulates matrix gla protein, anti-inflammatory cytokines, and inhibitors of metalloproteinases [22]. It is however also noteworthy that calcium supplements increase the risk of CVD events, especially myocardial infarction [23].

Hypercalcemia is the hallmark of vitamin D intoxication. Hypercalcemia promotes vascular calcification by the transition of contractile vascular smooth muscle cells into the osteoblast-like phenotype [24]. It has been stated that vitamin D intoxication is observed when circulating 25OHD levels are greater than 374 nmol/L [25]. However, with respect to the risk of hypercalcemia, others have selected 220 nmol/L as a healthy adult NOAEL (no observed adverse effect level) for circulating 25OHD [26]. Despite the aforementioned threshold levels, it is noteworthy that long-term results of vitamin D on plasma calcium are very limited and, according to the IOM, there continues to be large uncertainty about the progressive health effects for regular ingestion of even moderately high amounts of vitamin D in the long run [26].

4. Vitamin D Deficiency, Bone Disorders, and Cardiovascular Diseases

The consequences of vitamin D deficiency on the human musculoskeletal system have long been known and also well characterized in experimental animals. Therefore, experimental data on vitamin D deficiency and the skeleton during recent years were most of all confirmative, whereas experimental data on the cardiovascular system have provided important new insights on potential interaction between vitamin D and the cardiovascular system. Findings in experimental animals, infants, and adults are summarized below.

4.1. Experimental Data. Mice lacking the vitamin D receptor (VDR) develop hypocalcemia, severe hyperparathyroidism, elevated plasma levels of alkaline phosphatase, and the typical features of rickets. Normalization of impaired mineral homeostasis in VDR knockout mice fed a diet supplemented with high concentrations of calcium (2%) and phosphorus (1.25%) is reported to reverse the malformation of the bone and the growth retardation as well [27], indicating that the most important action of the VDR in skeletal growth, maturation, and remodeling is its role in intestinal calcium absorption [28]. As expected, targeted ablation of the CYP27B1 gene (1α-hydroxylase gene) in mice results in hypocalcemia, secondary hyperparathyroidism, retarded growth, and the skeletal abnormalities characteristic of rickets as well [29]. In CYP2R1 knockout mice, circulating 25OHD is reduced by more than 50% and it has been suggested that in some patients with rickets CYP2R1 mutations may be responsible for the disease [30].

In the cardiovascular system, VDR deletion results in elevated production of renin and angiotensin II, leading to hypertension and cardiac hypertrophy [31, 32]. Treatment of VDR knockout mice with the ACE inhibitor captopril reduces cardiac hypertrophy and normalizes atrial natriuretic peptide expression [33]. Cardiomyocyte-specific deletion of the VDR also results in cardiac hypertrophy, and treatment of neonatal cardiomyocytes with $1,25(OH)_2D$ is partially able to suppress hypertrophy [31]. Moreover, vitamin D deficiency stimulates renin expression in normal mice, whereas injection of $1,25(OH)_2D$ reduces renin synthesis [31]. This protective role of $1,25(OH)_2D$ on the cardiovascular system seems to be independent of plasma calcium and phosphate levels [34]. Deletion of the VDR as well as diets low in vitamin D content also stimulates osteoblast-like cell formation of vascular smooth muscle cells and aortic calcification [35].

4.2. Infancy. Rickets is the principal vitamin D-deficiency disease in infants. In the majority of studies in which circulating 25OHD has been measured in toddlers with rickets living in Europe, concentrations were <12.5 nmol/L [36]. However, higher 25OHD levels have also been reported [36] and dietary calcium deprivation rather than vitamin D deficiency may have been the cause of rickets in these cases. Some RCTs in children with 25OHD levels > 25 nmol/L but <50 nmol/L have demonstrated that the best therapeutic response is seen with a combination of calcium with vitamin D and if 25OHD levels achieve values > 40 to 50 nmol/L [37–39]. Collectively, data in infants support experimental and biochemical findings of jointed vitamin D and calcium effects on bone health. The risk of rickets progressively increases at circulating 25OHD < 40 nmol/L.

Some infants with rickets also develop cardiac problems: In a series of 61 cases of infants with rickets and heart failure [40], almost all patients had low circulating levels of 25OHD (mean values: 18.5 nmol/L), low plasma calcium concentrations, and low plasma phosphate concentrations, whereas PTH levels were markedly elevated. The vast majority of infants responded to treatment with calcium, vitamin D, and cardiotonics, indicating that vitamin D (and calcium) may have played an important role in the pathogenesis of the cardiac problems. The results are supported by an RCT in 80 infants with heart failure [41] and mean 25OHD levels of 35 nmol/L, in which treatment with 1000 IU vitamin D daily suppressed PTH levels, the proinflammatory cytokines interleukin-6 and tumor necrosis factor-α, and increased the anti-inflammatory cytokine interleukin-10 as well as left ventricular ejection fraction significantly.

4.3. Adulthood. It is well known that in adults prolonged and severe vitamin D deficiency (<12.5 nmol/L) can cause osteomalacia, a musculoskeletal disorder that is associated with diffuse joint and bone pain, muscle weakness, difficulty in walking, bone demineralization, and increased fracture risk. There is also evidence that 25OHD levels already below 25 nmol/L lead to osteomalacia in the long run [42]. Similar to rickets, osteomalacia is associated with hypocalcemia, hypophosphatemia, and severe hyperparathyroidism. Osteomalacia has been reported to be common in elderly women in the UK [43]. In Turkish immigrants in Germany, a high prevalence of vitamin D deficiency (78% < 50 nmol/L), secondary hyperparathyroidism (40% of those with low 25OHD levels), and generalized bone pain has also been reported, especially in veiled women [44]. Earlier data indicate that subclinical osteomalacia can already be corrected by relatively low doses of alfacalcidol (0.5 micrograms daily) or plain vitamin D (1000 IU daily) given for three months [45]. Moreover, 400 IU of vitamin D with 600 mg calcium daily was already adequate to increase bone mineral density significantly in low-income Bangladeshi women with low outdoor activities [46]. A histomorphometric analysis of iliac crest bone biopsies and circulating 25OHD in 675 patients demonstrated that pathologic bone mineralization was most prevalent in patients with 25OHD levels < 25 nmol/L [47]. The threshold for the absence of mineralization defects was 75 nmol/L. The investigators therefore concluded that together with a sufficient calcium intake, circulating 25OHD levels > 75 nmol/L should be ensured to maintain skeletal health. It is however noteworthy that the aforementioned investigation was an observational study and therefore cannot prove causality. Caution is necessary in recommending 75 nmol/L because a daily vitamin D supplement of 3800 to 5000 IU would be necessary to guarantee circulating 25OHD level of 75 nmol/L in almost all adults [48]. These doses would reach or exceed the UL and would be clearly above the IOM recommendation for older adults (Table 1).

Since the muscle is a target tissue for vitamin D, vitamin D deficiency is also discussed to contribute to an increased risk of falls (and fractures) in the elderly. Numerous meta-analyses of RCTs have summarized the results of vitamin D on the risk of falls/falling. Findings support the assumption that in the elderly the risk of falls/falling is influenced by baseline 25OHD levels, achieved 25OHD level, and calcium coadministration. Briefly, in a meta-analysis of 26 RCTs that enrolled 45,782 participants [49], vitamin D use was also associated with statistically significant reduction in the risk of falls (odds ratio 0.86 [(95% CI: 0.77–0.96]). This effect was more prominent in patients who were vitamin D deficient at baseline and in studies in which calcium was coadministered with vitamin D. In community dwellers [50], vitamin D did not reduce the rate of falls or risk of falling. However, it was concluded that it may do so in people with lower 25OHD levels before treatment. In patients of nursing care facilities, a group that is known to have a high prevalence of vitamin D deficiency, vitamin D supplementation reduced the rate of falls to 0.72 (95% CI, 0.55 to 0.95) [51]. In another meta-analysis of 8 RCTs [52], based on 2426 individuals, supplemental vitamin D in a dose of 700–1000 IU a day reduced the risk of falling among older individuals by 19% and to a similar degree as active forms of vitamin D. It was concluded from this meta-analysis that doses of supplemental vitamin D of less than 700 IU may not reduce the risk of falling among older individuals and that circulating 25OHD levels of 60 nmol/L should be achieved.

Data of RCTs on vitamin D and fracture risk support results on vitamin D and falls: the combined vitamin D and calcium administration was able to reduce fracture risk significantly only in institutionalized elderly individuals but not in community dwellers [53], probably because of lower baseline 25OHD levels in the former group of individuals. In pooled participant level data of RCTs, a 30% reduction in the risk of hip fracture and a 14% reduction in the risk of any nonvertebral fracture were shown if on the basis of actual intakes daily vitamin D intakes were at least 800 IU [54]. In line with the findings of the aforementioned meta-analysis on falls [52], a dose-response relationship was suggested with the highest and lowest fracture risk at 25OHD levels < 30 nmol/L and >61 nmol/L, respectively. However, it is noteworthy that the dose-response relationships on falls and fractures investigated by Bischoff-Ferrari et al. [52, 54] were only exploratory analyses of RCTs and can thus be subject to unexplained bias.

In total, results in infants and adults indicate a dose-response relationship between circulating 25OHD and the musculoskeletal system with the highest risk below 25 nmol/L and a low risk if a level of approximately 40 to 60 nmol/L is achieved.

Regarding vitamin D and CVD, it is noteworthy that data from RCTs on "hard" clinical endpoints are scarce. Therefore, epidemiological data have to be taken into account as well. In a meta-analysis of prospective cohort studies based on more than 20,000 individuals [55], adjusted risk of cardiovascular mortality was 57% higher in the lowest 25OHD category than in the highest 25OHD category. The Whitehall study [56], a large prospective cohort study of older men living in the UK, indicates that higher concentrations of 25OHD are inversely and approximately linearly (log-log scale) associated with age- and season-adjusted vascular mortality throughout the range of 40–90 nmol/L. After additional adjustment for prior disease and cardiovascular risk factors, a doubling in 25OHD concentration was associated with 20% [95% CI: 9–30%] lower vascular mortality. In a milestone publication of a European consortium of eight prospective studies [57], including seven general population cohorts, individual patient data and standardized 25OHD data were used to assess the association of 25OHD with all-cause and cause-specific mortality. Compared to participants with adequate 25OHD concentrations (75 to 99.99 nmol/L), the adjusted hazard ratios (with 95% Cl) for CVD mortality in the 25OHD groups with 40 to 49.99, 30 to 39.99, and <30 nmol/L were 1.65 (1.1.39–1.97), 1.61 (1.46–1.77), and 2.21 (1.50–3.26), respectively. In line with these findings, a 2017 meta-analysis of 34 cohort studies with more than 180,000 participants [58] reported a progressive increase of total CVD events at circulating 25OHD levels < 50 nmol/L but no association of 25OHD with CVD events at levels between 50 and 137 nmol/L. With respect to CVD mortality, the risk increased constantly at circulating 25OHD levels < 100 nmol/L [58].

Despite these promising epidemiological data regarding an effect of vitamin D status on CVD outcome, cohort studies are subject to residual confounding. Therefore, a Danish approach using an observational study design together with a Mendelian randomization analysis [59] is vitally important. Mendelian randomization takes advantage of lifelong differences in vitamin D status attributable to genetic variants and is hence not confounded by lifestyle factors. In the Danish investigation, the odds ratio for an observational multivariable-adjusted 20 nmol/L lower 25OHD concentration was 1.13 (95% CI: 1.03 to 1.24) for cardiovascular mortality but was 0.77 (95% CI: 0.55 to 1.08) for a genetically determined 20 nmol/L lower 25OHD level. Similarly, the observational multivariable-adjusted hazard ratios for a 25 nmol/L decrease in 25OHD were significantly higher for ischemic heart disease and myocardial infarction, whereas the hazard ratios for a genetically 25 nmol/L decrease were not [60]. Results are an indication that no premature conclusions should be drawn solely based on observational data.

Several RCTs have investigated surrogate parameters of cardiovascular risk such as blood pressure and arterial stiffness. Regarding blood pressure, a meta-analysis incorporating individual patient data of 46 RCTs came to the conclusion that vitamin D supplementation is ineffective as an agent for lowering blood pressure [61]. In RCTs with initial 25OHD levels > 40 nmol/L, even a clear increase in 25OHD levels did not influence systolic or diastolic blood pressure [62, 63]. However, in a Mendelian randomization approach including up to 108,173 individuals from 35 studies [64], each 10% increase in genetically determined 25OHD concentration was associated with a significant change of −0·29 mm Hg in diastolic blood pressure, a significant change of −0·37 mm Hg in systolic blood pressure, and an 81% decreased odds of hypertension, indicating that in the long run vitamin D might have a small but significant

beneficial effect on blood pressure. A meta-analysis of RCTs on arterial stiffness [65] reported nonsignificant reductions in pulse wave velocity (standardized mean difference = −0·10; 95% CI: −0·24, 0·04) and augmentation index (−0·15; 95% CI: −0·32, 0·02), the latter being a measure of the enhancement of central aortic pressure, by vitamin D supplementation in the range of 1000 to 5700 IU/day. Out of the included 18 studies, 11 had mean 25OHD levels < 50 nmol/L, 4 between 50 and 75 nmol/L, and 2 > 75 nmol/L at recruitment, whereas one study provided no 25OHD data.

Regarding CVD events, a meta-analysis of RCTs could not demonstrate a beneficial vitamin D effect on myocardial infarction or stroke [66] and these results were also confirmed by another more recent meta-analysis [67]. However, this recent meta-analysis [67] reported a 17% reduction in heart failure events by vitamin D supplementation. Nevertheless, it is noteworthy that results were largely influenced by a secondary analysis of only one large trial. In a very recent large RCT in elderly patients with initial 25OHD levels of 63.7 nmol/L [68], monthly high-dose vitamin D supplementation did not prevent CVD events. Moreover, a systematic Cochrane review on vitamin D supplementation for prevention of mortality in adults [69] showed no beneficial effect on CVD mortality.

Collectively, surrogate parameters of cardiovascular risk do not exclude the possibility of small beneficial vitamin D effects on CVD risk. However, the dose-response relationship is yet poorly understood and there is currently no convincing evidence that potential beneficial vitamin D effects on the cardiovascular system lead to a reduction of CVD events. More RCTs in individuals with deficient 25OHD levels (i.e., <30 nmol/L) are needed.

5. Harmful Vitamin D Effects on the Musculoskeletal and Cardiovascular System

5.1. Vitamin D and the Musculoskeletal System. Although calcium release from the bone is considered to be the most important cause of hypercalcemia seen in vitamin D intoxication [70], adverse effects of toxic vitamin D doses on the musculoskeletal system are almost completely lacking in experimental animals or infants. In adults, however, some recent investigations have reported adverse effects on the musculoskeletal system at higher circulating 25OHD levels or at higher vitamin D doses: a population-based prospective study in older men [71] reported a U-shaped association of circulating 25OHD levels with fracture risk, with the highest risk for patients not only in the lowest 25OHD quantile (≤36 nmol/L) but also in the highest quantile (>72 to ≤148 nmol/L) (reference group: >59 to ≤72 nmol/L). Results are confirmed by a large placebo-controlled trial in 2256 community-dwelling women, aged 70 years or older [72]. Compared with women in the placebo group, bolus administration of vitamin D (500,000 IU vitamin D3 once a year, equivalent to 1370 IU/daily, for 3 years) resulted in a higher rate of falls (83.4 versus 72.7 per 100 person-years, $P = 0.03$) and a higher rate of fractures (4.9 versus 3.9 per 100 person-years, $P = 0.047$). The increased likelihood of falls and fractures in the vitamin D group was exacerbated in the 3-month period immediately following the annual dose. Levels of 25OHD increased in the vitamin D group at 1 month after dosing to approximately 120 nmol/L and to approximately 90 nmol/L at 3 months. Another study also reported an increase in fracture associated with vitamin D treatment [73]. Participants (4354 men, 5086 women) 75 years or older received an annual injection of 300,000 IU vitamin D2 (equivalent to 820 IU/daily) or placebo. In men, treatment had no effect on fractures. However, women treated with vitamin D had a 21% higher risk of nonvertebral fractures, an 80% higher risk of hip/femur fractures, and a 59% higher risk of hip/femur/wrist/forearm fractures. Two recent RCTs could confirm the higher risk of falls by bolus administration of vitamin D. In a cohort of 200 community-dwelling men and women, 70 years and older [74], the incidence of falls was higher in the group receiving 60,000 IU vitamin D monthly (equivalent to 2000 IU vitamin D daily) and in the group receiving 24,000 IU vitamin D plus 300 μg calcifediol monthly (equivalent to 800 IU vitamin D plus 10 μg calcifediol daily) than in the group receiving 24,000 IU vitamin D monthly (equivalent to 800 IU vitamin D daily) (incidence 66.9%, 66.1%, and 47.9%, resp.; $P = 0.048$). In addition, the total mean number of falls tended to be higher in the two former groups than in the latter group. Seniors reaching the highest quartile of 25OHD level at the 12-month follow-up (112–247 nmol/L) had a 5.5-fold higher odds of falling compared with those reaching the lowest quartile of 25OHD (53.2 to 75.6 nmol/L). In another study in 107 long-term care residents aged 60 and older [75], falls were more common in a high-dose vitamin D group receiving a monthly supplement of 100,000 IU vitamin D3 (equivalent to 3333 IU daily) versus a standard-dose vitamin D group receiving 400 to 1000 IU daily (1.47 versus 0.63 per person-years; $P < .001$). Fractures were uncommon and similar in both groups. Mean circulating 25OHD levels during the trial were 80 nmol/L in the high-dose group and 63 nmol/L in the standard-dose group. In total, vitamin D effects on the musculoskeletal system seem to follow a U-shaped association, with deleterious effects at low circulating 25OHD concentrations (i.e., <50 nmol/L) and also at high 25OHD concentrations. Especially, individuals achieving 25OHD levels > 100 nmol/L seem to be at an increased risk.

5.2. Vitamin D and the Cardiovascular System. Numerous historical and recent studies have demonstrated that supraphysiological doses of vitamin D result in vascular calcification in experimental animals and these results have already been summarized elsewhere [4, 76, 77]. Moreover, harmful cardiovascular effects of toxic vitamin D doses (resulting in 25OHD > 374 nmol/L) are well established in infants and adults [4, 6, 77, 78], but the question arises whether levels already between 100 nmol/L and 374 nmol/L are also associated with an increased CVD risk [79].

In the aforementioned milestone cohort study of a European consortium [57], 25OHD levels > 100 nmol/L were not associated with an increased risk of CVD mortality. However, the majority of samples exceeding the threshold of 100 nmol/L originated from a German cohort of

Table 2: Suggested dose-response relationship of circulating 25-hydroxyvitamin D with musculoskeletal and cardiovascular disease.

25-Hydroxyvitamin D concentration	Musculoskeletal system	Cardiovascular system
<12.5 nmol/L	Rickets ↑↑, osteomalacia ↑↑ Elderly people: falls ↑↑, fractures ↑↑	CVD events ↑ (?)
12.5–24.99 nmol/L	Rickets ↑, osteomalacia ↑ Elderly people: falls ↑↑, fractures ↑↑	CVD events ↑ (?)
25.0–49.99 nmol/L	Elderly people: falls ↑, fractures ↑	CVD surrogate parameters probably adversely affected
50.0–100.0 nmol/L	Adequate muscle and bone function	Adequate cardiovascular function
>100 nmol/L	Elderly people: falls ↑, fractures ↑	CVD events ↑ (?)

CVD: cardiovascular disease events; (?): probably; ↑: elevated; ↑↑: markedly elevated.

apparently healthy, middle-aged individuals and may thus not be representative for individuals in the clinical setting. In two huge Israeli and Danish data analyses in patients from the general practise sector [80, 81], an inverse J-shaped association of circulating 25OHD with CVD morbidity and mortality was reported. Morbidity and mortality were lowest at 25OHD levels between 50 and 90 nmol/L and increased again above this range. In another prospective cohort study in cardiac surgical patients [82], a U-shaped association between circulating 25OHD and the risk of major adverse cardiac and cerebrovascular events has been reported. Risk was highest at both circulating 25OHD levels < 30 nmol/L and >100 nmol/L. A recent RCT in advanced heart failure provided further evidence for adverse vitamin D effects in CVD patients [83]. A daily vitamin D supplement of 4000 IU for 3 years resulted in a greater need for mechanical circulatory support implants, especially in patients with initial circulating 25OHD concentrations ≥ 30 nmol/L. They also achieved median in-study 25OHD levels > 100 nmol/L. The underlying mechanism for this effect remains unclear at present but may be related to elevated plasma calcium levels. In this study, the incidence of hypercalcemia (plasma calcium > 2.75 mmol/L) was in the vitamin D and placebo group (6.2% and 3.1%, resp., $P = .192$). Generally, it seems that oral vitamin D doses resulting in mean circulating 25OHD levels of 75 to 160 nmol/L do not lead to hypercalcemia [79]. However, in the aforementioned RCT, vitamin D administration resulted in a significant increase in plasma calcium, although mean calcium levels remained within the reference range [82]. A similar effect has already been reported in an earlier RCT in heart failure [84]. Importantly, the ARIC (Atherosclerosis Risk in Communities) study reported that high plasma calcium was independently associated with greater risk of incident heart failure [85]. Heart failure incidence was lowest at calcium levels of 2.25 mmol/L and increased progressively up to 2.75 mmol/L [85]. Moreover, a meta-analysis of observational data indicates a statistically positive association between plasma calcium and CVD [86].

6. Interactions of Vitamin D with the Musculoskeletal and Cardiovascular System

An increase in plasma calcium does not only result from excessive vitamin D doses but can also be due to other reasons. Briefly, hypokinesia and immobilization are associated with a significant increase in plasma calcium and phosphate and a decrease in circulating $1,25(OH)_2D$ levels [87, 88]. Similarly, postmenopausal bone loss is associated with a significant rise in plasma calcium [89]. According to the Utah paradigm of bone biology [90], the increase in plasma calcium and decrease in circulating $1,25(OH)_2D$ in postmenopausal women and individuals with sedentary lifestyle can be explained by a loss of bone mass due to estrogen deficiency or muscle loss, subsequently leading to an influx of calcium into soft tissues such as vessels and kidneys.

Vascular calcification has been identified as a risk factor for CVD mortality [91] and a predictor of poorer 5-year survival [92]. The inverse relationship between the amount of vascular and skeletal calcium can explain why vascular calcification is often associated with osteoporosis [93, 94]. While vitamin D supplementation appears logical in case of inadequate vitamin D supply to increase the amount of intestinally absorbed calcium and thus to prevent musculoskeletal diseases, such a measure appears questionable when plasma calcium levels are already elevated due to immobilization-induced or estrogen deficiency-induced calcium release from the bone. Therefore, scepticism is necessary regarding an American Geriatrics Society consensus statement [95]. They recommend up to 4000 IU daily of vitamin D supplementation for prevention of falls in older adults. The effect of moderately high daily vitamin D doses on the cardiovascular system is far from clear. Since this amount may further increase plasma calcium levels (see before), caution is needed in administering vitamin D doses of 4000 IU in the clinical setting.

It is however intriguing that physical activity and remobilization have hypocalcemic effects [96] and are associated with an increase in circulating $1,25(OH)_2D$ [87, 88, 97]. Although physically active individuals have higher 25OHD levels than individuals with sedentary lifestyle, indicating an increase in intestinal calcium absorption, the surplus of absorbed calcium is usually excreted via sweat or deposed in the skeleton [98]. This effect of physical activity on calcium metabolism is thus in line with findings that traditionally living individuals with abundant UVB exposure have a lifelong low CVD risk [99], although circulating 25OHD levels in these groups clearly exceed 100 nmol/L [100]. However, the high circulating 25OHD levels in these groups cannot a priori be considered as safe for an aging westernized society with

sedentary lifestyle. Likely, results on circulating 25OHD and CVD outcomes obtained in Mendelian randomization studies in patients with a high CVD risk should not be extrapolated to young healthy individuals. Table 2 presents a potential dose-response relationship of circulating 25OHD with musculoskeletal and cardiovascular outcomes.

7. Conclusions

There is accumulating evidence that circulating 25OHD levels < 40–60 nmol/L are nonlinearly related to an increased risk of musculoskeletal diseases and probably also to an increased CVD risk. The classification of the North American IOM [6] and of several European Nutrition Societies [10] of circulating 25OHD levels > 50 nmol/L as adequate is in line with these findings. Recent results demonstrate that a daily vitamin D supplement of 800 IU is able to achieve circulating 25OHD levels in almost all young female adults in winter [101]. Elderly people usually require on average a daily dose of ≤400 IU to achieve 25OHD levels > 50 nmol/L [102]. These data concur with official recommendations of an oral intake of 800 IU vitamin D daily beyond infancy in the absence of skin synthesis of vitamin D [10].

The threshold of harmful vitamin D effects is probably influenced by the level of physical activity. In the clinical setting, caution is needed in administering vitamin D doses resulting in circulating 25OHD levels > 100 nmol/L. Some statements, such as a daily vitamin D intake of up to 4000 IU for the prevention of falls [95] or that a daily intake of up to 10,000 IU vitamin D is safe [14], should therefore be reconsidered. In the future, RCTs with multiple outcomes and multivariate meta-analyses of RCTs are needed to assess the health effects of vitamin D supplements on the musculoskeletal and cardiovascular system.

Conflicts of Interest

The author declares that he has no conflicts of interest.

References

[1] https://www.ncbi.nlm.nih.gov/pubmed/?term=vitamin+D, assessed February 27th, 2017.

[2] K. Rajakumar, "Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective," *Pediatrics*, vol. 112, no. 2, pp. e132–e135, 2003.

[3] M. F. Holick, "The D-lightful vitamin D for child health," *Journal of Parenteral and Enteral Nutrition*, vol. 36, Supplement 1, pp. 9S–19S, 2012.

[4] G. Jahreis and V. Hesse, "Vitamin D-induced tissue calcinosis and arteriosclerosis changes. I: a contribution to the 60 year history of vitamin D research with special reference to childhood," *Pädiatrie und Grenzgebiete*, vol. 29, no. 3, pp. 203–211, 1990.

[5] C. F. Munns, N. Shaw, M. Kiely et al., "Global consensus recommendations on prevention and management of nutritional rickets," *The Journal of Clinical Endocrinology and Metabolism*, vol. 101, no. 2, pp. 394–415, 2016.

[6] A. C. Ross, C. L. Taylor, A. L. Yaktine, and H. B. Del Valle, *Institute of Medicine of the National Academies, Food and Nutrition Board. Dietary Reference Intakes Calcium Vitamin D. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium*, The National Academies Press, Washington, D.C, 2010.

[7] S. Pilz, H. Dobnig, A. Tomaschitz et al., "Low 25-hydroxyvitamin D is associated with increased mortality in female nursing home residents," *The Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 4, pp. E653–E657, 2012.

[8] C. P. Benziger, G. A. Roth, and A. E. Moran, "The global burden of disease study and the preventable burden of NCD," *Global Heart*, vol. 11, pp. 393–397, 2016.

[9] A. M. Briggs, M. J. Cross, D. G. Hoy et al., "Musculoskeletal health conditions represent a global threat to healthy aging: a report for the 2015 World Health Organization world report on ageing and health," *The Gerontologist*, vol. 56, Supplement 2, pp. S243–S255, 2016.

[10] Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, and Schweizerische Vereinigung für Ernährung, *Referenzwerte für die nährstoffzufuhr*, Neuer Umschau Buchverlag, 1. Aufl Neustadt an der Weinstraße, 2013, (5. Korrigierter Nachdruck).

[11] European Food Safety Authority, "Scientific opinion on the tolerable upper intake level of vitamin D," *EFSA Journal*, vol. 10, p. 2813, 2012.

[12] A. Zittermann and S. Pilz, "Vitamin D in clinic and practice," *Aktuel Ernaehrungsmed*, vol. 41, pp. 300–316, 2016.

[13] S. Docio, J. A. Riancho, A. Pérez, J. M. Olmos, J. A. Amado, and J. González-Macías, "Seasonal deficiency of vitamin D in children: a potential target for osteoporosis-preventing strategies?," *Journal of Bone and Mineral Research*, vol. 13, no. 4, pp. 544–548, 1998.

[14] M. F. Holick, N. C. Binkley, H. A. Bischoff-Ferrari et al., "Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline," *The Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 7, pp. 1911–1930, 2011.

[15] J. J. Scialla and M. Wolf, "Roles of phosphate and fibroblast growth factor 23 in cardiovascular disease," *Nature Reviews. Nephrology*, vol. 10, no. 5, pp. 268–278, 2014.

[16] A. Zittermann, K. Scheld, and P. Stehle, "Seasonal variations in vitamin D status and calcium absorption do not influence bone turnover in young women," *European Journal of Clinical Nutrition*, vol. 52, no. 7, pp. 501–506, 1998.

[17] A. Devine, S. G. Wilson, I. M. Dick, and R. L. Prince, "Effects of vitamin D metabolites on intestinal calcium absorption and bone turnover in elderly women," *The American Journal of Clinical Nutrition*, vol. 75, no. 2, pp. 283–288, 2002.

[18] J. F. Aloia, R. Dhaliwal, A. Shieh et al., "Vitamin D supplementation increases calcium absorption without a threshold effect," *The American Journal of Clinical Nutrition*, vol. 99, no. 3, pp. 624–631, 2014.

[19] L. Steingrimsdottir, O. Gunnarsson, O. S. Indriason, L. Franzon, and G. Sigurdsson, "Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake," *JAMA*, vol. 294, no. 18, pp. 2336–2341, 2005.

[20] N. E. Koundourakis, P. D. Avgoustinaki, N. Malliaraki, and A. N. Margioris, "Muscular effects of vitamin D in young

athletes and non-athletes and in the elderly," *Hormones*, vol. 15, no. 4, pp. 471–488, 2016.

[21] A. Zittermann, S. S. Schleithoff, and R. Koerfer, "Vitamin D and vascular calcification," *Current Opinion in Lipidology*, vol. 18, no. 1, pp. 41–46, 2007.

[22] A. Zittermann and J. F. Gummert, "Sun, vitamin D, and cardiovascular disease," *Journal of Photochemistry and Photobiology. B*, vol. 101, no. 2, pp. 124–129, 2010.

[23] M. J. Bolland, A. Avenell, J. A. Baron et al., "Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis," *BMJ*, vol. 341, article c3691, 2010.

[24] S. Huybers and R. J. Bindels, "Vascular calcification in chronic kidney disease: new developments in drug therapy," *Kidney International*, vol. 72, no. 6, pp. 663–665, 2007.

[25] M. F. Holick, "Vitamin D deficiency," *The New England Journal of Medicine*, vol. 357, no. 3, pp. 266–281, 2007.

[26] J. N. Hathcock, A. Shao, R. Vieth, and R. Heaney, "Risk assessment for vitamin D," *The American Journal of Clinical Nutrition*, vol. 85, no. 1, pp. 6–18, 2007.

[27] R. Masuyama, Y. Nakaya, S. Tanaka et al., "Dietary phosphorus restriction reverses the impaired bone mineralization in vitamin D receptor knockout mice," *Endocrinology*, vol. 142, no. 1, pp. 494–497, 2001.

[28] M. Amling, M. Priemel, T. Holzmann et al., "Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses," *Endocrinology*, vol. 140, no. 11, pp. 4982–4987, 1999.

[29] D. K. Panda, D. Miao, M. L. Tremblay et al., "Targeted ablation of the 25-hydroxyvitamin D 1alpha -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 13, pp. 7498–7503, 2001.

[30] T. D. Thacher and M. A. Levine, "CYP2R1 mutations causing vitamin D-deficiency rickets," *The Journal of Steroid Biochemistry and Molecular Biology*, 2016.

[31] Y. C. Li, J. Kong, M. Wei, Z. F. Chen, S. Q. Liu, and L. P. Cao, "1,25-Dihydroxyvitamin D_3 is a negative endocrine regulator of the renin-angiotensin system," *The Journal of Clinical Investigation*, vol. 110, no. 2, pp. 229–238, 2002.

[32] W. Xiang, J. Kong, S. Chen et al., "Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-ngiotensin systems," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 288, no. 1, pp. E125–E132, 2005.

[33] S. Chen, C. S. Law, C. L. Grigsby et al., "Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy," *Circulation*, vol. 124, no. 17, pp. 1838–1847, 2011.

[34] C. Zhou, F. Lu, K. Cao, D. Xu, D. Goltzman, and D. Miao, "Calcium-independent and $1,25(OH)_2D_3$-dependent regulation of the renin-angiotensin system in 1α-hydroxylase knockout mice," *Kidney International*, vol. 74, no. 2, pp. 170–179, 2008.

[35] N. Schmidt, C. Brandsch, H. Kühne, A. Thiele, F. Hirche, and G. I. Stangl, "Vitamin D receptor deficiency and low vitamin D diet stimulate aortic calcification and osteogenic key factor expression in mice," *PLoS One*, vol. 7, no. 4, article e35316, 2012.

[36] J. M. Pettifor, "Vitamin D deficiency and nutritional rickets in children," in *Vitamin D*, D. Feldman, J. W. Pike and F. H. Glorieux, Eds., pp. 1065–1083, Elsevier Academic Press, San Diego CA, USA, 2005.

[37] K. Balasubramanian, J. Rajeswari, Gulab et al., "Varying role of vitamin D deficiency in the etiology of rickets in young children vs. adolescents in northern India," *Journal of Tropical Pediatrics*, vol. 49, no. 4, pp. 201–206, 2003.

[38] V. Aggarwal, A. Seth, R. K. Marwaha et al., "Management of nutritional rickets in Indian children: a randomized controlled trial," *Journal of Tropical Pediatrics*, vol. 59, no. 2, pp. 127–133, 2013.

[39] T. D. Thacher, P. R. Fischer, and J. M. Pettifor, "Vitamin D treatment in calcium-deficiency rickets: a randomised controlled trial," *Archives of Disease in Childhood*, vol. 99, no. 9, pp. 807–811, 2014.

[40] A. T. Elidrissy, M. Munawarah, and K. M. Alharbi, "Hypocalcemic rachitic cardiomyopathy in infants," *Journal of Saudi Heart Association*, vol. 25, no. 1, pp. 25–33, 2013.

[41] S. A. Shedeed, "Vitamin D supplementation in infants with chronic congestive heart failure," *Pediatric Cardiology*, vol. 33, no. 5, pp. 713–719, 2012.

[42] B. Basha, D. S. Rao, Z. H. Han, and A. M. Parfitt, "Osteomalacia due to vitamin D depletion: a neglected consequence of intestinal malabsorption," *The American Journal of Medicine*, vol. 108, no. 4, pp. 296–300, 2000.

[43] J. Anderson, A. E. R. Campbell, A. Dunn, and J. B. M. Runciman, "Osteomalacia in elderly women," *Scottish Medical Journal*, vol. 82, pp. 429–435, 1966.

[44] M. Z. Erkal, J. Wilde, Y. Bilgin et al., "High prevalence of vitamin D deficiency, secondary hyperparathyroidism and generalized bone pain in Turkish immigrants in Germany: identification of risk factors," *Osteoporosis International*, vol. 17, no. 8, pp. 1133–1140, 2006.

[45] D. J. Hosking, G. A. Campbell, J. R. Kemm, R. E. Cotton, and R. V. Boyd, "Safety of treatment for subclinical osteomalacia in the elderly," *British Medical Journal*, vol. 289, pp. 785–787, 1984.

[46] M. Z. Islam, A. A. Shamim, H. T. Viljakainen et al., "Effect of vitamin D, calcium and multiple micronutrient supplementation on vitamin D and bone status in Bangladeshi premenopausal garment factory workers with hypovitaminosis D: a double-blinded, randomised, placebo-controlled 1-year intervention," *The British Journal of Nutrition*, vol. 104, no. 2, pp. 241–247, 2010.

[47] M. Priemel, C. von Domarus, T. O. Klatte et al., "Bone mineralization defects and vitamin D deficiency: histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients," *Journal of Bone and Mineral Research*, vol. 25, no. 2, pp. 305–312, 2010.

[48] J. F. Aloia, M. Patel, R. Dimaano et al., "Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration," *The American Journal of Clinical Nutrition*, vol. 87, no. 6, pp. 1952–1958, 2008.

[49] M. H. Murad, K. B. Elamin, N. O. Abu Elnour et al., "Clinical review: the effect of vitamin D on falls: a systematic review and meta-analysis," *The Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 10, pp. 2997–3006, 2011.

[50] L. D. Gillespie, M. C. Robertson, W. J. Gillespie et al., "Interventions for preventing falls in older people living in the

community," *Cochrane Database of Systematic Reviews*, vol. 12, no. 9, article CD007146, 2012.

[51] I. D. Cameron, L. D. Gillespie, M. C. Robertson et al., "Interventions for preventing falls in older people in care facilities and hospitals," *Cochrane Database of Systematic Reviews*, vol. 12, article CD005465, 2012.

[52] H. A. Bischoff-Ferrari, B. Dawson-Hughes, H. B. Staehelin et al., "Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials," *BMJ*, vol. 339, article b3692, 2009.

[53] M. Chung, J. Lee, T. Terasawa, J. Lau, and T. A. Trikalinos, "Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the U.S. Preventive Services Task Force," *Annals of Internal Medicine*, vol. 155, no. 12, pp. 827–838, 2011.

[54] H. A. Bischoff-Ferrari, W. C. Willett, E. J. Orav et al., "A pooled analysis of vitamin D dose requirements for fracture prevention," *The New England Journal of Medicine*, vol. 367, no. 1, pp. 40–49, 2012.

[55] H. Fan, W. Yu, H. Cao et al., "Meta-analysis of circulating 25-hydroxyvitamin D levels and risk of cardiovascular and all-cause mortality in elderly population," *International Journal of Cardiology*, vol. 176, no. 3, pp. 1025–1029, 2014.

[56] J. Tomson, J. Emberson, M. Hill et al., "Vitamin D and risk of death from vascular and non-vascular causes in the Whitehall study and meta-analyses of 12,000 deaths," *European Heart Journal*, vol. 34, no. 18, pp. 1365–1374, 2013.

[57] M. Gaksch, R. Jorde, G. Grimnes et al., "Vitamin D and mortality: individual participant data meta-analysis of standardized 25-hydroxyvitamin D in 26916 individuals from a European consortium," *PLoS One*, vol. 12, no. 2, article e0170791, 2017.

[58] R. Zhang, B. Li, X. Gao et al., "Serum 25-hydroxyvitamin D and the risk of cardiovascular disease: dose-response meta-analysis of prospective studies," *The American Journal of Clinical Nutrition*, vol. 105, no. 4, pp. 810–819, 2017.

[59] S. Afzal, P. Brøndum-Jacobsen, S. E. Bojesen, and B. G. Nordestgaard, "Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts," *BMJ*, vol. 349, article g6330, 2014.

[60] P. Brøndum-Jacobsen, M. Benn, S. Afzal, and B. G. Nordestgaard, "No evidence that genetically reduced 25-hydroxyvitamin D is associated with increased risk of ischaemic heart disease or myocardial infarction: a Mendelian randomization study," *International Journal of Epidemiology*, vol. 44, no. 2, pp. 651–661, 2015.

[61] L. A. Beveridge, A. D. Struthers, F. Khan et al., "Effect of vitamin D supplementation on blood pressure: a systematic review and meta-analysis incorporating individual patient data," *JAMA Internal Medicine*, vol. 175, no. 5, pp. 745–754, 2015.

[62] R. Scragg, J. Wishart, A. Stewart et al., "No effect of ultraviolet radiation on blood pressure and other cardiovascular risk factors," *Journal of Hypertension*, vol. 29, no. 9, pp. 1749–1756, 2011.

[63] S. Pilz, M. Gaksch, K. Kienreich et al., "Effects of vitamin D on blood pressure and cardiovascular risk factors: a randomized controlled trial," *Hypertension*, vol. 65, no. 6, pp. 1195–1201, 2015.

[64] K. S. Vimaleswaran, A. Cavadino, D. J. Berry et al., "Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study," *The Lancet Diabetes and Endocrinology*, vol. 2, no. 9, pp. 719–729, 2014.

[65] A. J. Rodríguez, D. Scott, V. Srikanth, and P. Ebeling, "Effect of vitamin D supplementation on measures of arterial stiffness: a systematic review and meta-analysis of randomized controlled trials," *Clinical Endocrinology*, vol. 84, no. 5, pp. 645–657, 2016.

[66] M. B. Elamin, N. O. Abu Elnour, K. B. Elamin et al., "Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis," *The Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 7, pp. 1931–1942, 2011.

[67] J. A. Ford, G. S. MacLennan, A. Avenell et al., "Cardiovascular disease and vitamin D supplementation: trial analysis, systematic review, and meta-analysis," *The American Journal of Clinical Nutrition*, vol. 100, no. 3, pp. 746–755, 2014.

[68] R. Scragg, A. W. Stewart, D. Waayer et al., "Effect of monthly high-dose vitamin D supplementation on cardiovascular disease in the vitamin D assessment study : a randomized clinical trial," *JAMA Cardiology*, vol. 2, no. 6, pp. 608–616, 2017.

[69] G. Bjelakovic, L. L. Gluud, D. Nikolova et al., "Vitamin D supplementation for prevention of mortality in adults," *Cochrane Database of Systematic Reviews*, vol. 10, no. 1, article CD007470, 2014.

[70] G. Jones, "Pharmacokinetics of vitamin D toxicity," *The American Journal of Clinical Nutrition*, vol. 88, no. 2, pp. 582S–586S, 2008.

[71] K. Bleicher, R. G. Cumming, V. Naganathan et al., "U-shaped association between serum 25-hydroxyvitamin D and fracture risk in older men: results from the prospective population-based CHAMP study," *Journal of Bone and Mineral Research*, vol. 29, no. 9, pp. 2024–2031, 2014.

[72] K. M. Sanders, A. L. Stuart, E. J. Williamson et al., "Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial," *JAMA*, vol. 303, no. 18, pp. 1815–1822, 2010.

[73] H. Smith, F. Anderson, H. Raphael, P. Maslin, S. Crozier, and C. Cooper, "Effect of annual intramuscular vitamin D on fracture risk in elderly men and women: a population-based, randomized, double-blind, placebo controlled trial," *Rheumatology*, vol. 46, no. 12, pp. 1852–1857, 2007.

[74] H. A. Bischoff-Ferrari, B. Dawson-Hughes, E. J. Orav et al., "Monthly high-dose vitamin D treatment for the prevention of functional decline: a randomized clinical trial," *JAMA Internal Medicine*, vol. 176, no. 2, pp. 175–183, 2016.

[75] A. A. Ginde, P. Blatchford, K. Breese et al., "High-dose monthly vitamin D for prevention of acute respiratory infection in older long-term care residents: a randomized clinical trial," *Journal of the American Geriatrics Society*, vol. 65, no. 3, pp. 496–503, 2017.

[76] A. Zittermann, S. S. Schleithoff, and R. Koerfer, "Protective and toxic effects of vitamin D on vascular calcification: clinical implications," *Molecular Aspects of Medicine*, vol. 29, no. 6, pp. 423–432, 2008.

[77] A. Zittermann, "Vitamin D and cardiovascular disease," *Anticancer Research*, vol. 34, no. 9, pp. 4641–4648, 2014.

[78] V. Hesse and G. Jahreis, "Vitamin D-induced tissue calcinosis and arteriosclerosis changes. II. Current knowledge and conclusions for preventive vitamin D administration in infancy and early childhood," *Pädiatrie und Grenzgebiete*, vol. 29, no. 3, pp. 213–219, 1990.

[79] A. Zittermann, S. Prokop, J. F. Gummert, and J. Börgermann, "Safety issues of vitamin D supplementation," *Anti-Cancer Agents in Medicinal Chemistry*, vol. 13, no. 1, pp. 4–10, 2013.

[80] Y. Dror, S. M. Giveon, M. Hoshen, I. Feldhamer, R. D. Balicer, and B. S. Feldman, "Vitamin D levels for preventing acute coronary syndrome and mortality: evidence of a nonlinear association," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 5, pp. 2160–2167, 2013.

[81] D. Durup, H. L. Jørgensen, J. Christensen et al., "A reverse J-shaped association between serum 25-hydroxyvitamin D and cardiovascular disease mortality: the CopD study," *The Journal of Clinical Endocrinology and Metabolism*, vol. 100, no. 6, pp. 2339–2346, 2015.

[82] A. Zittermann, J. Kuhn, J. Dreier, C. Knabbe, J. F. Gummert, and J. Börgermann, "Vitamin D status and the risk of major adverse cardiac and cerebrovascular events in cardiac surgery," *European Heart Journal*, vol. 34, no. 18, pp. 1358–1364, 2013.

[83] A. Zittermann, J. B. Ernst, S. Prokop et al., "Effect of vitamin D on all-cause mortality in heart failure (EVITA): a 3-year randomized clinical trial with 4,000 IU vitamin D daily," *European Heart Journal*, vol. 38, no. 29, pp. 2279–2286, 2017.

[84] A. Dalbeni, G. Scaturro, M. Degan, P. Minuz, and P. Delva, "Effects of six months of vitamin D supplementation in patients with heart failure: a randomized double-blind controlled trial," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 24, no. 8, pp. 861–868, 2014.

[85] P. L. Lutsey, A. Alonso, E. D. Michos et al., "Serum magnesium, phosphorus, and calcium are associated with risk of incident heart failure: the Atherosclerosis Risk in Communities (ARIC) study," *The American Journal of Clinical Nutrition*, vol. 100, no. 3, pp. 756–764, 2014.

[86] I. R. Reid, G. D. Gamble, and M. J. Bolland, "Circulating calcium concentrations, vascular disease and mortality: a systematic review," *Journal of Internal Medicine*, vol. 279, no. 6, pp. 524–540, 2016.

[87] Y. G. Zorbas, K. K. Kakuris, V. A. Deogenov, and K. B. Yerullis, "Phosphate homeotasis in healthy subjects during prolonged periodic and continuous hypokinesia," *Clinical Biochemistry*, vol. 40, no. 7, pp. 460–466, 2007.

[88] K. Scheld, A. Zittermann, M. Heer et al., "Nitrogen metabolism and bone metabolism markers in healthy adults during 16 weeks of bed rest," *Clinical Chemistry*, vol. 47, no. 9, pp. 1688–1695, 2001.

[89] B. E. Nordin and K. J. Polley, "Metabolic consequences of the menopause. A cross-sectional, longitudinal, and intervention study on 557 normal postmenopausal women," *Calcified Tissue International*, vol. 41, Supplement 1, pp. S1–59, 1987.

[90] H. Schiessl, H. M. Frost, and W. S. Jee, "Estrogen and bone-muscle strength and mass relationships," *Bone*, vol. 22, no. 1, pp. 1–6, 1998.

[91] J. Honye, D. J. Mahon, A. Jain et al., "Morphological effects of coronary balloon angioplasty in vivo assessed by intravascular ultrasound imaging," *Circulation*, vol. 85, no. 3, pp. 1012–1025, 1992.

[92] J. R. Margolis, J. T. Chen, Y. Kong, R. H. Peter, V. S. Behar, and J. A. Kisslo, "The diagnostic and prognostic significance of coronary artery calcification. A report of 800 cases," *Radiology*, vol. 137, no. 3, pp. 609–616, 1980.

[93] L. M. Banks, B. Lees, J. E. MacSweeney, and J. C. Stevenson, "Effect of degenerative spinal and aortic calcification on bone density measurements in postmenopausal women: links between osteoporosis and cardiovascular disease?," *European Journal of Clinical Investigation*, vol. 24, no. 12, pp. 813–817, 1994.

[94] E. I. Barengolts, M. Berman, S. C. Kukreja, T. Kouznetsova, C. Lin, and E. V. Chomka, "Osteoporosis and coronary atherosclerosis in asymptomatic postmenopausal women," *Calcified Tissue International*, vol. 62, no. 3, pp. 209–213, 1998.

[95] American Geriatrics Society Workgroup on Vitamin D Supplementation for Older Adults, "Recommendations abstracted from the American Geriatrics Society consensus statement on vitamin D for prevention of falls and their consequences," *Journal of the American Geriatrics Society*, vol. 62, no. 1, pp. 147–152, 2014.

[96] T. Klausen, L. Breum, H. A. Sørensen, S. Schifter, and B. Sonne, "Plasma levels of parathyroid hormone, vitamin D, calcitonin, and calcium in association with endurance exercise," *Calcified Tissue International*, vol. 52, no. 3, pp. 205–208, 1993.

[97] A. Zittermann, O. Sabatschus, S. Jantzen et al., "Exercise-trained young men have higher calcium absorption rates and plasma calcitriol levels compared with age-matched sedentary controls," *Calcified Tissue International*, vol. 67, no. 3, pp. 215–219, 2000.

[98] A. Zittermann, J. Börgermann, J. F. Gummert, and S. Pilz, "Future directions in vitamin D and cardiovascular research," *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 22, no. 7, pp. 541–546, 2012.

[99] H. Kaplan, R. C. Thompson, B. C. Trumble et al., "Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study," *Lancet*, vol. 389, no. 10080, pp. 1730–1739, 2017.

[100] M. F. Luxwolda, R. S. Kuipers, I. P. Kema, E. Veervan der, D. A. Dijck-Brouwer, and F. A. Muskiet, "Vitamin D status indicators in indigenous populations in East Africa," *European Journal of Nutrition*, vol. 52, pp. 1115–1125, 2013.

[101] S. Pilz, A. Hahn, C. Schön, M. Wilhelm, and R. Obeid, "Effect of two different multimicronutrient supplements on vitamin D status in women of childbearing age: a randomized trial," *Nutrients*, vol. 9, no. 1, 2017.

[102] A. Zittermann, J. B. Ernst, J. F. Gummert, and J. Börgermann, "Vitamin D supplementation, body weight and human serum 25-hydroxyvitamin D response: a systematic review," *European Journal of Nutrition*, vol. 53, no. 2, pp. 367–374, 2014.

Hindawi
International Journal of Endocrinology
Volume 2017, Article ID 3946569, 9 pages
https://doi.org/10.1155/2017/3946569

Research Article

The Association between Bone Quality and Atherosclerosis: Results from Two Large Population-Based Studies

V. Lange,[1] M. Dörr,[2,3] U. Schminke,[4] H. Völzke,[3,5] M. Nauck,[1,3] H. Wallaschofski,[1,3] and A. Hannemann[1]

[1]*Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany*
[2]*Department for Internal Medicine B, University Medicine Greifswald, Greifswald, Germany*
[3]*German Centre for Cardiovascular Research, Partner Site Greifswald, Greifswald, Germany*
[4]*Department for Neurology, University Medicine Greifswald, Greifswald, Germany*
[5]*Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany*

Correspondence should be addressed to A. Hannemann; anke.hannemann@uni-greifswald.de

Received 20 April 2017; Accepted 3 July 2017; Published 9 August 2017

Academic Editor: Astrid Fahrleitner-Pammer

Objective. It is highly debated whether associations between osteoporosis and atherosclerosis are independent of cardiovascular risk factors. We aimed to explore the associations between quantitative ultrasound (QUS) parameters at the heel with the carotid artery intima-media thickness (IMT), the presence of carotid artery plaques, and the ankle-brachial index (ABI). *Methods*. The study population comprised 5680 men and women aged 20–93 years from two population-based cohort studies: Study of Health in Pomerania (SHIP) and SHIP-Trend. QUS measurements were performed at the heel. The extracranial carotid arteries were examined with B-mode ultrasonography. ABI was measured in a subgroup of 3853 participants. Analyses of variance and linear and logistic regression models were calculated and adjusted for major cardiovascular risk factors. *Results*. Men but not women had significantly increased odds for carotid artery plaques with decreasing QUS parameters independent of diabetes mellitus, dyslipidemia, and hypertension. Beyond this, the QUS parameters were not significantly associated with IMT or ABI in fully adjusted models. *Conclusions*. Our data argue against an independent role of bone metabolism in atherosclerotic changes in women. Yet, in men, associations with advanced atherosclerosis, exist. Thus, men presenting with clinical signs of osteoporosis may be at increased risk for atherosclerotic disease.

1. Introduction

Osteoporosis and atherosclerosis substantially impact the elderly, leading to increased morbidity and mortality [1, 2]. Osteoporosis, on the one side, is a chronic disease characterized by low bone mass, microarchitectural deterioration of bone tissue, and an increased fracture risk [1]. Based on these diagnostic criteria, 27.6 million women and men in the European Union are affected by osteoporosis [1]. Atherosclerosis, on the other side, is characterized by loss of elasticity of the artery walls, wall thickening, and plaque formation [2]. Cardiovascular disease due to atherosclerosis represents a major economic burden on the European health care system, with estimated annual costs of 192 billion euros [3]. Moreover, cardiovascular disease is the leading cause of death worldwide, with more than 17.5 million deaths in 2012 [4].

Osteoporosis and atherosclerosis share common risk factors like aging, dyslipidemia, oxidative stress, inflammation, hypertension, and diabetes [2, 5]. Furthermore, they share molecular pathways involving, for example, bone and vascular mineralization or inflammatory processes [6]. Despite these common risk factors, epidemiologic studies suggested an independent association between low bone mass and atherosclerosis [5, 7]. Thus, a population-based study including [5] 2726 postmenopausal women and 2543 men from

Norway reported associations between low bone mineral density (BMD) and echogenic calcified atherosclerotic plaques. Further, lower volumetric trabecular lumbar BMD was associated with advanced carotid plaque in 1833 postmenopausal women and men from a subsample of the Multi-Ethnic Study of Atherosclerosis [7]. In that study [7], also, associations of lower volumetric trabecular lumbar BMD with lower ankle-brachial index (ABI) and increased internal carotid artery intima-media thickness (IMT) were found in men but not in women. A male-specific inverse association between femoral neck BMD and the 10-year risk for coronary heart disease was also described in a study including 5415 men and 7409 women from the general population in Korea [8]. On the other side, associations between BMD and pulse wave velocity [9] as well as between BMD loss and coronary artery calcification [10] were described for women only. The previous study results [5, 7–10] appear even more controversial when considering that competing studies reported no associations between a low BMD and carotid IMT as well as pulse wave velocity and ABI [11] or that associations between peripheral artery disease and bone loss turned nonsignificant after age adjustment [12].

Taken together, it is still uncertain whether low bone quality is associated with atherosclerotic changes independent of major cardiovascular risk factors like diabetes mellitus, dyslipidemia, or hypertension or whether sex-specific differences exist. Besides, there are only few data in the association between bone quality at the heel, measured by quantitative ultrasound (QUS), and atherosclerosis [13, 14]. We therefore aimed to explore the associations between QUS-based parameters, with carotid artery IMT, the presence of carotid artery plaques, and the ABI in the general population.

2. Subjects and Methods

2.1. Study Populations. The present analysis is based on data from two population-based cohort studies in northeast Germany: the second follow-up of the Study of Health in Pomerania (SHIP-2) and SHIP-Trend. Details on the study design, protocols, and sampling methods have been reported elsewhere [15, 16]. In short, the baseline examinations in the SHIP cohort were performed between 1997 and 2001 with a total of 4308 men and women aged 20–81 years. The second follow-up (SHIP-2) was performed between 2008 and 2012 with 2333 subjects aged 30–93 years being re-examined. The baseline examinations in the SHIP-Trend cohort were performed in parallel with SHIP-2 between 2008 and 2012 with a total of 4420 adult men and women aged 20–84 years. All investigations were carried out in accordance with the Declaration of Helsinki, including written informed consent of all participants. The study methods were approved by an institutional review board (ethics committee at the University of Greifswald).

Data from SHIP-2 and SHIP-Trend were pooled for the present analyses. From the resulting population of $N = 6753$, all subjects with missing data in QUS, IMT, plaque, or confounder variables were excluded as well as subjects with extremely high values in the QUS variables, pregnant women, subjects with estimated glomerular filtration rate (eGFR) below 30 ml/min/1.73m^2 or missing eGFR, and those who reported with intake of bisphosphonates, selective estrogen receptor modulators, parathyroid hormone, steroids, or strontium ranelate (for details, see Supplemental Figure 1 available online at https://doi.org/10.1155/2017/3946569). The resulting study population comprised 5680 subjects. Among these subjects, two-thirds (67.8%) participated in the ABI examination, resulting in a subsample of 3853 subjects.

2.2. Interview and Physical Examination. All SHIP-2 and SHIP-Trend participants were offered a large range of standardized medical examinations, biomaterial sampling, and an extensive computer-aided personal interview. During the personal interview, information on sociodemographic characteristics, lifestyle, and medical histories were collected. All participants were asked to bring their medications taken seven days prior to the time of examination. Medication data were obtained online using the IDOM program (online drug-database leaded medication assessment) and classified using the Anatomical-Therapeutic-Chemical (ATC) classification system. Intake of bisphosphonates was defined as ATC M05BA-BB, selective estrogen receptor modulators as ATC G03XC, PTH preparations as ATC H05AA, strontium ranelate as ATC M05BX03, glucocorticoids for systemic use as ATC H02AB and H02BX, vitamin D preparations as ATC A11CC, and cardioprotective medication as ATC C. Participants were defined as physically inactive if they reported less than one hour of regular physical activity per week during summer and winter. Risky alcohol consumption was defined as alcohol intake at or above 30 g/day in men and 20 g/day in women. All women older than 60 years of age and women between 40 and 60 years of age without self-reported menstrual cycling were classified as postmenopausal. Years since menopause were calculated as the difference between current age and age at last menstruation. Standardized measurements of body height and weight were performed with calibrated scales. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Systolic and diastolic blood pressures were measured three times on the right arm of seated subjects, using a digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan). The mean of the second and third measurements was used for statistical analyses. Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg or self-reported intake of antihypertensive medication. Diabetes mellitus was defined when a respective physician's diagnosis or intake of antidiabetic medication (ATC A10) was reported, when HbA1c was $\geq$6.5% or serum glucose concentrations were $\geq$11.1 mmol/l. Dyslipidemia was defined as total cholesterol concentration $\geq$ 6.2 mmol/l or LDL-cholesterol concentration $\geq$ 4.1 mmol/l or HDL-cholesterol concentration $<$ 1.04 mmol/l or triglycerides $\geq$ 1.7 mmol/l or intake of lipid-modifying agents (ATC C10).

2.3. QUS. QUS at the heel was performed using the Achilles InSight device (GE Medical Systems Ultrasound, GE Healthcare, Chalfont St. Giles, U.K.), a water-based bone

ultrasonometer, as reported previously [17]. Two devices without systematic differences were used during the course of the study. The measurements were performed successively on both feet of the seated participants by trained and certified examiners. Alcohol was used as a coupling agent. The system measures the frequency-dependent attenuation of the sound waves (broadband ultrasound attenuation (BUA)) and the speed of sound waves (SOS) as they pass through the heel (os calcis). BUA and SOS were combined to form the stiffness index according to the following formula: $\text{stiffness index} = (0.67 \times \text{BUA}) + (0.28 \times \text{SOS}) - 420$. The system automatically compares individual stiffness index results to values obtained in a healthy young reference population. Indices below the reference mean minus 2.5 standard deviations were taken to indicate a high osteoporotic fracture risk, indices above the reference mean minus 2.5 standard deviations but below the reference mean minus 1 standard deviations were taken to indicate a medium osteoporotic fracture risk, and indices above the reference mean minus 1 standard deviations were taken to indicate a low osteoporotic fracture risk [17].

2.4. IMT and Plaques. Certified medical assistants examined the extracranial carotid arteries with B-mode ultrasonography (vivid-i, GE Medical Systems, Waukesha, WI, USA) using a broad-bandwidth linear array transducer with an operating frequency of 13 MHz. Longitudinal scans of the distal straight portion of the far wall of the common carotid artery (CCA) of both sides were recorded. CCA-IMT was assessed on-screen using a semiautomated edge tracking software, which measures the distance between the lumen-intima and media-adventitia interfaces at an arterial segment of 1 cm in length located directly proximal to the widening of the artery at the bifurcation. The "mean CCA-IMT," which was used for statistical analyses, was calculated as the average of the mean values of 250 measurement points of each side. The carotid arteries were further evaluated in longitudinal and cross-sectional scans for the presence of atherosclerotic plaques. Each arterial segment (i.e., the left or right common carotid artery, internal carotid artery, external carotid artery, and the carotid bifurcation) was categorized into either affected by plaque or plaque-free. If at least one arterial segment was classified as being affected by plaque, "carotid plaques" were defined as being present. Further, the number of arterial sites affected by plaque ranging between zero and eight was recorded.

2.5. ABI. The Doppler method was used to determine systolic blood pressure in both arms (brachial artery) and both ankles (anterior and posterior tibial arteries) for the calculation of the ABI. After at least ten minutes of rest in the supine position, the measurements were started. The measurements were performed with the "Dopplex D900" (Huntleigh Healthcare Ltd., Cardiff, U.K.) and a blood pressure cuff (Welch Allyn, Skaneateles Falls, USA). The calculation of the ABI followed the guidelines of the American Heart Association [18]. The higher of the anterior and posterior tibial artery systolic blood pressure of each leg was divided by the higher of the right or left brachial artery systolic blood pressure. The lower of the right or left leg ABIs was used for statistical analyses.

2.6. Laboratory Measurements. Blood samples were taken between 7 a.m. and 1 p.m. from participants in the supine position. Creatinine, glucose, total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol serum concentrations were measured on the Dimension Vista (Siemens Healthcare Diagnostics, Eschborn, Germany). Hba1c concentrations were measured with high performance liquid chromatography on a DIAMAT analyzer (Bio-Rad Laboratories, Munich, Germany). Serum 25-hydroxy vitamin D (25OHD) concentrations were measured in a subgroup of 3540 (62.3%) SHIP-Trend participants with the IDS-iSYS 25-hydroxy vitamin D assay on the IDS-iSYS Multidiscipline Automated Analyser (Immunodiagnostic Systems Limited, Frankfurt am Main, Germany). The eGFR was calculated according to the 4-variable modification of diet in renal disease equation [19].

2.7. Statistical Analyses. Due to previously reported marked differences between men and women in the association between bone metabolism and atherosclerotic changes, all analyses were stratified by sex. Continuous data are expressed as median (1st–3rd quartiles) and nominal data as percentage. The Kruskal-Wallis test or the χ^2 test was used for group comparisons. A value of $p < 0.05$ was considered statistically significant.

Analyses of variance (ANOVA) and linear and logistic regression analyses were performed to assess the associations of the QUS-based parameters (exposures: BUA, SOS, stiffness index, and risk for osteoporotic fractures) with the cardiovascular parameters (outcomes: IMT, carotid plaque, the number of arterial segments, and ABI). In all analyses, a one standard deviation decrease in the continuous exposure variables (BUA 13.7 and 14.7 dB/MHz; SOS 37.5 and 33.5 m/s; and stiffness index 18.1 and 17.5 in men and women, resp.) was modelled. In models with the categorical exposure variable, osteoporotic fracture risk, a low osteoporotic fracture risk was used as a reference category. The outcomes IMT and ABI entered the regression models as continuous variables, while carotid plaque was dichotomized (present/not present). Finally, the number of arterial segments affected by plaque was used as a continuous variable. It was transformed (log (number of segments + 1)) before being entered in the ANOVA or linear regression models. We report adjusted means with 95% confidence intervals from the ANOVA, β-coefficients with standard errors and p values from the linear regression models and odds ratios with 95% confidence intervals from the logistic regression models. Results from unadjusted and fully adjusted models, including age, BMI, smoking status, physical inactivity, risky alcohol consumption, diabetes mellitus, dyslipidemia, hypertension, and, in women, intake of estrogens (oral contraceptives or hormone replacement therapy) and years since menopause, are presented.

Among the SHIP-2 and SHIP-Trend participants, a subpopulation underwent the ABI examination. To account for the possible selection bias according to nonparticipation,

inverse probability weights using sex, age, smoking, education, equivalence household income, blood pressure, antihypertensive medication, diabetes mellitus, HbA1c, lipids, and BMI as explanatory variables were applied [20]. All statistical analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

The majority of men (68.2%) and women (61.2%) in our study population had a low QUS-based osteoporotic fracture risk. On the other side, 4.4% of men and 6.5% of women had a high and 27.4% and 32.3% a medium QUS-based osteoporotic fracture risk, respectively. The presence of cardiovascular risk factors differed between the three fracture risk groups. Men with a high fracture risk were older, had lower BMI, were more often smokers or physically inactive, and were more often risky alcohol consumers than men with a low or medium fracture risk. Women with a high risk were also older than those with a low or medium risk, had more often diabetes, dyslipidemia, and hypertension but did not differ with respect to BMI, physical inactivity, and risky alcohol consumption. Besides, the CCA-IMT, the presence of plaques, and the number of arterial segments affected by plaque significantly increased over the three risk groups in men and women (Table 1).

In unadjusted linear regression analyses, inverse associations of BUA, SOS, stiffness index, or the QUS-based osteoporotic fracture risk with the CCA-IMT were found. In men, for example, a decrease in the stiffness index by 18.1 points was associated with an increase in CCA-IMT of 0.016 cm. After adjustment for age (data not shown) and also in fully adjusted models, however, the associations were not confirmed (Table 2).

Regarding plaques, unadjusted logistic regression analyses demonstrated significantly increased odds for the presence of plaques with decreasing QUS-based parameters in both sexes. In fully adjusted models, these associations were confirmed in men but not in women (Table 3). Men with a high fracture risk had significantly increased odds for plaque occurrence compared to men with a low risk. In men, also, the number of arterial segments affected by plaque significantly increased over the risk categories (Figure 1) in unadjusted as well as in the fully adjusted ANOVA. In women, similar findings were made in unadjusted analyses, whereas in fully adjusted models, the results disappeared.

In the subpopulation with ABI measurement, the proportions of men and women with high, medium, or low fracture risk were comparable to the proportions in the whole population (men and women high risk: 69.2% and 62.1%; medium risk: 26.3% and 32.0%; and low risk: 4.5% and 5.9%; for more details, see Supplemental Table 1). The ABI was similar between the three risk groups [median (1st–3rd quartiles) for men and women with a high risk: 1.12 (1.07–1.19) and 1.11 (1.07–1.18), medium risk: 1.13 (1.07–1.19) and 1.13 (1.08–1.19), and low risk: 1.12 (1.04–1.21) and 1.13 (1.08–1.18), resp.]. Fully adjusted, sex-specific linear regression models (Supplemental Table 2) revealed no significant association between the QUS parameters and the ABI in men or women.

4. Discussion

In the present study, we demonstrated associations between decreased bone quality, defined by heel QUS parameters, and the presence of atherosclerotic carotid artery plaque in men. These associations were independent of major cardiovascular risk factors, including dyslipidemia, diabetes, and hypertension. In contrast, our data do not provide evidence for relevant independent associations of heel bone quality with CCA-IMT or ABI.

Traditionally, osteoporosis and atherosclerosis have been regarded as independent processes sharing common risk factors, for example, aging [21]. Recent evidence from cell culture as well as epidemiological studies, however, points to an age-independent association between osteoporosis and atherosclerosis [6, 22]. Defects in bone mineralization and arterial calcification were attributed of having a similar pathogenesis [6], and associations between bone quality and atherosclerotic changes independent of cardiovascular risk factors have been proposed.

Following this hypothesis, the previous studies [23, 24] demonstrated associations between decreased BMD and increased IMT in elderly individuals. For example, the San Antonio Family Osteoporosis Study demonstrated that decreased BMD at various sites is correlated with carotid artery IMT in older women and men [23]. Among the SHIP-2 and SHIP-Trend participants, highly significant inverse associations between the QUS-based parameters and the carotid artery IMT were observed in unadjusted models but turned nonsignificant after adjustment for cardiovascular risk factors. Comparable observations were made by Frost et al. [25], who found associations between spine BMD and IMT, but the relationship was not significant after adjustment for age, mean arterial pressure, and triglycerides.

While our data thus argues against independent associations between bone metabolism and IMT, it provides evidence for a male-specific relation with carotid artery plaques. CCA-IMT and plaques are biologically and genetically distinct markers of atherosclerosis. They differ with respect to specific pattern of risk factors, their pathogenesis, and their ability to predict cardiovascular and cerebrovascular events [26]. Numerous factors including inflammation, protein metabolism, and oxidative stress were proposed to promote development of disease in both osteoporotic and atherosclerotic changes [7]. These factors are likely involved in the pathogenesis of focal atherosclerotic plaques but are less important for an arterial wall thickening of the CCA, which may explain the strong association between QUS-based bone quality and plaque occurrence and the nonsignificant association between bone quality and IMT. Corresponding observations of associations between bone quality and the presence of plaques were reported from various studies [5, 25, 27, 28]. For example, the Tromsø study [5] showed that BMD was associated with calcified echogenic plaque independent of potential confounders, mediators, and shared risk factors in men and women [5]. Also, Hyder et al.

Table 1: Characteristics of the IMT study population.

Characteristics	Risk for osteoporotic fractures—men				Risk for osteoporotic fractures—women			
	Low ($n = 1887$)	Medium ($n = 759$)	High ($n = 122$)	p	Low ($n = 1783$)	Medium ($n = 940$)	High ($n = 189$)	p
Age, years	52 (41–64)	56 (45–68)	63 (52–71)	<0.01	47 (38–58)	58 (47–67)	67 (59–75)	<0.01
BMI, kg/m^2	28.2 (25.8–31.1)	27.8 (25.1–30.5)	27.6 (24.5–30.7)	<0.01	26.5 (23.3–30.8)	26.4 (23.3–30.3)	26.7 (23.7–29.5)	0.59
Current smoker, %	24.1	31.6	38.5	<0.01	26.1	21.4	17.5	<0.01
Physically inactive, %	48.6	57.8	63.9	<0.01	51.1	48.2	50.3	0.35
Risky alcohol consumption, %	13.3	16.1	20.5	0.02	3.0	2.6	2.7	0.81
25OHD, ng/ml*	23.4 (17.9–29.6)	22.7 (16.9–28.2)	20.4 (16.2–28.9)	0.08	22.4 (16.4–29.8)	22.8 (16.8–29.2)	20.5 (15.0–28.1)	0.25
Diabetes mellitus, %	12.8	16.5	14.8	0.04	8.8	11.4	16.4	<0.01
Dyslipidemia, %	67.4	71.4	71.3	0.10	48.6	58.5	67.7	<0.01
Hypertension, %	71.1	74.3	75.4	0.18	43.2	55.5	72.0	<0.01
BUA, dB/MHz	123 (116–131)	105 (101–109)	94 (89–97)	<0.01	116 (109–125)	99 (94–103)	86 (82–90)	<0.01
SOS, m/s	1576 (1560–1597)	1532 (1521–1543)	1500 (1489–1509)	<0.01	1576 (1562–1597)	1539 (1528–1548)	1509 (1499–1516)	<0.01
Stiffness index	103 (95–114)	80 (75–84)	63 (58–66)	<0.01	99 (91–110)	77 (73–81)	61 (57–64)	<0.01
Osteoporosis, %†	1.3	2.9	8.2	<0.01	1.9	8.5	21.7	<0.01
IMT, cm	0.61 (0.51–0.73)	0.63 (0.54–0.75)	0.66 (0.56–0.80)	<0.01	0.54 (0.48–0.64)	0.61 (0.51–0.71)	0.68 (0.57–0.78)	<0.01
Plaques, %	43.1	55.2	77.1	<0.01	28.3	44.0	64.6	<0.01
Number of arterial segments with plaque	0 (0–2)	1 (0–2)	2 (1–3)	<0.01	0 (0-1)	0 (0–2)	1 (0–2)	<0.01

BMI: body mass index; BUA: broadband ultrasound attenuation; SOS: speed of sound; IMT: intima-media thickness; 25OHD: 25-hydroxy vitamin D. Data are median (1st–3rd quartiles) or proportions. Group differences were tested with the Kruskal-Wallis or chi-squared tests. *25OHD: men—1013 missing, women—1127 missing. †Self-reported osteoporosis: men—51 missing, women—63 missing.

Table 2: Associations between a decrease in QUS-based parameters and IMT.

Exposure	Adjustment	Men			Women		
		β-Coefficient	SE	p	β-Coefficient	SE	p
BUA	Unadjusted	0.009	0.003	<0.01	0.034	0.003	<0.01
SOS		0.020	0.003	<0.01	0.038	0.002	<0.01
Stiffness index		0.016	0.003	<0.01	0.039	0.002	<0.01
Risk: medium versus low		0.022	0.007	<0.01	0.053	0.005	<0.01
Risk: high versus low		0.052	0.148	<0.01	0.113	0.010	<0.01
BUA	Fully adjusted	−0.002	0.002	0.32	−0.003	0.002	0.13
SOS		−0.003	0.002	0.28	−0.000	0.002	0.87
Stiffness index		−0.003	0.002	0.25	−0.002	0.002	0.34
Risk: medium versus low		−0.004	0.004	0.34	−0.003	0.004	0.50
Risk: high versus low		−0.010	0.008	0.19	−0.007	0.008	0.38

BUA: broadband ultrasound attenuation; IMT: intima-media thickness; QUS: quantitative ultrasound; SD: standard deviation; SE: standard error; SOS: speed of sound. β-Coefficients, standard errors (SE), and p values from linear regression models. For BUA, SOS, and stiffness index, a one standard deviation decrease was modelled. A one standard deviation of BUA for men and women: 13.7 and 14.7 dB/MHz; SOS: 37.5 and 33.5 m/s; stiffness index: 18.1 and 17.5. Full adjustment for age, body mass index, smoking status, physical inactivity, risky alcohol consumption, diabetes mellitus, dyslipidemia, hypertension, and, in women, additionally intake of estrogens (oral contraceptives or hormone replacement therapy) and years since menopause.

Table 3: Associations between a decrease in QUS-based parameters and plaques.

Exposure	Adjustment	Odds ratio (95% confidence interval)	
		Men	Women
BUA	Unadjusted	1.32 (1.22–1.42)	1.68 (1.54–1.82)
SOS		1.46 (1.35–1.59)	1.74 (1.59–1.89)
Stiffness index		1.43 (1.33–1.55)	1.80 (1.65–1.96)
Risk: medium versus low		1.62 (1.37–1.93)	1.99 (1.69–2.35)
Risk: high versus low		4.43 (2.87–6.81)	4.61 (3.36–6.32)
BUA	Fully adjusted	1.23 (1.11–1.37)	0.98 (0.88–1.10)
SOS		1.20 (1.08–1.34)	1.01 (0.90–1.12)
Stiffness index		1.24 (1.11–1.38)	1.00 (0.90–1.11)
Risk: medium versus low		1.24 (0.99–1.55)	0.90 (0.73–1.20)
Risk: high versus low		2.93 (1.70–5.06)	0.93 (0.63–1.38)

BUA: broadband ultrasound attenuation; QUS: quantitative ultrasound; SOS: speed of sound. Odds ratios and 95% confidence intervals from logistic regression models. For BUA, SOS, and stiffness index, a one standard deviation decrease was modelled. One standard deviation of BUA for men and women: 13.7 and 14.7 dB/MHz; SOS: 37.5 and 33.5 m/s; stiffness index: 18.1 and 17.5. Full adjustment for age, body mass index, smoking status, physical inactivity, risky alcohol consumption, diabetes mellitus, dyslipidemia, hypertension, and, in women, additionally intake of estrogens (oral contraceptives or hormone replacement therapy) and years since menopause.

investigated in 904 postmenopausal women and 929 men an independent association between echogenically measured carotid plaque and lower volumetric trabecular lumbar BMD [7]. While our results were restricted to men, other studies reported associations between carotid plaques and BMD only in women [10, 27] or were restricted to women [25, 29, 30]. Nevertheless, also, male-specific associations between BMD and plaques [7, 8, 31, 32] were previously reported.

The sex differences in the examined associations may be explained by differences in bone metabolism between men and women. Men have a higher peak of bone mass than women [33], and osteoporosis emerges later in life and is more often due to secondary causes [34]. At the same time, osteoporosis is strongly associated with comorbidities and frailty in men than in women [35] and men have a higher fracture-related mortality than women [36]. Thus, men presenting with clinical signs of osteoporosis may be generally more frail and present with a larger number of comorbidities than men without such signs. This probably explains the increased risk of cardiovascular pathologies with decreasing bone quality, independent of age, and further cardiovascular risk factors. In these men, heightened awareness regarding atherosclerotic changes may be of clinical importance.

In previous studies, bone quality was rarely assessed by QUS, instead dual-X-ray-absorptiometry (DXA) was performed. DXA provides information on BMD and is the recommended measurement in the diagnosis and monitoring of osteoporosis [37, 38]. However, also, QUS provides reliable results to predict fracture risk [39, 40] and has the advantage of being radiation-free and easy to handle. To our knowledge, there are only few studies using QUS to

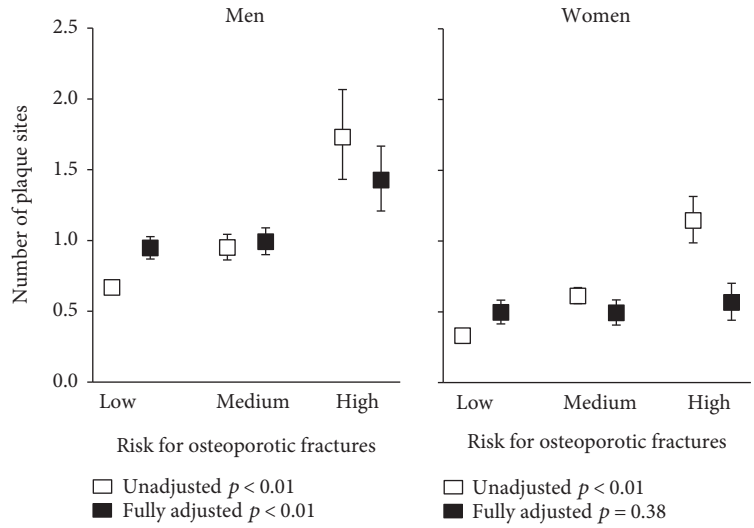


Figure 1: Adjusted mean number of plaque sites according to QUS-based osteoporotic fracture risk by sex. ANOVA was adjusted for age, body mass index, smoking status, physical inactivity, risky alcohol consumption, diabetes mellitus, dyslipidemia, hypertension, and, in women, additionally intake of estrogens (oral contraceptives or hormone replacement therapy) and years since menopause. The number of arterial segments affected by plaque was transformed (log (number of plaque sites + 1)) before being entered in the model and back-transformed for display in the figure.

investigate the association between bone quality and atherosclerosis [13, 41–43]. These studies reported conflicting results, but predominantly suggest an association between QUS and atherosclerotic changes, which is in line with our results.

Next to IMT and plaques, we assessed the relation between bone stiffness and ABI, as a marker of vascular calcification and increased vessel stiffness [44]. It is known that decreased blood flow to the lower limbs caused by PAD leads to compromising the bone quality [45]. In line with this, the Rotterdam Study [46], including 5268 individuals, reported a significantly increased risk for PAD in women with a low femoral neck BMD even after adjusting for age. Our study failed to show an association between bone stiffness and ABI. Yet, PAD as defined by ABI ≤ 0.9 was rare in our study population; only 1.53% of participants entailed these values, and early stages of PAD may not impair bone health [12]. This may have prevented us from detecting a respective association. Moreover, our results are in line with the majority of previous studies that report no [43, 47] or only weak [12, 48] associations between BMD and arterial stiffness.

Our study has several strengths and limitations. Strengths result from the large sample including men and women over a large age range (20–93 years). Further, all study participants underwent intensive medical examinations with highly standardized procedures, assuring high data quality. Moreover, we adjusted our models for interfering covariates to assess the impact of comorbidities.

Besides these strengths, our study has its limitations. First, the cross-sectional design does not allow assessing causality between the measures. Second, cardioprotective drugs were taken by a large proportion (40.7%) of our study population. The intake of such medication reduces the cardiovascular risk and may lead to an underestimation of the effect of the examined associations. Third, BMD measurements were not available; thus, our study is not directly comparable to other studies using BMD. However, the QUS-based results provide complementary evidence and, in a population-based research setting, offer the advantage of being simpler, less expensive, and free of ionizing radiation. Fourth, ABI measurements were only available in a subsample. To rule out that nonparticipation resulted in a selection bias, we weighted the respective data based on social-demographic and health-related variables. Fifth, 25OHD concentrations were unavailable in nearly 40% of the study population, and vitamin D intake was reported by only 29 subjects. Therefore, we refrained from including that information in the analyses. Sixth, our study was performed exclusively in Caucasian European subjects; thus, our results may not be directly transferrable to other regions or ethnicities.

In conclusion, our data argue against an independent role of bone metabolism in atherosclerotic changes in women. Yet, in men, associations with atherosclerotic changes, especially formation of plaques, seem present. Thus, men presenting with clinical signs of osteoporosis may be at increased risk for atherosclerotic disease. Further studies are needed to understand the relation between calcified plaque and decreased bone quality.

Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgments

SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (Grant nos.

01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, and the Social Ministry of the Federal State of Mecklenburg-West Pomerania. This work is also part of the research project Greifswald Approach to Individualized Medicine (GANI_MED), which is funded by the Federal Ministry of Education and Research and the Ministry of Cultural Affairs of the Federal State of Mecklenburg-West Pomerania (03IS2061A). Dr. A. Hannemann is supported by a Gollwitz-Meier scholarship.

References

[1] E. Hernlund, A. Svedbom, M. Ivergard et al., "Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA)," *Archives of Osteoporosis*, vol. 8, p. 136, 2013.

[2] T. M. Doherty, L. A. Fitzpatrick, D. Inoue et al., "Molecular, endocrine, and genetic mechanisms of arterial calcification," *Endocrine Reviews*, vol. 25, pp. 629–672, 2004.

[3] S. Allender, P. Scarborough, and V. Peto, "European cardiovascular disease statistics: 2008 edition, European Heart Network," April 2017, https://www.bhforguk/publications/statistics/european-cardiovascular-disease-statistics-2008.

[4] World Health Organization, "Cardiovascular diseases (CVDs): key facts," March 2017, http://www.whoint/mediacentre/factsheets/fs317/en/.

[5] L. Jorgensen, O. Joakimsen, G. K. Rosvold Berntsen, I. Heuch, and B. K. Jacobsen, "Low bone mineral density is related to echogenic carotid artery plaques: a population-based study," *American Journal of Epidemiology*, vol. 160, pp. 549–556, 2004.

[6] T. M. Doherty, K. Asotra, L. A. Fitzpatrick et al., "Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, pp. 11201–11206, 2003.

[7] J. A. Hyder, M. A. Allison, E. Barrett-Connor et al., "Bone mineral density and atherosclerosis: the multi-ethnic study of atherosclerosis, abdominal aortic calcium study," *Atherosclerosis*, vol. 209, pp. 283–289, 2010.

[8] H. T. Lee, J. Shin, S. Y. Min et al., "Relationship between bone mineral density and a 10-year risk for coronary artery disease in a healthy Korean population: the Korea National Health and Nutrition Examination Survey 2008-2010," *Coronary Artery Disease*, vol. 26, pp. 66–71, 2015.

[9] N. L. Kim, H. M. Jang, S. K. Kim, K. D. Ko, I. C. Hwang, and H. S. Suh, "Association of arterial stiffness and osteoporosis in healthy men undergoing screening medical examination," *Journal of Bone Metabolism*, vol. 21, pp. 133–141, 2014.

[10] N. Campos-Obando, M. Kavousi, J. E. Roeters van Lennep et al., "Bone health and coronary artery calcification: the Rotterdam study," *Atherosclerosis*, vol. 241, pp. 278–283, 2015.

[11] D. K. Liang, X. J. Bai, B. Wu et al., "Associations between bone mineral density and subclinical atherosclerosis: a cross-sectional study of a Chinese population," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, pp. 469–477, 2014.

[12] D. von Muhlen, M. Allison, S. K. Jassal, and E. Barrett-Connor, "Peripheral arterial disease and osteoporosis in older adults: the Rancho Bernardo study," *Osteoporosis International*, vol. 20, pp. 2071–2078, 2009.

[13] K. Hirose, H. Tomiyama, R. Okazaki et al., "Increased pulse wave velocity associated with reduced calcaneal quantitative osteo-sono index: possible relationship between atherosclerosis and osteopenia," *The Journal of Clinical Endocrinology and Metabolism*, vol. 88, pp. 2573–2578, 2003.

[14] P. Pennisi, S. S. Signorelli, S. Riccobene et al., "Low bone density and abnormal bone turnover in patients with atherosclerosis of peripheral vessels," *Osteoporosis International*, vol. 15, pp. 389–395, 2004.

[15] U. John, B. Greiner, E. Hensel et al., "Study of health in Pomerania (SHIP): a health examination survey in an east German region: objectives and design," *Sozial- und Präventivmedizin*, vol. 46, pp. 186–194, 2001.

[16] H. Volzke, D. Alte, C. O. Schmidt et al., "Cohort profile: the study of health in Pomerania," *International Journal of Epidemiology*, vol. 40, pp. 294–307, 2011.

[17] R. M. Berg, H. Wallaschofski, M. Nauck et al., "Positive association between adipose tissue and bone stiffness," *Calcified Tissue International*, vol. 97, pp. 40–49, 2015.

[18] V. Aboyans, M. H. Criqui, P. Abraham et al., "Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association," *Circulation*, vol. 126, pp. 2890–2909, 2012.

[19] A. S. Levey, T. Greene, J. W. Kusek, and G. J. Beck, "A simplified equation to predict glomerular filtration rate from serum creatinine," *Journals of the American Society of Nephrology*, vol. 11, 2000, Abstract A0828.

[20] L. Li, C. Shen, X. Li, and J. M. Robins, "On weighting approaches for missing data," *Statistical Methods in Medical Research*, vol. 22, pp. 14–30, 2013.

[21] D. Hamerman, "Osteoporosis and atherosclerosis: biological linkages and the emergence of dual-purpose therapies," *QJM: Monthly Journal of the Association of Physicians*, vol. 98, pp. 467–484, 2005.

[22] P. Anagnostis, A. Karagiannis, A. I. Kakafika, K. Tziomalos, V. G. Athyros, and D. P. Mikhailidis, "Atherosclerosis and osteoporosis: age-dependent degenerative processes or related entities?," *Osteoporosis International*, vol. 20, pp. 197–207, 2009.

[23] J. R. Shaffer, C. M. Kammerer, D. L. Rainwater et al., "Decreased bone mineral density is correlated with increased subclinical atherosclerosis in older, but not younger, Mexican American women and men: the San Antonio family osteoporosis study," *Calcified Tissue International*, vol. 81, pp. 430–441, 2007.

[24] J. Tamaki, M. Iki, Y. Hirano et al., "Low bone mass is associated with carotid atherosclerosis in postmenopausal women: the Japanese population-based osteoporosis (JPOS) cohort study," *Osteoporosis International*, vol. 20, pp. 53–60, 2009.

[25] M. L. Frost, R. Grella, S. C. Millasseau et al., "Relationship of calcification of atherosclerotic plaque and arterial stiffness to bone mineral density and osteoprotegerin in postmenopausal women referred for osteoporosis screening," *Calcified Tissue International*, vol. 83, pp. 112–120, 2008.

[26] P. J. Touboul, M. G. Hennerici, S. Meairs et al., "Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011," *Cerebrovascular Diseases*, vol. 34, pp. 290–296, 2012.

[27] S. N. Kim, H. S. Lee, H. S. Nam et al., "Carotid intima-media thickness is inversely related to bone density in female but not in male patients with acute stroke," *Journal of Neuroimaging*, vol. 26, pp. 83–88, 2016.

[28] O. Uyama, Y. Yoshimoto, Y. Yamamoto, and A. Kawai, "Bone changes and carotid atherosclerosis in postmenopausal women," *Stroke*, vol. 28, pp. 1730–1732, 1997.

[29] H. Sumino, S. Ichikawa, S. Kasama et al., "Elevated arterial stiffness in postmenopausal women with osteoporosis," *Maturitas*, vol. 55, pp. 212–218, 2006.

[30] M. Varri, T. P. Tuomainen, R. Honkanen et al., "Carotid intima-media thickness and calcification in relation to bone mineral density in postmenopausal women-the OSTPRE-BBA study," *Maturitas*, vol. 78, pp. 304–309, 2014.

[31] J. J. Carr, T. C. Register, F. C. Hsu et al., "Calcified atherosclerotic plaque and bone mineral density in type 2 diabetes: the diabetes heart study," *Bone*, vol. 42, pp. 43–52, 2008.

[32] J. H. Magnus and D. L. Broussard, "Relationship between bone mineral density and myocardial infarction in US adults," *Osteoporosis International*, vol. 16, pp. 2053–2062, 2005.

[33] D. A. Bailey, A. D. Martin, H. A. McKay, S. Whiting, and R. Mirwald, "Calcium accretion in girls and boys during puberty: a longitudinal analysis," *Journal of Bone and Mineral Research*, vol. 15, pp. 2245–2250, 2000.

[34] S. P. Tuck and H. K. Datta, "Osteoporosis in the aging male: treatment options," *Clinical Interventions in Aging*, vol. 2, pp. 521–536, 2007.

[35] M. Laurent, E. Gielen, F. Claessens, S. Boonen, and D. Vanderschueren, "Osteoporosis in older men: recent advances in pathophysiology and treatment," *Best Practice & Research. Clinical Endocrinology & Metabolism*, vol. 27, pp. 527–539, 2013.

[36] J. R. Center, T. V. Nguyen, D. Schneider, P. N. Sambrook, and J. A. Eisman, "Mortality after all major types of osteoporotic fracture in men and women: an observational study," *Lancet*, vol. 353, pp. 878–882, 1999.

[37] J. A. Kanis, E. V. McCloskey, H. Johansson et al., "European guidance for the diagnosis and management of osteoporosis in postmenopausal women," *Osteoporosis International*, vol. 24, pp. 23–57, 2013.

[38] Y. Lu, H. K. Genant, J. Shepherd et al., "Classification of osteoporosis based on bone mineral densities," *Journal of Bone and Mineral Research*, vol. 16, pp. 901–910, 2001.

[39] Dachverband Osteologie e.V. Prophylaxe, Diagnostik und Therapie der Osteoporose bei Männern ab dem 60. Lebensjahr und bei postmenopausalen Frauen, "S3-Leitlinie des Dachverbands der Deutschsprachigen Wissenschaftlichen Osteologischen Gesellschaften (DVO) e.V. 2014," April 2017, http://www.dv-osteologie.org/dvo_leitlinien/osteoporose-leitlinie-2014.

[40] J. Shams, A. B. Spitzer, A. M. Kennelly, and L. L. Tosi, "Bone quality: educational tools for patients, physicians, and educators," *Clinical Orthopaedics and Related Research*, vol. 469, pp. 2248–2259, 2011.

[41] S. Yamada, M. Inaba, H. Goto et al., "Significance of intima-media thickness in femoral artery in the determination of calcaneus osteo-sono index but not of lumbar spine bone mass in healthy Japanese people," *Osteoporosis International*, vol. 16, pp. 64–70, 2005.

[42] M. M. Pinheiro, C. M. Castro, and V. L. Szejnfeld, "Low femoral bone mineral density and quantitative ultrasound are risk factors for new osteoporotic fracture and total and cardiovascular mortality: a 5-year population-based study of Brazilian elderly women," *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, vol. 61, pp. 196–203, 2006.

[43] S. C. van Dijk, R. T. de Jongh, A. W. Enneman et al., "Arterial stiffness is not associated with bone parameters in an elderly hyperhomocysteinemic population," *Journal of Bone and Mineral Metabolism*, vol. 34, pp. 99–108, 2016.

[44] K. I. Paraskevas, I. Kotsikoris, S. A. Koupidis, A. D. Giannoukas, and D. P. Mikhailidis, "Ankle-brachial index: a marker of both peripheral arterial disease and systemic atherosclerosis as well as a predictor of vascular events," *Angiology*, vol. 61, pp. 521–523, 2010.

[45] M. Laroche, L. Moulinier, P. Leger, D. Lefebvre, B. Mazieres, and H. Boccalon, "Bone mineral decrease in the leg with unilateral chronic occlusive arterial disease," *Clinical and Experimental Rheumatology*, vol. 21, pp. 103–106, 2003.

[46] M. van der Klift, H. A. Pols, A. E. Hak, J. C. Witteman, A. Hofman, and C. E. de Laet, "Bone mineral density and the risk of peripheral arterial disease: the Rotterdam study," *Calcified Tissue International*, vol. 70, pp. 443–449, 2002.

[47] Y. Q. Wang, P. T. Yang, H. Yuan et al., "Low bone mineral density is associated with increased arterial stiffness in participants of a health records based study," *Journal of Thoracic Disease*, vol. 7, pp. 790–798, 2015.

[48] S. Y. Wong, T. Kwok, J. Woo et al., "Bone mineral density and the risk of peripheral arterial disease in men and women: results from Mr. and Ms Os, Hong Kong," *Osteoporosis International*, vol. 16, pp. 1933–1938, 2005.

Hindawi
International Journal of Endocrinology
Volume 2017, Article ID 4138783, 8 pages
https://doi.org/10.1155/2017/4138783

Clinical Study

Angiotensin-Converting Enzyme Inhibition and Parathyroid Hormone Secretion

Sarah Zaheer,[1] Jenifer M. Brown,[2] Molly Connors,[1] Jonathan S. Williams,[1] Gail K. Adler,[1] and Anand Vaidya[1]

[1]*Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*
[2]*Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*

Correspondence should be addressed to Sarah Zaheer; szaheer@bwh.harvard.edu and Anand Vaidya; anandvaidya@bwh.harvard.edu

Received 19 April 2017; Accepted 31 May 2017; Published 20 July 2017

Academic Editor: Nicolas Verheyen

Background. Prior studies suggest that renin-angiotensin-aldosterone system (RAAS) inhibitors decrease parathyroid hormone (PTH) secretion. *Objective*. To evaluate the effect of angiotensin-converting enzyme inhibitors (ACEi) on serum PTH in participants with and without primary hyperparathyroidism (P-HPT). *Methods*. An open-label, single-arm, pilot study whereby participants with and without P-HPT had PTH were evaluated before and after 1 week of maximally tolerated lisinopril therapy. *Results*. A total of 12 participants with, and 15 participants without, P-HPT successfully completed the protocol. Following 1 week of lisinopril, participants with P-HPT had a decrease in systolic blood pressure (SBP) (−6.4 mmHg, $P < 0.01$), an increase in plasma renin activity (PRA) (+1.50 ng/mL/h, $P = 0.06$), and a decrease in PTH (79.5 (21.6) to 70.9 (19.6) pg/mL, $\Delta = -8.6$ pg/mL, $P = 0.049$); however, serum and urine calcium did not change. In contrast, although 1 week of lisinopril significantly decreased SBP and increased PRA among participants without P-HPT, there were no changes in PTH or calcium. *Conclusion*. In this short pilot investigation, 1 week of maximally titrated ACEi did not impact PTH in participants without P-HPT, but resulted in a modest and marginally significant reduction of PTH but not calcium, among participants with P-HPT. This trial is registered with ClinicalTrials.gov NCT01691781.

1. Introduction

Renin-angiotensin-aldosterone system (RAAS), the key hormonal regulator of sodium and volume homeostasis, also plays a major role in the pathogenesis of cardiovascular disease [1, 2]. Pharmacologic inhibition of the RAAS is a cornerstone of treatment for hypertension, coronary artery disease, and heart failure [3–5].

Parathyroid hormone (PTH) is a well-established regulator of calcium and skeletal homeostasis. Observational studies have shown that higher PTH levels are independently associated with higher blood pressure and increased risk for incident hypertension, cardiovascular mortality, and structural cardiac dysfunction [6–10]. Though several hypotheses have been proposed to explain these observations, there is compelling evidence that an interaction exists between the RAAS and PTH that may explain these observational findings [11–14].

Individuals with primary aldosteronism have higher PTH levels when compared to matched individuals with essential hypertension [13, 15–17]. Even in individuals without primary aldosteronism, higher serum aldosterone levels are independently associated with higher PTH levels [18]. We previously reported that infusion of angiotensin II in individuals without primary hyperparathyroidism (P-HPT) increased PTH levels by >30% [19]. Since we [19], and others [16], have shown that both the angiotensin type I receptor (ATR1) and the mineralocorticoid receptor (MR) are expressed in parathyroid tissue, we hypothesized that these findings may have been mediated by activation of the

parathyroid AT1R and/or MR. Some studies suggest that this interaction may be modifiable: among individuals without P-HPT, ACE inhibitors and angiotensin receptor blockers have been associated with lower PTH [18, 19]. Further, studies in participants without P-HPT suggest that MR antagonists associate with lower PTH [19, 20]; however, a recent placebo-controlled and randomized clinical trial reported that MR antagonist therapy did not influence PTH levels in subjects with P-HPT [21].

Based on this accumulation of suggestive but also inconsistent evidence of a modifiable interaction between the RAAS and PTH, we conducted a single-arm, open-label pilot study to assess whether ACE inhibitors decrease PTH levels in patients with P-HPT. The impetus to evaluate the clinical applicability of ACE inhibitors in P-HPT is based on the hypothesis that they may have a dual benefit in lowering the risk for both adverse skeletal [22] and cardiovascular outcomes [23, 24].

2. Materials and Methods

2.1. Study Participants. Participants were recruited from the greater Boston area, and the trial was conducted at the Clinical Research Center at Brigham and Women's Hospital in Boston, Massachusetts. All participants provided informed consent, and the study protocol was approved and monitored by our institutional human research and ethics committee (NCT01691781). Participants with P-HPT were recruited from Endocrinology and Endocrine Surgery Clinics at Brigham and Women's Hospital and affiliated hospitals. Participants without P-HPT were recruited from healthy volunteers. Participants underwent a screening visit with a study physician to determine eligibility.

Participants with primary hyperparathyroidism were included if they had a biochemical diagnosis of P-HPT confirmed by their endocrinologist, were normotensive or had mild (stage I) hypertension that was untreated or treated with a single antihypertensive agent, were between the ages of 18 and 80 years, and had normal estimated glomerular filtration rate (eGFR > 60 mL/min/1.73m^2). Participants without P-HPT were included based on the same inclusion criteria except they could not have a known diagnosis of P-HPT.

Exclusion criteria for all participants were presence of chronic kidney disease defined as eGFR <60 mL/min/1.73m^2, stage 2 or 3 hypertension or use of >1 antihypertensive medication, type 2 diabetes not controlled by diet or metformin alone or A1c > 7.5%, history of liver or heart failure, use of antipsychotic medication or lithium, presence of chronic inflammatory condition treated with prescribed nonsteroidal anti-inflammatory drugs (NSAIDs), use of prescribed doses of potassium supplements, illness requiring overnight hospitalization in the last 6 months, or pregnancy/breast-feeding. Participants with P-HPT who were in the midst of planning a parathyroidectomy were also excluded.

The initial objective of this pilot study was to enroll participants over a project period of up to four years, or until a maximum of 15 participants without P-HPT, or 30 participants with P-HPT, were enrolled.

2.2. Antihypertensive Medication Washout Protocol. All enrolled participants who were on a single antihypertensive medication underwent a medication washout period before initiation of study procedures to avoid interference with RAAS and calcium-regulatory physiology. Angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin receptor blockers, and mineralocorticoid receptor antagonists were stopped for 2 months, beta-blockers and diuretics were stopped for 1 month, and calcium-channel blockers were stopped for 2 weeks prior to the start of any study procedures. During the medication washout period, participants were given home sphygmomanometers to measure their daily blood pressure at home and report blood pressure readings to study staff. If blood pressures exceeded 159/99 mmHg for more than 1 week, a study physician considered either withdrawing the participant from the study or initiating amlodipine to lower blood pressure during the washout period. If amlodipine was started, it was discontinued 2 weeks prior to starting the study procedures. Participants whose blood pressure could not be maintained below 159/99 mmHg were withdrawn from the study. Participants were also asked to discontinue use of NSAIDs, decongestants, and over-the-counter cold and flu remedies for 2 weeks prior to study initiation.

2.3. Calcium and Vitamin D Washout. Enrolled participants on vitamin D therapy were required to be on a stable dose for at least 2 months prior to study initiation. All calcium supplements were discontinued for the duration of the study, and study participants received a standardized calcium supplementation as part of the study diet (below).

2.4. Dietary Control. All participants were placed on a liberal sodium diet for 5 days prior to their study visit to ensure standardization of sodium balance, which can dramatically influence RAAS activity. This diet consisted of their usual ad lib diet, supplemented with 150 mEq of sodium per day. Dietary potassium was also supplemented during the study diet weeks, given its crucial role in RAAS regulation, with 50 mEq of potassium chloride daily. Participants without P-HPT were given daily dietary calcium supplementation (1000 mg of calcium carbonate) to ensure similar intake between participants; participants with P-HPT did not receive any dietary calcium supplementation during the study.

2.5. Study Visits. The study schematic is demonstrated in Figure 1. After 5 days of standardized dietary intake as outlined above, participants arrived to the outpatient research center at 8 am for study visit 1. They completed a 24-hour urine collection for creatinine, sodium, calcium, phosphate, and aldosterone, ending just prior to their arrival at the outpatient center. Participants arrived fasting overnight except for water and were instructed to lie supine for 1 hour to control for postural effects of the RAAS. Blood pressure was measured every 10 minutes with a Dinamap Pro Monitor (GE Medical). After 1 hour of supine posture, blood was drawn to measure PTH, calcium (total and ionized), plasma renin activity (PRA), and aldosterone. Participants were then

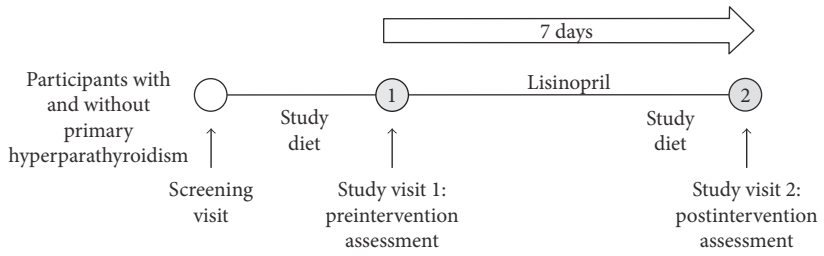


Figure 1: Study schema.

discharged home with a 7-day supply of lisinopril and asked to resume their typical diet, until resuming the outlined dietary protocol 5 days prior to study visit 2. The lisinopril dose was increased to a tolerated maximum as dictated by blood pressure (see Lisinopril Dosing Protocol below).

After 7 days of lisinopril therapy, participants returned, having fasted overnight, to the outpatient research center at 8 am to repeat all study procedures conducted at baseline (study visit 2) (Figure 1). Upon study completion, participants resumed all medications that they were taking prior to study initiation.

2.6. Lisinopril Dosing Protocol. The goal of the intervention was to treat each participant with the maximum dose of lisinopril that could be tolerated without development of hypotension. Lisinopril was dosed twice daily to ensure that the pharmacologic effect was sustained over 24 hours. All participants were required to monitor their blood pressure at home while on lisinopril with a home sphygmomanometer for safety and titration purposes. Home blood pressure readings were reported daily to study staff. Lisinopril dosing was adjusted based on home blood pressure readings, described in Supplemental Figure 1 available online at https://doi.org/10.1155/2017/4138783.

Study staff spoke with participants by phone or electronic mail every 1-2 days during the 7-day lisinopril intervention to ensure they were compliant with the medication dosing regimen and to evaluate blood pressure readings and other symptoms that may be suggestive of adverse effects.

2.7. Laboratory Measurements. PTH (Beckman Coulter, Fullerton Ca), 25OHD (DiaSorin Inc., Stillwater, MN), plasma renin activity (DiaSorin, Stillwater, MN) and serum aldosterone (Siemens, Los Angeles, CA), serum and urinary electrolytes (including total and ionized calcium and phosphate), and urinary aldosterone excretion were measured at each study visit.

2.8. Statistical Analysis. Mean (standard deviation (SD)) descriptive values are reported. Paired *t*-tests were used to compare the main outcome variable, PTH, before and after intervention with lisinopril. Paired *t*-tests were also used to compare other biochemical and hemodynamic parameters. The change in PTH was assessed in the full study population and then according to P-HPT status. Subgroup analyses explored whether the change in PTH by lisinopril differed among those with and without vitamin D deficiency and in those with hypercalcemic versus normocalcemic P-HPT. Data analysis was performed using SAS statistical software (SAS Institute, Cary, NC).

Table 1: Screening demographic and biochemical characteristics of study population.

	Participants without primary HPT $N = 15$	Participants with primary HPT $N = 12$
Age, years	39.6 (12.7)	51.4 (15.6)
Female, number (%)	6 (40)	5 (42)
White, number (%)	8 (53)	9 (75)
SBP, mmHg	120.0 (18.0)	121.4 (12.6)
DBP, mmHg	78.1 (12.0)	76.7 (5.2)
Number with hypertension	1	2
Number on antihypertensive therapy	0	3
PTH, pg/mL	21.8 (5.7)	94.8 (29.0)
25(OH)D, ng/mL	23.0 (8.6)	28.8 (10.7)
Serum calcium	9.6 (0.3)	11.0 (0.4)
Serum creatinine, mg/dL	0.84 (0.10)	0.83 (0.13)
Serum potassium, mmol/L	4.5 (0.5)	4.5 (0.3)

Values are mean (SD) unless otherwise noted. HPT: hyperparathyroidism; SBP: systolic blood pressure; DBP: diastolic blood pressure; PTH: parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D.

3. Results

3.1. Study Participants. A total of 12 participants with P-HPT and 15 without P-HPT successfully completed the study protocol (Supplemental Figure 2). Participants with P-HPT were older and had higher calcium and PTH levels, though no differences in vitamin D or creatinine levels were observed (Table 1).

3.2. Changes in Blood Pressure and RAAS Activity with Lisinopril Intervention. Participants with P-HPT had lisinopril titrated to a maximum daily dose of 16.9 (12.8) mg (range 2.5–30 mg), and participants without P-HPT had

Table 2: Blood pressure and RAAS activity before and after lisinopril intervention.

	Preintervention	Postintervention	Delta	*P* value
With P-HPT, N = 12				
SBP, mmHg	123.7 (15.7)	117.3 (14.7)	−6.4	0.006
DBP, mmHg	74.5 (8.7)	70.1 (7.7)	−4.4	0.003
MAP, mmHg	92.7 (12.4)	86.8 (11.1)	−5.9	0.002
Supine serum aldosterone, ng/dL	4.6 (3.0)	4.3 (3.2)	−0.3	0.50
PRA, ng/mL·hr	0.5 (0.4)	1.9 (2.7)	+1.5	0.06
Aldosterone-to-renin ratio	26.3 (39.0)	26.2 (40.5)	−0.15	0.98
24 h urinary aldosterone excretion rate, ng/dL·μg/TV	11.7 (18.3)	7.8 (8.9)	−3.9	0.22
24 h urinary sodium excretion, mmol/day	239.8 (59.1)	239.6 (94.1)	−0.2	0.99
Without P-HPT, N = 15				
SBP, mmHg	117.4 (15.1)	108.2 (12.2)	−9.2	<0.0001
DBP, mmHg	72.1 (11.2)	66.3 (9.3)	−5.8	0.0002
MAP, mmHg	87.7 (12.6)	79.9 (9.7)	−7.8	0.003
Supine serum aldosterone, ng/dL	3.4 (1.6)	4.7 (5.0)	+1.3	0.26
PRA, ng/mL·hr	0.5 (0.4)	4.4 (6.1)	+3.9	0.02
Aldosterone-to-renin ratio	13.6 (11.7)	4.2 (5.5)	−9.4	0.0002
24 h urinary aldosterone excretion rate, ng/dL·μg/TV	5.7 (3.5)	6.1 (9.1)	+0.4	0.86
24 h urinary sodium excretion, mmol/day	231.9 (82.2)	203.4 (74.9)	−28.5	0.21

Values are mean (SD) unless otherwise noted. *P* values are paired *t*-tests. RAAS: renin-angiotensin-aldosterone system; HPT: hyperparathyroidism; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PRA: plasma renin activity.

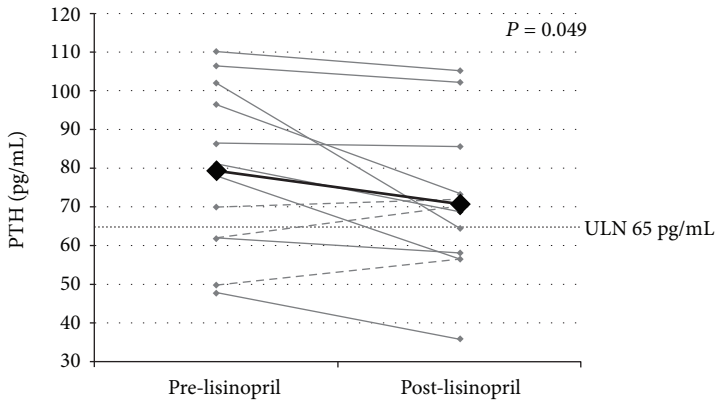


Figure 2: Change in PTH levels with lisinopril in primary HPT participants. Grey lines: participants with lower PTH post-lisinopril. Dashed grey lines: participants with higher PTH post-lisinopril. Black line: mean PTH. PTH, parathyroid hormone; HPT, hyperparathyroidism; ULN, upper limit of normal.

lisinopril titrated to a maximum dose of 11.3 (7.3) mg (range 5–40 mg), $P = 0.17$. Lisinopril significantly decreased blood pressure in both groups (Table 2). Despite high dietary sodium intake that suppressed PRA, one week of lisinopril therapy resulted in substantial increases in PRA (Table 2). Serum and urinary aldosterone levels were expectantly suppressed on the high dietary sodium intake and did not change with 1 week of maximally tolerated lisinopril.

3.3. The Impact of Lisinopril on PTH and Calcium Parameters. Among participants with P-HPT, there was a modest (9.5%) decrease in PTH after 1 week of lisinopril therapy (79.5 (21.6) pg/mL to 70.9 (19.6) pg/mL, $\Delta = -8.6$ pg/mL, $P = 0.049$, Figure 2). Of the 12 participants with P-HPT, 9/12 demonstrated a decrease in PTH following lisinopril, whereas 3/12 had an increase in PTH. Most decrements in PTH were modest (<10% of baseline) (Figure 2). Serum calcium, ionized calcium, and 24-hour urinary calcium and phosphate excretion did not significantly change with lisinopril therapy (Table 3). Among participants without P-HPT, there were no significant changes in PTH, serum calcium, ionized calcium, or 24-hour urinary calcium after lisinopril therapy (Table 3).

3.4. Subgroup Analyses. We assessed the influence of lisinopril on PTH in "normocalcemic" P-HPT versus

Table 3: Markers of PTH and calcium metabolism before and after lisinopril intervention; primary HPT, without HPT, and total cohort.

	Preintervention	Postintervention	Delta	*P* value
Primary HPT, N = 12				
PTH, pg/mL	79.5 (21.6)	70.9 (19.6)	−8.6	0.049
Serum total calcium, mg/dL	10.6 (0.5)	10.5 (0.4)	−0.05	0.48
Ionized calcium, mmol/L	1.38 (0.06)	1.37 (0.04)	−0.009	0.51
24-hour urinary calcium excretion, mg/day	310.6 (80.8)	324.8 (100.1)	+14.2	0.51
24-hour urine phosphate excretion, mg/day	998.4 (338.0)	975.7 (454.5)	−22.7	0.84
Without primary HPT, N = 15				
PTH, pg/mL	33.3 (25.4)	32.5 (16.7)	−0.78	0.80
Serum total calcium, mg/dL	9.4 (0.4)	9.5 (0.3)	+0.03	0.80
Ionized calcium, mmol/L	1.21 (0.07)	1.21 (0.07)	−0	1.0
24-hour urinary calcium excretion, mg/day	171.0 (90.6)	153.2 (70.4)	−17.8	0.34
24-hour urine phosphate excretion, mg/day	681.8 (406.9)	597.9 (241.6)	−165.4	0.26
Total cohort, N = 27				
PTH, pg/mL	53.8 (33.0)	49.6 (26.3)	−4.2	0.10
Serum total calcium, mg/dL	9.9 (0.7)	9.9 (0.6)	−0.004	0.96
Ionized calcium, mmol/L	1.29 (0.10)	1.28 (0.10)	−0.007	0.60
24-hour urinary calcium excretion, mg/day	233.0 (110.4)	229.4 (120.2)	−3.60	0.80
24-hour urine phosphate excretion, mg/day	822.5 (404.0)	765.8 (394.2)	−56.7	0.37

Values are mean (SD) unless otherwise noted. *P* values are paired *t*-tests. HPT: hyperparathyroidism; PTH: parathyroid hormone.

"hypercalcemic" P-HPT and observed no differences (Supplemental Table 1). Similarly, there was no apparent difference in PTH changes with lisinopril among participants with low versus high 25-hydroxyvitamin D levels (Supplemental Table 2), although sample sizes were small.

4. Discussion

In this single-arm pilot study examining the effect of ACE inhibition on PTH levels in normal and primary hyperparathyroidism participants, we found that one week of lisinopril therapy titrated to maximally tolerated blood pressure lowering resulted in a modest and marginally statistically significant lowering of PTH levels among participants with P-HPT without any detectable change in calcium. Although lisinopril therapy similarly lowered blood pressure and raised renin activity in participants without P-HPT, there was no change in PTH or calcium levels detected. Given the many prior studies suggesting a RAAS-PTH interaction that may potentially be modifiable and clinically meaningful [11, 15, 16, 18, 25], our current findings, in addition to another recently reported study [21], suggest that short-term therapy (1–8 weeks) with RAAS inhibitors (ACE inhibitors and MR antagonists) are unlikely to induce a robust and clinically meaningful reduction in PTH in patients with P-HPT. Although our study did not assess whether a small and sustained lowering of PTH by ACE inhibitors over many years could impart benefit, this is a worthy consideration given the accruing association between PTH and cardiovascular and skeletal outcomes.

Numerous observational studies have reported an association between elevated PTH levels and cardiovascular disease [7, 10, 26, 27], which may be due to an interaction between PTH and calcium regulation and the RAAS [12, 13]. Studies investigating effects of parathyroidectomy in P-HPT have repeatedly demonstrated improvements in cardiovascular function [28–31] and decreases in RAAS activity [32, 33]. Our prior results among individuals *without* P-HPT suggested that the hypothesized interaction between PTH and the RAAS may be modifiable; the *chronic* use of ACE inhibitors and angiotensin receptor blockers is associated with lower PTH levels in a large cross-sectional study [18], and the administration of a single dose of captopril 25 mg has been shown to lower PTH levels within hours [19]. Thus, there was considerable enthusiasm to investigate whether ACE inhibition to lower RAAS activity could induce clinically meaningful PTH reductions in P-HPT.

The results of our current study are best interpreted in the context of the aforementioned prior observational data and also the recently published EPATH trial [21], a relatively large, randomized, and placebo-controlled trial that evaluated the effect of eplerenone on PTH levels in P-HPT participants. In EPATH, 110 P-HPT participants were randomized to eplerenone (up to 25–50 mg/d) versus placebo for 8 weeks. Though eplerenone induced significant reductions in blood pressure, no significant change in PTH or calcium was detected. This study has several advantages over our study, in that it had a larger sample size, longer duration of treatment, and use of placebo-control. However, a key difference was that EPATH investigated MR antagonism and not ACE inhibition as in the current study.

As previously noted, both normal and adenomatous parathyroid cells express AT1R in addition to MR [19], and prior human studies have suggested that increases in either angiotensin II and/or aldosterone may increase PTH secretion [19]. Our current study focused on the influence

of ACE inhibition, which primarily lowers angiotensin II generation, and consequently aldosterone secretion. Therefore, ACE inhibitors may decrease PTH levels via decreased stimulation of *both* AT1R and MR, as opposed to MR antagonism alone, which may paradoxically increase angiotensin II generation. However, one potential limitation of lowering aldosterone secretion with ACE inhibitors is the phenomenon of aldosterone "escape," or normalization over time [34, 35], which may or may not be a limitation of MR antagonism.

In this regard, the current study design went to great lengths to ensure that confounders of the RAAS were controlled, including dietary control of sodium and potassium, eliminating confounding medications that modulate the RAAS, and control of body posture. Lisinopril was consumed twice daily to best ensure a continuous duration of action. Further, a dose titration protocol was utilized to obtain the highest tolerated biological effect. In this context, we successfully induced blood pressure reductions and renin elevations, providing confidence that the effect of ACE inhibition was evident, yet it only induced a modest and marginally significant reduction in PTH among those with P-HPT. To some degree, these findings support our prior observations that chronic ACE inhibitor use is associated with lower PTH [18], and that a single dose of ACE inhibitor acutely lowers PTH [19]. On the other hand, the current study does not address whether a longer duration of ACE inhibitor therapy in P-HPT could have induced a sustained and durable reduction in PTH, and perhaps even in calcium. Therefore, the findings of the current study suggest that at best, ACE inhibitor use is likely to have a modest effect with unclear long-term clinical value. In this regard, it may be possible that dual blockade with both an ACE inhibitor and an MR antagonist may lead to more robust and sustained effects of PTH lowering, as both AT1R and MR would be inhibited and the potential for reversal of efficacy due to aldosterone escape with ACE inhibition would be lessened with the concurrent use of MR antagonism. However, larger clinical trials with close attention to the safety of dual blockade are needed to evaluate this further.

There are limitations to this study that are worth discussing. The most notable are the small sample size, the lack of a placebo control, and the short duration, all of which contribute to the variability of our data. Since we previously observed that ACE inhibitor mediated lowering of aldosterone and PTH [19], the current study was a natural extension of the prior. However, the current results raise the possibility that while acute ACE inhibition lowered PTH by 9.7% [19], continued ACE inhibition over the course of one week resulted in at least some re-equilibration of RAAS and PTH changes. Though we did observe a marginally significant 9.5% PTH reduction in the P-HPT group, our study was not designed to evaluate whether this PTH lowering effect would be durable over a longer period of time, or if it would result in meaningful reductions in serum calcium and cardiovascular and skeletal risk. We did not directly measure angiotensin II but instead relied on a rise in PRA to indicate ACE inhibition. And lastly, we observed no differences in aldosterone levels; however, this should not be surprising since our dietary sodium protocol suppressed levels to a near nadir. Thus, it may also be considered that while the dietary sodium standardization helped to minimize confounding of intra- and interindividual RAAS measurements, it may have also dampened the efficacy of ACE inhibition on PTH regulation.

In conclusion, this pilot intervention study demonstrated that maximally-tolerated and short-term ACE inhibitor therapy induced only modest and marginally significant decreases in PTH among participants with P-HPT, but not in participants without P-HPT. However, we caveat this conclusion by noting the limitations of our study, including the small sample size, short study duration, and lack of placebo control. Whether ACE inhibition for patients with P-HPT could induce a sustained and clinically significant reduction in PTH would require a study design with longer duration of therapy and a larger sample size.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to acknowledge the funding sources. Sarah Zaheer was supported by F32 HL132477. Anand Vaidya was supported by the National Institutes of Diabetes and Digestive and Kidney Disease of the National Institutes of Health under Award no. R01 DK107407, by the National Heart, Lung, and Blood Institute under Award no. K23 HL111771, and by Grant 2015085 from the Doris Duke Charitable Foundation. Gail K. Adler was supported by the National Heart, Lung, and Blood Institute under Award no. K24 HL103845.

References

[1] C. M. Ferrario and W. B. Strawn, "Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease," *The American Journal of Cardiology*, vol. 98, pp. 121–128, 2006.

[2] J. H. Laragh and J. E. Sealey, "The plasma renin test reveals the contribution of body sodium-volume content (V) and renin-angiotensin (R) vasoconstriction to long-term blood pressure," *American Journal of Hypertension*, vol. 24, pp. 1164–1180, 2011.

[3] B. Pitt, F. Zannad, W. J. Remme et al., "The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized aldactone evaluation study investigators," *The New England Journal of Medicine*, vol. 341, pp. 709–717, 1999.

[4] S. Yusuf, P. Sleight, J. Pogue, J. Bosch, R. Davies, and G. Dagenais, "Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The heart outcomes prevention evaluation study investigators," *The New England Journal of Medicine*, vol. 342, pp. 145–153, 2000.

[5] J. L. Probstfield and K. D. O'Brien, "Progression of cardiovascular damage: the role of renin-angiotensin system blockade," *The American Journal of Cardiology*, vol. 105, pp. 10A–20A, 2010.

[6] A. J. van Ballegooijen, I. Reinders, M. Visser et al., "Serum parathyroid hormone in relation to all-cause and cardiovascular mortality: the Hoorn study," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, pp. E638–E645, 2013.

[7] A. J. van Ballegooijen, B. Kestenbaum, M. C. Sachs et al., "Association of 25-hydroxyvitamin D and parathyroid hormone with incident hypertension: MESA (multi-ethnic study of atherosclerosis)," *Journal of the American College of Cardiology*, vol. 63, pp. 1214–1222, 2014.

[8] A. J. van Ballegooijen, M. Visser, M. F. Cotch et al., "Serum vitamin D and parathyroid hormone in relation to cardiac structure and function: the ICELAND-MI substudy of AGES-Reykjavik," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, pp. 2544–2552, 2013.

[9] S. Pilz, A. Tomaschitz, C. Drechsler et al., "Parathyroid hormone level is associated with mortality and cardiovascular events in patients undergoing coronary angiography," *European Heart Journal*, vol. 31, pp. 1591–1598, 2010.

[10] L. Morfis, P. Smerdely, and L. G. Howes, "Relationship between serum parathyroid hormone levels in the elderly and 24 h ambulatory blood pressures," *Journal of Hypertension*, vol. 15, pp. 1271–1276, 1997.

[11] A. Tomaschitz, S. Pilz, J. Rus-Machan et al., "Interrelated aldosterone and parathyroid hormone mutually modify cardiovascular mortality risk," *International Journal of Cardiology*, vol. 184, pp. 710–716, 2015.

[12] A. Tomaschitz, E. Ritz, B. Pieske et al., "Aldosterone and parathyroid hormone interactions as mediators of metabolic and cardiovascular disease," *Metabolism: Clinical and Experimental*, vol. 63, pp. 20–31, 2014.

[13] G. P. Rossi, F. Ragazzo, T. M. Seccia et al., "Hyperparathyroidism can be useful in the identification of primary aldosteronism due to aldosterone-producing adenoma," *Hypertension*, vol. 60, pp. 431–436, 2012.

[14] G. P. Rossi, "Hyperparathyroidism, arterial hypertension and aortic stiffness: a possible bidirectional link between the adrenal cortex and the parathyroid glands that causes vascular damage?" *Hypertension Research : Official Journal of the Japanese Society of Hypertension*, vol. 34, pp. 286–288, 2011.

[15] S. Pilz, K. Kienreich, C. Drechsler et al., "Hyperparathyroidism in patients with primary aldosteronism: cross-sectional and interventional data from the GECOH study," *The Journal of Clinical Endocrinology and Metabolism*, vol. 97, pp. E75–E79, 2012.

[16] C. Maniero, A. Fassina, V. Guzzardo et al., "Primary hyperparathyroidism with concurrent primary aldosteronism," *Hypertension*, vol. 58, pp. 341–346, 2011.

[17] C. Maniero, A. Fassina, T. M. Seccia et al., "Mild hyperparathyroidism: a novel surgically correctable feature of primary aldosteronism," *Journal of Hypertension*, vol. 30, pp. 390–395, 2012.

[18] J. Brown, I. H. de Boer, C. Robinson-Cohen et al., "Aldosterone, parathyroid hormone, and the use of renin-angiotensin-aldosterone system inhibitors: the multi-ethnic study of atherosclerosis," *The Journal of Clinical Endocrinology and Metabolism*, vol. 100, pp. 490–499, 2015.

[19] J. M. Brown, J. S. Williams, J. M. Luther et al., "Human interventions to characterize novel relationships between the renin-angiotensin-aldosterone system and parathyroid hormone," *Hypertension*, vol. 63, pp. 273–280, 2014.

[20] E. Rossi, C. Sani, F. Perazzoli, M. C. Casoli, A. Negro, and C. Dotti, "Alterations of calcium metabolism and of parathyroid function in primary aldosteronism, and their reversal by spironolactone or by surgical removal of aldosterone-producing adenomas," *American Journal of Hypertension*, vol. 8, pp. 884–893, 1995.

[21] A. Tomaschitz, N. Verheyen, A. Meinitzer et al., "Effect of eplerenone on parathyroid hormone levels in patients with primary hyperparathyroidism: results from the EPATH randomized, placebo-controlled trial," *Journal of Hypertension*, vol. 34, pp. 1347–1356, 2016.

[22] C. Marcocci and F. Cetani, "Clinical practice. Primary hyperparathyroidism," *The New England Journal of Medicine*, vol. 365, pp. 2389–2397, 2011.

[23] K. Stamatelopoulos, F. Athanasouli, T. Pappa et al., "Hemodynamic markers and subclinical atherosclerosis in postmenopausal women with primary hyperparathyroidism," *The Journal of Clinical Endocrinology and Metabolism*, vol. 99, pp. 2704–2711, 2014.

[24] P. Andersson, E. Rydberg, and R. Willenheimer, "Primary hyperparathyroidism and heart disease—a review," *European Heart Journal*, vol. 25, pp. 1776–1787, 2004.

[25] L. Brunaud, A. Germain, R. Zarnegar et al., "Serum aldosterone is correlated positively to parathyroid hormone (PTH) levels in patients with primary hyperparathyroidism," *Surgery*, vol. 146, pp. 1035–1041, 2009.

[26] F. D. Rosenthal and S. Roy, "Hypertension and hyperparathyroidism," *British Medical Journal*, vol. 4, pp. 396–397, 1972.

[27] A. J. van Ballegooijen, I. Reinders, M. Visser, and I. A. Brouwer, "Parathyroid hormone and cardiovascular disease events: a systematic review and meta-analysis of prospective studies," *American Heart Journal*, vol. 165, pp. 655.e5–664.e5, 2013.

[28] G. Agarwal, G. Nanda, A. Kapoor et al., "Cardiovascular dysfunction in symptomatic primary hyperparathyroidism and its reversal after curative parathyroidectomy: results of a prospective case control study," *Surgery*, vol. 154, pp. 1394–1403, 2013.

[29] C. A. Feldstein, M. Akopian, D. Pietrobelli, A. Olivieri, and D. Garrido, "Long-term effects of parathyroidectomy on hypertension prevalence and circadian blood pressure profile in primary hyperparathyroidism," *Clinical and Experimental Hypertension*, vol. 32, pp. 154–158, 2010.

[30] A. Heyliger, V. Tangpricha, C. Weber, and J. Sharma, "Parathyroidectomy decreases systolic and diastolic blood pressure in hypertensive patients with primary hyperparathyroidism," *Surgery*, vol. 146, pp. 1042–1047, 2009.

[31] J. J. Sancho, J. Rouco, R. Riera-Vidal, and A. Sitges-Serra, "Long-term effects of parathyroidectomy for primary hyperparathyroidism on arterial hypertension," *World Journal of Surgery*, vol. 16, pp. 732–735, 1992.

[32] L. Kovacs, M. I. Goth, I. Szabolcs, O. Dohan, A. Ferencz, and G. Szilagyi, "The effect of surgical treatment on secondary hyperaldosteronism and relative hyperinsulinemia in primary hyperparathyroidism," *European Journal of Endocrinology*, vol. 138, pp. 543–547, 1998.

[33] R. Pacifici, H. M. Perry 3rd, W. Shieber, E. Biglieri, D. M. Droke, and L. V. Avioli, "Adrenal responses to subtotal parathyroidectomy for primary hyperparathyroidism," *Calcified Tissue International*, vol. 41, pp. 119–123, 1987.

[34] J. Staessen, P. Lijnen, R. Fagard, L. J. Verschueren, and A. Amery, "Rise in plasma concentration of aldosterone during long-term angiotensin II suppression," *The Journal of Endocrinology*, vol. 91, pp. 457–465, 1981.

[35] C. A. Farquharson and A. D. Struthers, "Gradual reactivation over time of vascular tissue angiotensin I to angiotensin II conversion during chronic lisinopril therapy in chronic heart failure," *Journal of the American College of Cardiology*, vol. 39, pp. 767–775, 2002.