Journal of Steroid Biochemistry & Molecular Biology 175 (2018) 157–163

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Original Article

Effects of Vitamin D3 on asymmetric- and symmetric dimethylarginine in arterial hypertension

M.R. Grübler[a,b,*], M. Gaksch[a], K. Kienreich[a], N.D. Verheyen[c], J. Schmid[c], C. Müllner[a], G. Richtig[d], H. Scharnagl[e], C. Trummer[a], V. Schwetz[a], A. Meinitzer[e], B. Pieske[f], W. März[e,g], A. Tomaschitz[c,h], S. Pilz[a,i]

[a] Department of Internal Medicine, Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria
[b] Swiss Cardiovascular Center Bern, Department of Cardiology, Bern University Hospital, University of Bern, 3007 Bern, Switzerland
[c] Department of Cardiology, Medical University of Graz, Graz, Austria
[d] Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria
[e] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria
[f] Department of Cardiology, Campus Virchow, Charité University, Berlin, Germany
[g] Synlab Academy, Synlab Services GmbH, Mannheim, Germany
[h] Bad Gleichenberg Clinic, Bad Gleichenberg, Austria
[i] Department of Epidemiology and Biostatistics, EMGO Institute for Health and Care Research, VU University Medical Centre, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form 14 October 2016
Accepted 23 December 2016
Available online 24 December 2016

Keywords:
Vitamin D deficiency
Arterial hypertension
Endocrinology
Cardiovascular disease
Endothelial dysfunction
Vitamin D

ABSTRACT

Background and aims: Accumulating evidence has proposed a correlation between vitamin D (25(OH)D) insufficiency and cardiovascular (CV) disease. Vitamin D associated effects on endothelial function have been suggested to be a possible culprit. The present study investigated the association of vitamin D3 treatment on markers of endothelial dysfunction in patients with arterial hypertension.
Methods and results: The Styrian Vitamin D Hypertension Trial is a double-blind, placebo-controlled, single-centre study conducted at the Medical University of Graz, Austria. A total of 200 study participants with arterial hypertension and 25(OH)D levels below 30 ng/mL were enrolled. The study participants were randomized to receive 2800 IU of vitamin D3 per day as oily drops (n = 100) or placebo (n = 100) for a duration of eight weeks. The present study uses an analysis of covariance (ANCOVA) to investigate the effect of vitamin D3 treatment on symmetric (SDMA) and asymmetric dimethylarginine (ADMA). A total of 187 participants (mean [SD] age 60.0 [11.3] years; 47% women; 25(OH)D 21.2 [5.6] ng/mL; mean systolic blood pressure of 131.4 [8.9] mmHg on a median of 2 antihypertensive drugs) completed the trial. Mean treatment effect was −0.004 (95%CI [−0.03 to 0.04]; P = 0.819) on ADMA and 0.001 (95%CI [−0.05 to 0.05]; P = 0.850) on SDMA. In the subgroup analysis patients with a 25(OH)D concentration <20 ng/mL had a significant increase in their log L-arginine/ADMA ratio (mean treatment effect 18.4 95%CI [1.84–34.9]μmol/L/μmol/L; P = 0.030). ClinicalTrials.gov Identifier: NCT02136771 EudraCT number: 2009-018125-70
Conclusions: Vitamin D3 supplementation in hypertensive patients with low 25-hydroxyvitamin D has no significant effect on ADMA and SDMA.

1. Introduction

Vitamin D is a steroid hormone classically known to be responsible for bone mineralization [1,2]. It's deficiency is known to cause a childhood diseases called rickets, which is characterized by skeletal deformities [2]. Definition of normal vitamin D levels is based on the concentration of 25-hydroxyvitamin D (25[OH]D) – in this article referred to as vitamin D – which is the pro-hormone of the active metabolite, 1,25-dihydroxyvitamin D that subsequently activates the vitamin D receptor (VDR) [1,3]. Intriguingly, the VDR has also been found in extra-skeletal tissues, like myocardium and vasculature [4,5]. So the question raised whether there are non-bone related health effects of vitamin D insufficiency. Beside reports of children with heart failure and rickets which both improved with vitamin D supplementation [6], vitamin D

* Corresponding author at: Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria/Swiss Cardiovascular Center Bern, Department of Cardiology, Bern University Hospital, 3007 Bern, Switzerland.
E-mail address: martin.gruebler@gmx.net (M.R. Grübler).

http://dx.doi.org/10.1016/j.jsbmb.2016.12.014
0960-0760/

insufficiency was broadly described to be a risk factor for cardiovascular (CV) diseases [2,4,7–10]. Low 25(OH)D concentrations have been even proposed to actually cause or mediate CV disease, but might also be only an epiphenomenon of poor health and low physical activity [2,9–12]. Nevertheless, in animal models the vitamin D receptor (VDR) activation led to improved endothelial function [2,7–10]. As endothelial dysfunction is a major component of CV disease[13] an interaction with vitamin D could explain – at least partially – the increased CV mortality seen in 25(OH)D insufficient patients [7,8,13–17]. Asymmetrical dimethylarginine (ADMA) is a marker of endothelial derangement and has been validated previously in cell based and clinical models. ADMA is a competitive inhibitor of NO-synthase which catalyses the production of nitric oxide, one of the most potent endogenous vasodilators. [18–22] Previous studies on vitamin D and ADMA reported cross sectional associations between them [23–25]. *Ngo* et al. observed an inverse association between 25(OH)D concentration and ADMA [23]. This was further supported by similar findings in patients with hypogonadism [24], phenylketonuria [25], Polycystic ovary syndrome (PCOS) [26], and in individuals on long-term haemodialysis (HD)[27]. The effect also seems to be associated with aging [28]. In line with this, *Syal* and colleagues observed that patients with lower 25(OH)D levels had significantly reduced flow-mediated brachial artery dilation, what strengthens hypothesis of a vitamin D associated effect on endothelial function [29]. Some authors further proposed the L-arginine to ADMA ratio as a more sensitive marker for endothelial function [18–20,30–32]. Similar results are reported in regard to symmetrical dimethylarginine (SDMA), a sensitive marker for renal function [33], which may also have indirect effects on NO synthesis [34]. Interventional data in humans on the effects of vitamin D supplementation on ADMA and SDMA are however, missing. We report results from our randomized double blind clinical trial supplementing vitamin D or placebo in hypertensive patients with 25(OH)D insufficiency to address the question whether oral vitamin D treatment for 8 weeks has an effect on ADMA, L-arginine to ADMA ratio and SDMA serum concentrations.

2. Methods

2.1. Study design

The present study is a post-hoc analysis adhering to a stringent protocol and investigates the treatment effect of oral vitamin D3 on ADMA and SDMA in patients with arterial hypertension and vitamin D insufficiency. The methods of the Styrian Vitamin D Hypertension Trial have been already reported [35–38]. Briefly: It is a double blind, placebo-controlled study comparing the effect of 2800 IU vitamin D3 versus placebo on clinical and laboratory biomarkers in patients with arterial hypertension. The Medical University of Graz, Austria funded the study. The publication of this trial complies to the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement [39]. The RCT was registered at the www.clinicaltrialsregister.eu (EudraCT Number 2009-018125-70) as well as at clinicaltrials.gov (ClinicalTrials.gov Identifier NCT02136771). The difference between previous reports and this study is a) the outcomes, i.e. ADMA and SDMA, have not been reported previously b) baseline characteristics (Table 1) restricted to those participants who had measurements of ADMA and SDMA available and are thus unique c) a discussion of vitamin D effects on endothelial function were not part of previous publications.

2.2. Participants

All participants included in the present study were over 18 years old. Main inclusion criteria was a diagnosis of arterial hypertension and a 25(OH)D level ≤30 ng/mL (74.9 nmol/L multiply by 2.496 to convert ng/mL into nmol/L). Arterial hypertension was defined as an office BP of systolic ≥140 mmHg or diastolic ≥90 mmHg, a mean 24-h ABPM of systolic ≥125 mm Hg or diastolic ≥80 mmHg, a home BP of systolic ≥130 mm Hg or diastolic ≥85 mmHg, or ongoing antihypertensive treatment [40]. The exclusion criteria were published previously [35]. The study was approved by the ethics committee at the Medical University of Graz, Graz, Austria. All study participants provided written informed consent. The study complies with the Declaration of

Table 1
Baseline Characteristics of the Placebo and the Vitamin D group before randomization.

	Placebo n = 99 Mean ± SD Median (IQR)	Vitamin D n = 99 Mean ± SD Median (IQR)
Age (years)	59.5 ± 11.4	60.7 ± 10.8
Females (yes)	48%	46%
Mean 24 h systolic blood pressure	131.8 ± 9.7	132.0 ± 8.4
Asymmetric dimethylaginine (μmol/L)	0.73 ± 0.09	0.70 ± 0.15
Symmetric dimethylarginine (μmol/L)	0.71 ± 0.10	0.69 ± 0.16
L-arginine/ADMA ratio μmol/L/μmol/L	183.3 ± 58.7	183.1 ± 49.7
L-arginine (μmol/L)	131.0 ± 35.5	128.6 ± 31.6
Parathyroid hormone (pg/mL)	51.5 (39.5–65.8)	48.9 (40.0–61.7)
25-hydroxyvitamin D (ng/mL)	20.4 ± 5.7	21.8 ± 5.5
25-hydroxyvitamin D (nmol/L)	50.9 ± 14.2	54.4 ± 13.7
Serum total calcium (mmol/L)	2.37 ± 0.11	2.37 ± 0.10
Estimated glomerular filtration rate MDRD6 (mL/min/1.73m^2)	77.0 ± 17.9	79.9 ± 17.9
Number of different blood pressure lowering drugs	2 (1–3)	2 (1–3)
ACE inhibitor (yes)	38%	25%
AT1 receptor blocker (yes)	31%	33%
Calcium channel blocker (yes)	25%	27%
Thiazide diuretics (yes)	45%	39%
Loop diuretics (yes)	5%	5%
Beta blockers (yes)	49%	44%
Minteralocorticoid receptor blockers (yes)	4%	2%
Smoking (yes)	16.1%	7.4%

Helsinki. The study took place at the outpatient clinic at the Division of Endocrinology and Diabetology from 2011 to 2014 [35].

2.3. Intervention

Study medication was randomly filled into numbered bottles according to a computer generated randomization list. The randomization was conducted using web-based tool (http://www.randomizer.at/), with good clinical practice compliance. All eligible study patients were randomly assigned in a 1:1 ratio to receive 2800 IU vitamin D3 as oily drops per day (Oleovit D3, producer: Fresenius Kabi Austria, Austria) or a matching placebo. We performed a permuted block randomization (size of ten) and stratification according to gender. All investigators who enrolled patients were blinded during enrolment [35].

2.4. Outcome measure

The primary outcome (main endpoint) was 24-h systolic blood pressure as already published [35]. Sample size calculations were based assuming an effect size of −6 mmHg (E) systolic blood pressure with a SD of 12 mmHg (S). Based on that we calculated a standardized effect size (E/S) of 0.5. For a 2-sided α of 0.05 and a power (1−β) of 90%, we would have need at least a group size of 86 study participants [35]. The rationale for the present investigation was based on previous studies on vitamin D and ADMA reporting on inverse cross sectional associations for a wide range of 25(OH)D concentrations [23–27].

2.5. Measurements

Physical examinations, patient interviews and blood samplings were performed at study visits during workdays between 7 and 11 AM. After that, the patients left the hospital for 24-h BP measurements and 24-h urine collections. They were scheduled to return on the next (second) day, when the eligible study participants were randomized and started intake of the study medication. We measured serum symmetrical dimethylarginine (SDMA) in frozen serum (−80 °C) with a modified reversed-phase HPLC method. Within-day coefficients of variation (CVs) for SDMA were 4.6% (0.60 μmol/L) and 1.9% (1.0 μmol/L), and between-day CVs were 9.8% (0.60 μmol/L) and 6.1% (1.0 μmol/L). Limit of detection was 0.05 μmol/L. Serum asymmetric dimethylarginine (ADMA) was as well measured in frozen serum (−80 °C) with the

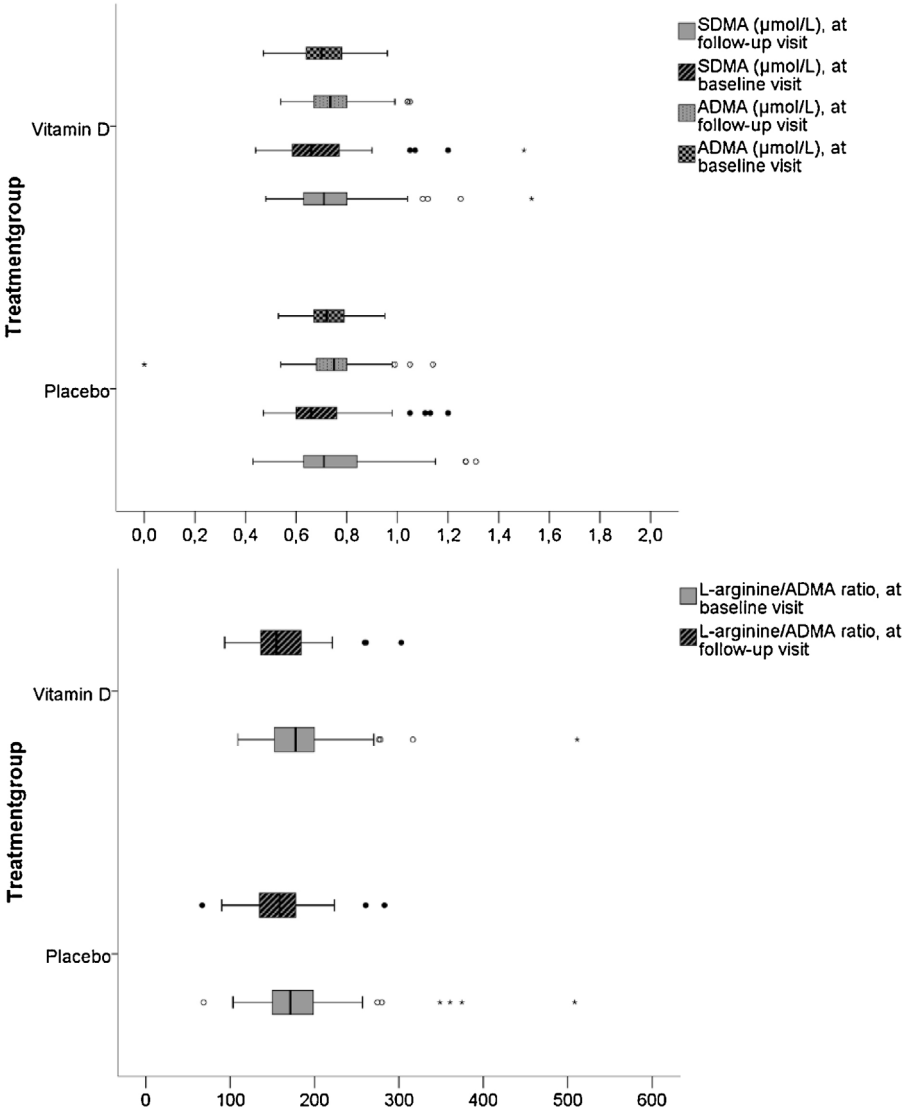

Fig 1. ANCOVA revealed no significant mean difference between placebo and control arm in log SDMA, ADMA or log L-arginine/ADMA ratio at the final visit (−0.004 95%CI [−0.03 to 0.04]ng/dL; P = 0.819 and 0.001 95%CI [−0.05 to 0.05] ng/dL; P = 0.850, respectively)..

reversed-phase HPLC method with slight modifications [41]. Within-day CVs for ADMA were 3.1% (0.62 μmol/L) and 1.0% (2.00 μmol/L), and between-day CVs were 9% (0.62 μmol/L) and 1.5% (2.00 μmol/L). Limit of detection was 0.05 μmol/L. Measurement of 25-hydroxyvitamin D [25(OH)D] was performed by means of a chemiluminescence assay (IDS-iSYS 25-hydroxyvitamin D S assay; Immunodiagnostic Systems Ltd., Boldon, UK) on an IDS-iSYS multidiscipline automated analyser with intra- and inter-assay CV of 6.2% and 11.6%, respectively [42]. Measurements of L-arginine have been conducted according to previously published methods [43,44]. Briefly, after precipitation of serum with perchloric acid following neutralization of the supernatant with sodium carbonate, the extracted amino acids were derivatized with o-phtalaldehyde and separated on a reversed phase column with gradient elution. Quantification were performed with ratios of fluorescence signals of the interesting amino acids to the internal standard norvaline in comparison to the appropriated calibration curves. Intra-assay and interassay CVs were all below 10%. More details on the laboratory methods used have been published previously [36,45–47].

2.6. Analysis

Continuous data following a normal distribution are reported as means with standard deviation and variables with a skewed distribution are shown as medians with interquartile range. Categorical data are presented as percentages. Where appropriate, skewed variables were log(e) transformed before use in parametric statistical analyses. Group comparisons at baseline were done by unpaired student's *t*-, Chi Square- or Man-Whitney *U* test. Analyses of outcome variables (ADMA, L-arginine to ADMA ratio, SDMA) were performed according to the intention-to-treat concept without data imputation. Analyses of Covariance (ANCOVA) with adjustments for baseline values were used to test for differences in the outcome parameters between the placebo and the treatment arm at the second visit. In sensitivity analysis we used multiple (data) imputation for missing values [48]. A P-value below 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 21.0 software (SPSS, Chicago, IL, USA) and Stata version 13 (StataCorp LP, College Station, Texas, USA).

3. Results

A total of 518 invited study participants gave written informed consent and were assessed for eligibility. The entire trial was performed between 2011 and August 2014. Baseline characteristics of all randomized study participants are shown in Table 1 (restricted to participants with baseline and follow-up values of ADMA, Arginin and SDMA). All parameters were available in at least 94% of the study participants. A total of 187 study participants (mean ± SD age: 60.0 ± 11.3 years; 47% females; baseline 25(OH)D: 21.2 ± 5.6 ng/mL; 52.9 ± 14.0nmol/L) completed the trial. There was a significant increase in 25(OH)D and a significant decrease in parathyroid hormone (PTH) levels with no effect on total calcium, as already reported [35]. ANCOVA revealed no significant treatment effect on ADMA −0.004 (95%CI [−0.03 to 0.04]; P = 0.819), log L-arginine/ADMA ratio 2.201 (95%CI [−7.89 to 12.30]; P = 0.668) or SDMA 0.001 (95%CI [−0.05 to 0.05]; P = 0.850). (Fig. 1 and Table 2) Including adjustments for active smoking and ACE inhibitor intake left the results materially unchanged. There was neither an effect seen on ADMA nor SDMA when including only participants with 25(OH)D levels ≤20.0 ng/mL/49.9 nmol/L (Fig. 2), ≤12.0 ng/mL/30.0 nmol/L and in treatment naïve patients (Table 2). In the subgroup analysis restricted to patients with 25(OH)D below 20 ng/mL, vitamin D 3 supplementation as compared to placebo significantly increased the L-arginine/ADMA ratio with a mean treatment effect of 18.4 (95%CI [1.3–35.5]; P = 0.030). No patient deceased during the study period and to the best of our knowledge there was no increased rate of adverse events (i.e. hypercalcemia or hospitalisations) in the active treatment arm of the trial.

4. Discussion

The present randomized controlled trial is the first to describe an effect of vitamin D3 on endothelial function in humans. In the subgroup analysis with patients below 20 ng/mL 25(OH)D there was a statistically significant increase in the L-arginine/ADMA ratio under vitamin D3 supplementation. As the effect is seen in a randomized controlled trial, the present study provides high quality clinical evidence for a possible link between vitamin D supplementation and CV events. Though we were unable to

Table 2
Baseline, Follow-Up and Changes From Baseline of ADMA, SDMA and L-arginine/ADMA ratio.

Characterisitics	Placebo		Vitamin D		mean change from baseline		Treatment Effect (95% CI)	P-Value
	Baseline	Follow-up	Baseline	Follow-up	Placebo	Vitamin D		
All patients randomized (Intention to treat) with follow-up n = 187								
ADMA (μmol/L)	0.73 ± 0.09	0.75 ± 0.13	0.71 ± 0.10	0.74 ± 0.11	0.02	0.03	−0.004 (−0.03 – 0.04)	0.819
L-arginine/ADMA ratio (μmol/L/μmol/L)	183.9 ± 58.8	157.3 ± 35.1	183.3 ± 49.9	161.1 ± 39.5	−26.63	−22.26	2.2 (−7.9 – 12.3)	0.668
SDMA (μmol/L)	0.70 ± 0.15	0.74 ± 0.18	0.69 ± 0.16	0.74 ± 0.17	0.04	0.06	0.001 (−0.05 – 0.05)	0.850
25(OH)D <20 ng/mL/ <49.9nmol/L n = 73								
ADMA (μmol/L)	0.72 ± 0.09	0.76 ± 0.17	0.69 ± 0.08	0.72 ± 0.09	0.04	0.02	−0.04 (−0.11 – 0.03)	0.256
L-arginine/ADMA ratio (μmol/L/μmol/L)	184.9 ± 57.7	146.6 ± 31.8	188.2 ± 68.6	165.2 ± 38.5	−38.32	−22.96	18.4 (1.3 – 35.5)	**.030**
SDMA (μmol/L)	0.66 ± 0.14	0.75 ± 0.20	0.66 ± 0.16	0.70 ± 0.14	0.09	0.05	−0.05 (−0.13 – 0.04)	0.347
25(OH)D <12 ng/mL/ < 30.0 nmol/L n = 15								
ADMA (μmol/L)	0.76 ± 0.10	0.76 ± 0.15	0.69 ± 0.04	0.68 ± 0.07	0.00	−0.01	−0.09 (−0.31 – 0.26)	0.210
L-arginine/ADMA ratio (μmol/L/μmol/L)	156.0 ± 28.8	153.5 ± 30.6	181.0 ± 70.5	179.6 ± 52.8	−2.48	−1.40	23.1 (−29.4 – 75.6)	0.353
SDMA (μmol/L)	0.70 ± 0.18	0.74 ± 0.28	0.65 ± 0.11	0.71 ± 0.19	0.04	0.07	−0.02 (−0.23 – 0.06)	0.997
Treatment naive patients* n = 38								
ADMA (μmol/L)	0.72 ± 0.07	0.72 ± 0.10	0.71 ± 0.10	0.71 ± 0.08	−0.02	−0.01	0.01 (−0.04 – 0.06)	0.797
L-arginine/ADMA ratio (μmol/L/μmol/L)	200.8 ± 67.8	160.9 ± 24.4	169.3 ± 20.8	160.8 ± 36.9	−39.90	−8.50	−3.9 (−20.8 – 13.0)	0.643
SDMA (μmol/L)	0.64 ± 0.11	0.72 ± 0.15	0.72 ± 0.15	0.74 ± 0.17	0.06	0.03	−0.02 (−0.08 – 0.15)	0.516

ADMA (μmol/L), asymetric dimethylarginine; SDMA (μmol/L), symetric dimethylarginin; 25(OH)D, 25-hydroxyvitamin D; 95% CI, 95% confidence intervall. A P-value ≤0.050 is considered stastically significant. Values represent participants with baseline and follow-up visit. Multiple data imputation for missing values is not included in this table.

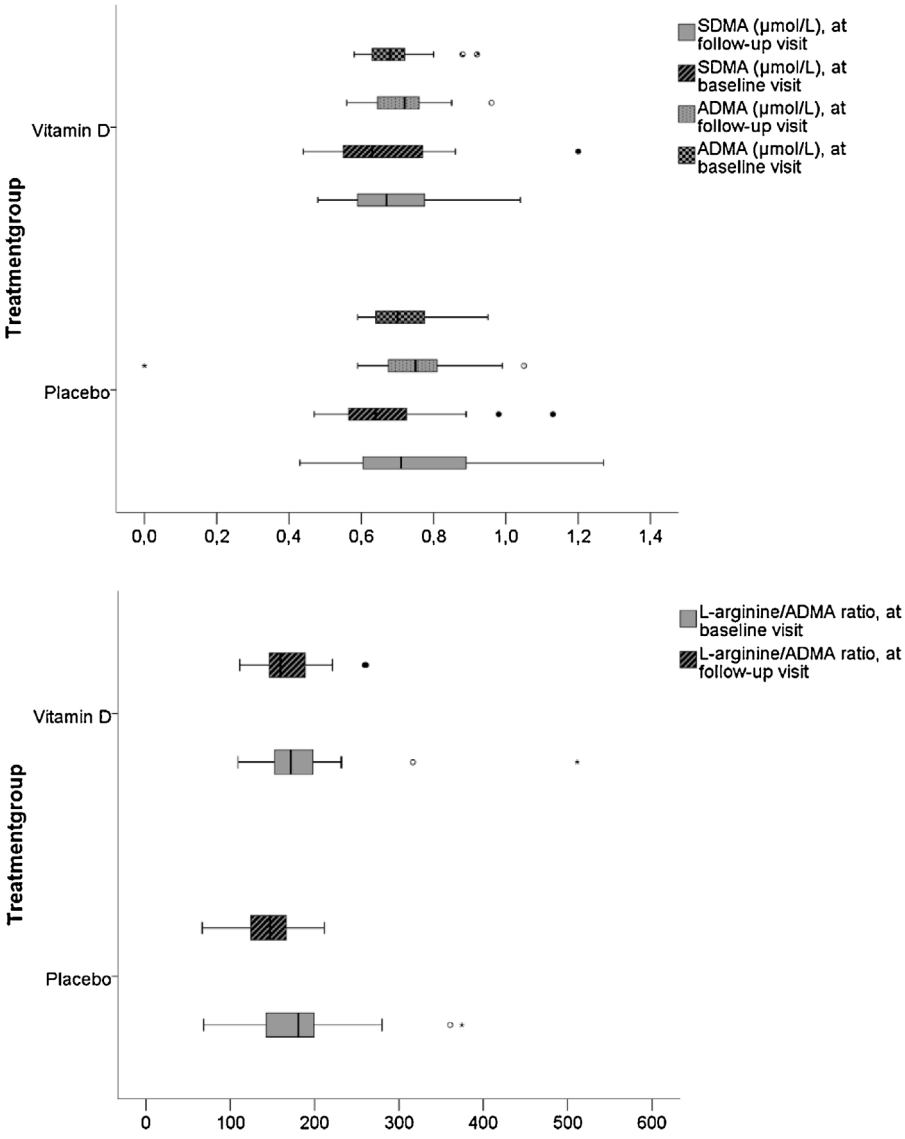

Fig. 2. In the subgroup of patients with a baseline 25(OH)D level below ≤20.0 ng/mL (n = 73) ANCOVA revealed no significant mean difference between placebo and control arm in log SDMA or ADMA at the final visit (−0.04 95%CI [−0.11 to 0.03]ng/dL; P = 0.256 and −0.05 95%CI [−0.13 to 0.04]ng/dL; P = 0.347, respectively). There was a statistically significant increase in log L-arginine/ADMA ratio (mean treatment effect 18.4 95%CI[1.84–34.9] µmol/L/µmol/L; P = 0.030).

observe a statistically significant reduction of ADMA or SDMA by oral vitamin D supplementation in patients with arterial hypertension it raises the question of appropriate cut-offs for the definition of vitamin D insufficiency. Though subgroup analysis can – in general – be only hypothesis generating[49], the present findings strengthen the assumption of a possible causal link between 25(OH)D and endothelial function. Therefore, this finding should be confirmed in future trials focussing on patients with vitamin D deficiency. The answer to this question is important because it would help us understand the (potential) mechanism mediating vitamin D deficiency and CV health. More so, from a clinical point of view trials are needed to define the role of treatment with vitamin D in cardiology and vascular medicine [50–52]. Nevertheless, the main findings of the present study are "negative", as we were unable to show an effect of vitamin D on ADMA, L-arginine/ADMA ratio or SDMA. Previous studies who described an association of vitamin D on ADMA and SDMA were cross sectional investigations and were therefore limited by possible unknown confounding or they may have been subject to reverse causality [23–25]. As the present investigation was randomized and both physicians and patients were blinded to the study medication unknown confounding is less likely [53]. Furthermore, the significant reduction of PTH indicates a good therapeutic effect of vitamin D3. Nevertheless, as this study is a post-hoc analysis someone has to be cautious interpreting its results [39]. Furthermore, the cross sectional evidence so far was in quite specific patient cohorts, amongst others hypogonadism[24], phenylketonuria[25], PCOS[26] and on long-term haemodialysis (HD)[27]. In line with our results, *Abu el Maaty* and colleagues described in 69 patients suffering from coronary artery disease no difference in ADMA or SDMA levels when comparing patients above vs. below 30 ng/mL of 25(OH)D [40].

4.1. Strengths and limitations

As the primary outcome of our RCT, namely the reduction in systolic blood pressure, could not be achieved, the question arises whether there was any vitamin D effect on the vasculature in the present trial. Still it should be noted that there was a statistical and clinical significant reduction in PTH, which by itself has been

linked to lower ADMA levels [54]. Furthermore, it has been already pointed out that – most likely – only patients who are truly deficient would benefit from vitamin D treatment warranting further trials on this topic such as the D-Cor study, an RCT restricted to individuals with 25(OH)D below 12 ng/mL (ClinicalTrials.gov Identifier: NCT02750293) [4,52]. Another potential limitation of our study may be the concurrent treatment with anti-hypertensive medication with potential impact on our outcome measures. Nevertheless we did see a significant increase in the L-arginine to ADMA ratio in patient who were vitamin D deficient (25(OH)D ≤20 ng/mL). Future trials should therefore focus on patients with vitamin D deficient patients, ideally also treatment naive. More so, future trials should aim to recruit larger samples as especially our subgroup analysis in participants with 25(OH)D ≤20.0 ng/mL is too small to draw final conclusions. The strengths of the present investigation clearly are the randomization and the blinding of both patients and treating physicians to the placebo-controlled intervention.

5. Conclusion

In summary, we did not observe an effect of vitamin D supplementation on ADMA or SDMA in patients with arterial hypertension and vitamin D insufficiency. In patients with a baseline 25(OH)D concentration below 20 ng/mL, we observed a statistically significant increase in the L-arginine to ADMA ratio under vitamin D supplementation. This finding warrants additional investigations and should be addressed in future trials focussing on patients with 25(OH) concentrations below 20 ng/mL.

Sources of funding

The Styrian Hypertension Study was supported by funding from the Austrian National Bank (Jubilaeumsfond: project #: 13878 and 13905).

Disclosures

None.

Acknowledgments

We thank all study participants. We also thank Fresenius Kabi for providing the study medication and BioPersMed (COMET K-project 825329, funded by the Austrian Federal Ministry of Transport, Innovation and Technology (BMVIT), the Austrian Federal Ministry of Economics and Labour/Federal Ministry of Economy, Family and Youth (BMWA/BMWFJ) and by the Styrian Business Promotion Agency (SFG)) for analysis equipment. We also thank the laboratory staff of the department of Endocrinology and Diabetology of the Medical University of Graz, Austria.

References

[1] M.F. Holick, McCollum award lecture, 1994: vitamin D–new horizons for the 21 st century, Am. J. Clin. Nutr. 60 (1994) 619–630.
[2] M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M.H. Murad, C.M. Weaver, Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited, J. Clin. Endocrinol. Metab. 97 (2012) 1153–1158, doi:http://dx.doi.org/10.1210/jc.2011-2601.
[3] M.F. Holick, Vitamin D status: measurement, interpretation, and clinical application, Ann. Epidemiol. 19 (2009) 73–78, doi:http://dx.doi.org/10.1016/j.annepidem.2007.12.001.
[4] G. Muscogiuri, B. Altieri, C. Annweiler, G. Balercia, H.B. Pal, B.J. Boucher, J.J. Cannell, C. Foresta, M.R. Grübler, K. Kotsa, L. Mascitelli, W. März, F. Orio, S. Pilz, G. Tirabassi, A. Colao, Vitamin D and chronic diseases: the current state of the art, Arch. Toxicol. (2016), doi:http://dx.doi.org/10.1007/s00204-016-1804-x.
[5] R. Bouillon, G. Carmeliet, L. Verlinden, E. van Etten, A. Verstuyf, H.F. Luderer, L. Lieben, C. Mathieu, M. Demay, Vitamin D and human health: lessons from vitamin D receptor null mice, Endocr. Rev. 29 (2008) 726–776, doi:http://dx.doi.org/10.1210/er.2008-0004.
[6] D. Carlton-Conway, R. Tulloh, L. Wood, D. Kanabar, Vitamin D deficiency and cardiac failure in infancy, J. R. Soc. Med. 97 (2004) 238–239.
[7] K. Kienreich, A. Tomaschitz, N. Verheyen, T. Pieber, M. Gaksch, M.R. Grübler, S. Pilz, Vitamin D and cardiovascular disease, Nutrients 5 (2013) 3005–3021, doi:http://dx.doi.org/10.3390/nu5083005.
[8] K. Kienreich, M. Grubler, A. Tomaschitz, J. Schmid, N. Verheyen, F. Rutters, J.M. Dekker, S. Pilz, Vitamin D, arterial hypertension & cerebrovascular disease, Indian J. Med. Res. 137 (2013) 669–679.
[9] H.M. Macdonald, A.D. Wood, W.D. Fraser, W.G. Simpson, Vitamin D status and ill health, Lancet Diabetes Endocrinol 2 (2016) e8–e9, doi:http://dx.doi.org/10.1016/s2213-8587(14)70047-6 (n.d).
[10] C.J. Rosen, J.S. Adams, D.D. Bikle, D.M. Black, M.B. Demay, J.E. Manson, M.H. Murad, C.S. Kovacs, The nonskeletal effects of vitamin D: an Endocrine Society scientific statement, Endocr. Rev. 33 (2012) 456–492, doi:http://dx.doi.org/10.1210/er.2012-1000.
[11] Y. Solak, A. Covic, M. Kanbay, What do we know and do not know about vitamin D?: A causal association between Vitamin D receptor genetic polymorphism and hypertension, J. Clin. Hypertens 16 (2014) 627–628, doi:http://dx.doi.org/10.1111/jch.12383.
[12] S. Pilz, K. Kienreich, F. Rutters, R. de Jongh, A.J. van Ballegooijen, M. Grübler, A. Tomaschitz, J.M. Dekker, Role of Vitamin D in the development of insulin resistance and type 2 diabetes, Curr. Diab. Rep. 13 (2012) 261–270, doi:http://dx.doi.org/10.1007/s11892-012-0358-4.
[13] T. Lim, A.D. Flaxman, G. Danaei, K. Shibuya, H. Adair-Rohani, M.A. AlMazroa, M. Amann, H.R. Anderson, K.G. Andrews, M. Aryee, C. Atkinson, L.J. Bacchus, A.N. Bahalim, K. Balakrishnan, J. Balmes, S. Barker-Collo, A. Baxter, M.L. Bell, J.D. Blore, F. Blyth, C. Bonner, G. Borges, R. Bourne, M. Boussinesq, M. Brauer, P. Brooks, N.G. Bruce, B. Brunekreef, C. Bryan-Hancock, C. Bucello, R. Buchbinder, F. Bull, R.T. Burnett, T.E. Byers, B. Calabria, J. Carapetis, E. Carnahan, Z. Chafe, F. Charlson, H. Chen, J.S. Chen, A.T.-A. Cheng, J.C. Child, K.E. Cohen, B.C. Cowie, S. Darby, S. Darling, A. Davis, L. Degenhardt, F. Dentener, D.C. Des Jarlais, K. Devries, M. Dherani, E.L. Ding, E.R. Dorsey, T. Driscoll, K. Edmond, S.E. Ali, R.E. Engell, P.J. Erwin, S. Fahimi, G. Falder, F. Farzadfar, A. Ferrari, M.M. Finucane, S. Flaxman, F.G.R. Fowkes, G. Freedman, M.K. Freeman, E. Gakidou, S. Ghosh, E. Giovannucci, G. Gmel, K. Graham, R. Grainger, B. Grant, D. Gunnell, H.R. Gutierrez, W. Hall, H.W. Hoek, A. Hogan, H.D. Hosgood III, D. Hoy, H. Hu, B.J. Hubbell, S.J. Hutchings, S.E. Ibeanusi, G.L. Jacklyn, R. Jasrasaria, J.B. Jonas, H. Kan, J.A. Kanis, N. Kassebaum, N. Kawakami, Y.-H. Khang, S. Khatibzadeh, J.-P. Khoo, C. Kok, F. Laden, R. Lalloo, et al., A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-: a systematic analysis for the Global Burden of Disease Study 2010, Lancet (2010) 2224–2260, doi:http://dx.doi.org/10.1016/S0140-6736(12)61766-8.
[14] B.M. Patel, A.A. Mehta, Aldosterone and angiotensin: role in diabetes and cardiovascular diseases, Eur. J. Pharmacol. 697 (2012) 1–12, doi:http://dx.doi.org/10.1016/j.ejphar.2012.09.034.
[15] D.A. Calhoun, Aldosterone and cardiovascular disease smoke and fire, Circulation 114 (2006) 2572–2574, doi:http://dx.doi.org/10.1161/CIRCULATIONAHA.106.668715.
[16] J.W. Funder, The nongenomic actions of aldosterone, Endocr. Rev. 26 (2005) 313–321, doi:http://dx.doi.org/10.1210/er.2005-0004.
[17] M. Gekle, C. Grossmann, Actions of aldosterone in the cardiovascular system: the good, the bad, and the ugly? Pflüg. Arch. Eur. J. Physiol. 458 (2009) 231–246, doi:http://dx.doi.org/10.1007/s00424-008-0616-0.
[18] M.B.P. Landim, A.C. Filho, A.C.P. Chagas, Asymmetric dimethylarginine (ADMA) and endothelial dysfunction: implications for atherogenesis, Clin. Sao Paulo Braz. 64 (2009) 471–478, doi:http://dx.doi.org/10.1590/S1807-59322009000500015.
[19] N. Lüneburg, V. Xanthakis, E. Schwedhelm, L.M. Sullivan, R. Maas, M. Anderssohn, U. Riederer, N.L. Glazer, R.S. Vasan, R.H. Böger, Reference intervals for plasma L-arginine and the L-arginine:asymmetric dimethylarginine ratio in the Framingham Offspring Cohort, J. Nutr. 141 (2011) 2186–2190, doi:http://dx.doi.org/10.3945/jn.111.148197.
[20] P. Vallance, A. Leone, A. Calver, J. Collier, S. Moncada, Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis, J. Cardiovasc. Pharmacol. 20 (Suppl. 12) (1992) S60–S62.
[21] D. Tousoulis, M. Georgakis, E. Oikonomou, N. Papageorgiou, M. Zaromitidou, G. Latsios, S. Papaioannou, G. Siasos, Asymmetric dimethylarginine: clinical significance and novel therapeutic approaches, Curr. Med. Chem. 22 (2015) 2871–2901, doi:http://dx.doi.org/10.2174/0929867322666150625095046.
[22] E. Gkaliagkousi, E. Gavriilaki, A. Triantafyllou, S. Douma, Clinical significance of endothelial dysfunction in essential hypertension, Curr. Hypertens. Rep. 17 (2015) 1–9, doi:http://dx.doi.org/10.1007/s11906-015-0596-3.
[23] D.T. Ngo, A.L. Sverdlov, J.J. McNeil, J.D. Horowitz, Does vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations? Am. J. Med. 123 (2010) 335–341, doi:http://dx.doi.org/10.1016/j.amjmed.2009.09.024.
[24] C. Meric, A. Sonmez, A. Aydogdu, S. Tapan, C. Haymana, Y. Basaran, K. Baskoy, E. Sertoglu, A. Taslipinar, E. Bolu, O. Azal, Osteoprotegerin, fibroblast growth factor 23, and vitamin D3 levels in male patients with hypogonadism, Horm. Metab. Res. Horm. Stoffwechselforschung Horm. Métabolisme 46 (2014) 955–958, doi:http://dx.doi.org/10.1055/s-0034-1387789.

[25] Y. Okano, H. Nagasaka, Optimal serum phenylalanine for adult patients with phenylketonuria, Mol. Genet. Metab. 110 (2013) 424–430, doi:http://dx.doi.org/10.1016/j.ymgme.2013.09.007.
[26] D.T.M. Ngo, W.P. Chan, S. Rajendran, T. Heresztyn, A. Amarasekera, A.L. Sverdlov, P.D. O'Loughlin, H.A. Morris, Y.Y. Chirkov, R.J. Norman, J.D. Horowitz, Determinants of insulin responsiveness in young women: impact of polycystic ovarian syndrome, nitric oxide, and vitamin D, Nitric Oxide Biol. Chem. Off. J. Nitric Oxide Soc. 25 (2011) 326–330, doi:http://dx.doi.org/10.1016/j.niox.2011.06.005.
[27] A. Bednarek-Skublewska, A. Smoleń, A. Jaroszyński, W. Załuska, A. Ksiazek, Effects of vitamin D3 on selected biochemical parameters of nutritional status inflammation, and cardiovascular disease in patients undergoing long-term hemodialysis, Pol. Arch. Med. Wewnętrznej 120 (2010) 167–174.
[28] A.L. Sverdlov, D.T.M. Ngo, W.P.A. Chan, Y.Y. Chirkov, J.D. Horowitz, Aging of the nitric oxide system: are we as old as our NO? J. Am. Heart Assoc. 3 (2014) e000973, doi:http://dx.doi.org/10.1161/JAHA.114.000973.
[29] S.K. Syal, A. Kapoor, E. Bhatia, A. Sinha, S. Kumar, S. Tewari, N. Garg, P.K. Goel, Vitamin D deficiency, coronary artery disease, and endothelial dysfunction: observations from a coronary angiographic study in Indian patients, J. Invasive Cardiol. 24 (2012) 385–389.
[30] M. Celik, A. Iyisoy, T. Celik, M.I. Yilmaz, U.C. Yuksel, H. Yaman, The relationship between L-arginine/ADMA ratio and coronary collateral development in patients with low glomerular filtration rate, Cardiol. J. 19 (2012) 29–35.
[31] R.H. Böger, The pharmacodynamics of L-arginine, J. Nutr. 137 (2007) 1650S–1655S.
[32] S.M. Bode-Böger, F. Scalera, L.J. Ignarro, The L-arginine paradox: importance of the L-arginine/asymmetrical dimethylarginine ratio, Pharmacol. Ther. 114 (2007) 295–306, doi:http://dx.doi.org/10.1016/j.pharmthera.2007.03.002.
[33] M.A. Abu El Maaty, S.I. Hassanein, R.S. Hanafi, M.Z. Gad, Insights on vitamin D's role in cardiovascular disease: investigating the association of 25-hydroxyvitamin D with the dimethylated arginines, J. Nutr. Sci. Vitaminol. (Tokyo) 59 (2013) 172–177.
[34] J.T. Kielstein, S.R. Salpeter, S.M. Bode-Boeger, J.P. Cooke, D. Fliser, Symmetric dimethylarginine (SDMA) as endogenous marker of renal function—a meta-analysis, Nephrol. Dial. Transplant. 21 (2006) 2446–2451, doi:http://dx.doi.org/10.1093/ndt/gfl292.
[35] S. Pilz, M. Gaksch, K. Kienreich, M. Grübler, N. Verheyen, A. Fahrleitner-Pammer, G. Treiber, C. Drechsler, B. ó Hartaigh, B. Obermayer-Pietsch, V. Schwetz, F. Aberer, J. Mader, H. Scharnagl, A. Meinitzer, E. Lerchbaum, J.M. Dekker, A. Zittermann, W. März, A. Tomaschitz, Effects of Vitamin D on blood pressure and cardiovascular risk factors a randomized controlled trial, Hypertension 65 (2015) 1195–1201, doi:http://dx.doi.org/10.1161/HYPERTENSIONAHA.115.05319.
[36] M.R. Grübler, M. Gaksch, K. Kienreich, N. Verheyen, J. Schmid, B.Ó. Hartaigh, G. Richtig, H. Scharnagl, A. Meinitzer, A. Fahrleitner-Pammer, W. März, A. Tomaschitz, S. Pilz, Effects of vitamin D supplementation on HbA1c and fasting glucose in hypertensive patients – a randomized controlled trial, Diabetes Obes. Metab.. (2016), doi:http://dx.doi.org/10.1111/dom.12709.
[37] M.R. Grübler, M. Gaksch, K. Kienreich, N. Verheyen, J. Schmid, B.W.J. Ó Hartaigh, G. Richtig, H. Scharnagl, A. Meinitzer, B. Pieske, A. Fahrleitner-Pammer, W. März, A. Tomaschitz, S. Pilz, Effects of Vitamin D supplementation on plasma aldosterone and renin-A randomized placebo-Controlled trial, J. Clin. Hypertens. Greenwich Conn. 18 (2016) 608–613, doi:http://dx.doi.org/10.1111/jch.12825.
[38] J.B. Ernst, A. Tomaschitz, M.R. Grübler, M. Gaksch, K. Kienreich, N. Verheyen, W. März, S. Pilz, A. Zittermann, Vitamin D supplementation and hemoglobin levels in hypertensive patients: a randomized controlled trial, Int. J. Endocrinol 2016 (2016) e6836402, doi:http://dx.doi.org/10.1155/2016/6836402.
[39] D. Moher, S. Hopewell, K.F. Schulz, V. Montori, P.C. Gøtzsche, P.J. Devereaux, D. Elbourne, M. Egger, D.G. Altman, CONSORT 2010 Explanation and Elaboration: updated guidelines for reporting parallel group randomised trials, BMJ 340 (2010) c869, doi:http://dx.doi.org/10.1136/bmj.c869.
[40] G. Mancia, R. Fagard, K. Narkiewicz, J. Redon, A. Zanchetti, M. Böhm, T. Christiaens, R. Cifkova, G.D. Backer, A. Dominiczak, M. Galderisi, D.E. Grobbee, T. Jaarsma, P. Kirchhof, S.E. Kjeldsen, S. Laurent, A.J. Manolis, P.M. Nilsson, L.M. Ruilope, R.E. Schmieder, P.A. Sirnes, P. Sleight, M. Viigimaa, B. Waeber, F. Zannad, J. Redon, A. Dominiczak, K. Narkiewicz, P.M. Nilsson, M. Burnier, M. Viigimaa, E. Ambrosioni, M. Caufield, A. Coca, M.H. Olsen, R.E. Schmieder, C. Tsioufis, P. van de Borne, J.L. Zamorano, S. Achenbach, H. Baumgartner, J.J. Bax, H. Bueno, V. Dean, C. Deaton, C. Erol, R. Fagard, R. Ferrari, D. Hasdai, A.W. Hoes, P. Kirchhof, J. Knuuti, P. Kolh, P. Lancellotti, A. Linhart, P. Nihoyannopoulos, M.F. Piepoli, P. Ponikowski, P.A. Sirnes, J.L. Tamargo, M. Tendera, A. Torbicki, W. Wijns, S. Windecker, D.L. Clement, A. Coca, T.C. Gillebert, M. Tendera, E.A. Rosei, E. Ambrosioni, S.D. Anker, J. Bauersachs, J.B. Hitij, M. Caulfield, M.D. Buyzere, S.D. Geest, G.A. Derumeaux, S. Erdine, C. Farsang, C. Funck-Brentano, V. Gerc, G. Germano, S. Gielen, H. Haller, A.W. Hoes, J. Jordan, T. Kahan, M. Komajda, D. Lovic, H. Mahrholdt, M.H. Olsen, J. Ostergren, G. Parati, J. Perk, J. Polonia, B.A. Popescu, Ž. Reiner, L. Rydén, Y. Sirenko, A. Stanton, H. Struijker-Boudier, C. Tsioufis, P. van de Borne, C. Vlachopoulos, M. Volpe, D.A. Wood, ESH/ESC Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC), Eur. Heart J. 34 (2013) 2159–2219, doi:http://dx.doi.org/10.1093/eurheartj/eht151.
[41] A. Meinitzer, M. Puchinger, B.M. Winklhofer-Roob, E. Rock, J. Ribalta, J.M. Roob, I. Sundl, G. Halwachs-Baumann, W. März, Reference values for plasma concentrations of asymmetrical dimethylarginine (ADMA) and other arginine metabolites in men after validation of a chromatographic method, Clin. Chim. Acta Int. J. Clin. Chem. 384 (2007) 141–148, doi:http://dx.doi.org/10.1016/j.cca.2007.07.006.
[42] J. Schmid, K. Kienreich, M. Gaksch, M. Grübler, R. Raggam, A. Meinitzer, F. Rutters, J.M. Dekker, W. März, N. Verheyen, The importance of assays in vitamin D status classification: a comparison of four automated 25-hydroxyvitamin D immunoassays, LaboratoriumsMedizin 37 (2013) 261–268.
[43] M. Roth, Fluorescence reaction for amino acids, Anal. Chem. 43 (1971) 880–882, doi:http://dx.doi.org/10.1021/ac60302a020.
[44] E.L. Schwarz, W.L. Roberts, M. Pasquali, Analysis of plasma amino acids by HPLC with photodiode array and fluorescence detection, Clin. Chim. Acta Int. J. Clin. Chem. 354 (2005) 83–90, doi:http://dx.doi.org/10.1016/j.cccn.2004.11.016.
[45] M.R. Grübler, K. Kienreich, M. Gaksch, N. Verheyen, A. Fahrleitner-Pammer, J. Schmid, J. Grogorenz, K. Ablasser, B. Pieske, A. Tomaschitz, S. Pilz, Aldosterone to active renin ratio is associated with nocturnal blood pressure in obese and treated hypertensive patients: the styrian hypertension study, J. Clin. Hypertens. 16 (2014) 289–294, doi:http://dx.doi.org/10.1111/jch.12274.
[46] B.ó. Hartaigh, M. Gaksch, K. Kienreich, M.R. Grübler, N. Verheyen, W. März, A. Tomaschitz, T.M. Gill, S. Pilz, Associations of daytime, nighttime, and 24-Hour heart rate with four distinct markers of inflammation in hypertensive patients: the styrian hypertension study, J. Clin. Hypertens. 16 (2014) 856–861, doi:http://dx.doi.org/10.1111/jch.12420.
[47] M.R. Grübler, K. Kienreich, M. Gaksch, N. Verheyen, B.Ó. Hartaigh, A. Fahrleitner-Pammer, W. März, J. Schmid, E.-M. Oberreither, J. Wetzel, C. Catena, L.A. Sechi, B. Pieske, A. Tomaschitz, S. Pilz, Aldosterone-to-Renin ratio is associated with reduced 24-Hour heart rate variability and QTc prolongation in hypertensive patients, Medicine (Baltimore) 95 (2016) e2794, doi:http://dx.doi.org/10.1097/MD.0000000000002794.
[48] I.R. White, N.J. Horton, J. Carpenter, S.J. Pocock, Strategy for intention to treat analysis in randomised trials with missing outcome data, BMJ 342 (2011) d40.
[49] R. Wang, S.W. Lagakos, J.H. Ware, D.J. Hunter, J.M. Drazen, Statistics in medicine — reporting of subgroup analyses in clinical trials, N. Engl. J. Med. 357 (2007) 2189–2194, doi:http://dx.doi.org/10.1056/NEJMsr077003.
[50] K.D. Cashman, K.G. Dowling, Z. Škrabáková, M. Gonzalez-Gross, J. Valtueña, S. De Henauw, L. Moreno, C.T. Damsgaard, K.F. Michaelsen, C. Mølgaard, R. Jorde, G. Grimnes, G. Moschonis, C. Mavrogianni, Y. Manios, M. Thamm, G.B. Mensink, M. Rabenberg, M.A. Busch, L. Cox, S. Meadows, G. Goldberg, A. Prentice, J.M. Dekker, G. Nijpels, S. Pilz, K.M. Swart, N.M. van Schoor, P. Lips, G. Eiriksdottir, V. Gudnason, M.F. Cotch, S. Koskinen, C. Lamberg-Allardt, R.A. Durazo-Arvizu, C.T. Sempos, M. Kiely, Vitamin D deficiency in europe: pandemic? Am. J. Clin. Nutr 103 (2016) 1033–1044, doi:http://dx.doi.org/10.3945/ajcn.115.120873.
[51] L. Mosekilde, P. Vestergaard, L. Rejnmark, The pathogenesis, treatment and prevention of osteoporosis in men, Drugs 73 (2012) 15–29, doi:http://dx.doi.org/10.1007/s40265-012-0003-1.
[52] S. Pilz, N. Verheyen, M.R. Grübler, A. Tomaschitz, W. März, Vitamin D and cardiovascular disease prevention, Nat. Rev. Cardiol. 13 (2016) 404–417, doi:http://dx.doi.org/10.1038/nrcardio.2016.73.
[53] P. Brennan, P. Croft, Interpreting the results of observational research: chance is not such a fine thing, BMJ 309 (1994) 727–730, doi:http://dx.doi.org/10.1136/bmj.309.6956.727.
[54] A.T. Amarasekera, A.L. Sverdlov, M.S. Roberts, J.D. Horowitz, D.T. Ngo, Elevated parathyroid hormone predicts high asymmetric dimethylarginine (ADMA) concentrations; independent of vitamin D status, Eur. Heart J. 34 (2013) P613, doi:http://dx.doi.org/10.1093/eurheartj/eht307.P613.