



Effect of Short-Term Vitamin D Supplementation on Blood Pressure, Arterial Health, and Stress Hormones in Healthy Volunteers [†]

Maha N. Abu Hajleh ¹ and Emad A. S. Al-Dujaili ^{2,*}

¹ Department of Pharmacy, Cosmetic Science, Pharmacological and Diagnostic Research Centre, Faculty of Allied Medical Sciences, Al-Ahliyya Amman University, Amman 19328, Jordan; mahaabuhajleh@hotmail.com

² Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh EH16 4JT, UK

* Correspondence: ealduja1@exseed.ed.ac.uk

[†] Presented at the 2nd International Electronic Conference on Nutrients, 15–31 March 2022; Available online: <https://iecn2022.sciforum.net/>.

Abstract: Purpose: Despite suggestive epidemiological findings and plausible mechanisms, data directly linking vitamin D supplementation with improvement in cardiovascular risk is limited. Moreover, little is known about the effect of vitamin D on cardiovascular health of young healthy people. The purpose of the current study was to investigate the effect of short-term supplementation with vitamin D₃ on blood pressure (BP), pulse wave velocity (PWV), body mass index (BMI), and salivary cortisol and cortisone levels in young healthy adults. Methods: The study applied a short, parallel placebo-controlled design. A total of 20 healthy, normotensive participants were instructed to consume 20 µg/d of vitamin D₃ for 2 w, and 10 volunteers received a placebo. BP, PWV, BMI, and salivary cortisol level were assessed at baseline and after 2 w time. Vitamin D and total energy intakes were also evaluated. Results: After 2 w of the supplementation there was a significant decrease in mean PWV by 0.475 ± 0.31 m/s ($p = 0.007$) with a negative correlation with vitamin D intake ($r = -0.43$), systolic BP by 5.3 ± 6.46 mmHg ($p = 0.035$) and diastolic BP by 3.4 ± 4.46 mmHg ($p = 0.002$). No significant change was observed in BMI. There was no significant effect on salivary cortisol ($p = 0.554$), but overall salivary cortisone increased from 5.33 ± 2.6 to 6.98 ± 3.3 nmole, $p = 0.042$. Salivary free cortisol/cortisone ratio was reduced from 0.952 ± 0.54 to 0.784 ± 0.68 , $p = 0.028$. Urinary free cortisol/cortisone ratio was reduced (1.71 ± 0.75 – 1.22 ± 0.53 , $p = 0.015$). Conclusions: Vitamin D₃ supplementation decreases both diastolic and systolic BP and improves arterial compliance but does not alter BMI or salivary cortisol levels. However, there was a reduction of salivary and urine free cortisol/cortisone ratio indicating an inhibition of 11βHSD type 1 enzyme activity. The results suggest that vitamin D₃ could have the potential to reduce the risk of hypertension and cardiovascular diseases in young healthy adults. Further research with controlled conditions is warranted to test the reproducibility of the obtained results.

Keywords: vitamin D; blood pressure; pulse wave velocity; cortisol; 11βHSD activity



Citation: Abu Hajleh, M.N.; Al-Dujaili, E.A.S. Effect of Short-Term Vitamin D Supplementation on Blood Pressure, Arterial Health, and Stress Hormones in Healthy Volunteers. *Biol. Life Sci. Forum* **2022**, *12*, 15. <https://doi.org/10.3390/IECN2022-12398>

Academic Editor: Alessandra Durazzo

Published: 14 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Vitamin D has been established to have multiple functions, more than just its long-established role in regulating calcium and bone homeostasis [1]. Observational studies reported a positive association between low levels of vitamin D and increased cardiovascular morbidity [2,3], and mortality [4,5]. Cardiovascular disease (CVD) is a leading cause of death worldwide and in western societies predicted to be the major cause of death by 2030. Vitamin D insufficiency and deficiency are globally neglected problems [6,7]. The deficiency is an inevitable consequence of low exposure to sunlight since cutaneous synthesis of vitamin D₃ (Figure 1) upon sufficient exposure to ultraviolet-B (UVB) radiation could provide

about 80% of body vitamin D [8]. Plasma vitamin D₃ status is estimated by evaluating the level of 25-hydroxyvitamin D (25-OH D), which is the primary circulating form of vitamin D in the body. Currently, there is still no global agreement regarding optimal vitamin D intake and status [9]. However, most of the existing evidence suggests the desirable concentration of 25(OH)D to be 70–80 nmol/L, this being a level which ensures the maximal suppression of circulating parathyroid hormone and prevents bone resorption [10]. Much higher levels are now recommended to boost the immune system against infection with COVID SARS-CoV-2 [11].

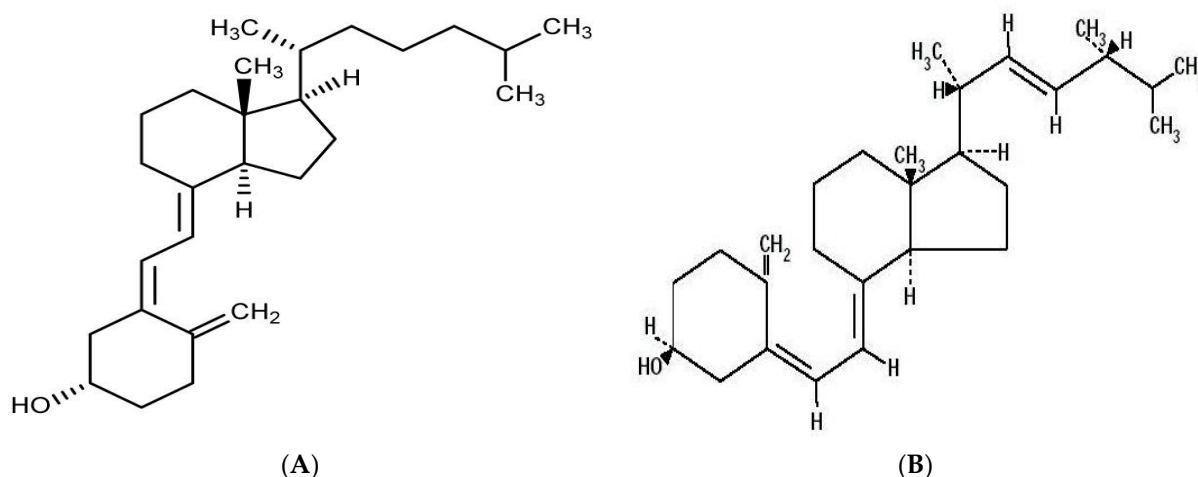


Figure 1. (A) Structure of vitamin D₃ (Cholecalciferol), and (B) Structure of Ergocalciferol (vitamin D₂).

Arterial compliance is an important independent predictor of cardiovascular risk, which is defined as the ability of an artery to change its diameter or volume with transmural pressure [12,13]. Adequate arterial compliance is important in reducing the cardiac load (decreased arterial stiffness). Measuring carotid–femoral pulse wave velocity (PWV) has emerged as the current ‘gold standard’ method of assessing arterial compliance and has been also recommended by the European Society of Hypertension guidelines as a favored non-invasive index of aortic stiffness [14]. PWV is inversely associated with arterial wall distensibility [15] and endothelium functions as well as the presence and extent of atherosclerotic plaques [16]. Supplementation with vitamin D was found to influence the ability of endothelium to synthesize mediators such as Nitric Oxide (NO) and thus improved endothelial function in deficient subjects and those with type2 diabetes [17], and decreased inflammatory markers (CRP, IL-6) in subjects with prolonged critical illness [18]. Researchers [19] have reported a negative correlation between circulating levels of 25-OH-D and vascular calcification (a process promoting vascular wall stiffening). A positive link between vitamin D deficiency and hypertension, the key risk factor for CVD, was reported by a large cross-sectional study where participants in the highest quintile of circulating 25-OH D levels had mean systolic BP of 3 mmHg lower than those in the lowest quintile [20]. Another study showed that 1,25-OH-D suppressed renin synthesis, thus down-regulating the renin–angiotensin–aldosterone system, which might have lowered BP [21]. An inverse relationship between low vitamin D status and obesity was also observed and studies showed that vitamin D inhibits preadipocytes differentiation and stimulates lipoprotein lipase synthesis and secretion [22,23]. However, observational studies provide little evidence of vitamin D effects on BP, arterial compliance, BMI, and cardiovascular health [10,13]. Glucocorticoids play an important role in maintaining extracellular fluid volume and normal BP [13]. Cortisol secretion is regulated by negative feedback on the pituitary responding to a low level of circulating cortisol as well as stress and circadian rhythm, resulting in its highest levels in the morning and its gradual decrease throughout the day [24]. Cortisol is also synthesized and secreted by the adrenal cortex in response to mental and physical

stress, and pathophysiological situations such as the excessive production of cortisol in Cushing's syndrome leading to hypertension that highlighted the importance of cortisol in the regulation of blood pressure [25,26]. Hypertension and diabetes are independent risk factor for the development of CVD and there are compelling arguments for Lowering BP to reduce the risk of CVD [27]. Hypertension is among the leading causes of cardiovascular morbidity and mortality [28]. Cardiovascular event as consequences of increased cortisol secretion and circulating levels in patients with essential hypertension [29], glucose intolerance, and insulin resistance have been reported [30]. It was also established that intracellular glucocorticoid reactivation was elevated in adipose tissue of humans, and 11 β hydroxysteroid dehydrogenase (11 β -HSD) is the principal enzyme responsible for regulating glucocorticoid metabolism [31]. The aim of this study was to investigate the effects of short-term vitamin D intake on CVD risk factors: BP, arterial health, and stress hormone status (cortisol secretion and metabolism) in healthy volunteers.

2. Methods and Results

2.1. Study Design

A placebo controlled randomized single blinded parallel design has been applied in this study. The experiment took place over 21 d with the total intervention period of 14 d over where participants were required to consume 20 μ g/d of vitamin D₃ (cholecalciferol; Simply Supplements, Guernsey, UK) or placebo (maltodextrin tablets) after meal. Vitamin D and placebo tablets were placed in opaque bottles and provided to all participants at the day of baseline measurements (Table 1). The inclusion criteria were as follows: females and males aged 18–60 years, normotensive and apparently healthy. The exclusion criteria included a vitamin D allergy or intolerance, being on vitamin D supplementation, taking any blood or cholesterol lowering medications, restricted calorie intake, smoking, pregnancy, and breastfeeding. A total of 30 volunteers (18 females and 12 males) agreed to take part in the study and breastfeeding. The volunteers (18 females and 12 males) agreed to take part in the study and were then randomized into the vitamin D group ($n = 20$) and placebo group ($n = 10$). The study was granted the necessary ethical approval from QMU Central University Ethical Committee (code: Honors/07004211/Vitamin D/BSc-NUT/DNBS/QMU Ethical Committee).

Table 1. Study protocol.

Week Number	Activities
Week 0: Supplement-free 1 d before starting supplementation	5-d wash-out period for all volunteers Completing 2-d diet diary Collection of three saliva samples and 24-h urine sample (Baseline data)
Week 1: Supplementation starts (Vitamin D3 or placebo)	First meeting (day 1); measure BP, PWV, weight, and height.
Week 2: Supplementation continues (Vitamin D3 or placebo).	Completing 2-d diet diary
1 d before supplementation terminates	Collection of three saliva samples and 24-h urine sample (Intervention data)
Last day of supplementation	Second meeting to measure BP, PWV, weight, and height. Urine sample collected

2.2. Data Collection

All subjects provided 2-d diet diaries, consisting of one weekday and one weekend day, completed before and during the final days of the intervention phase. The subjects were advised to maintain their usual diets, not to take any additional vitamin D supplements, or expose themselves excessively to sun during the study period. A 5-d wash-out period prior to commencing of intervention had to be applied to as an initiation phase. Collection of saliva samples (AM, noon, and PM) and 24-h urine samples were carried out by participants 1 d before the onset of the supplementation and on day 13 of the intervention period.

Participants were given six labelled tubes for saliva collection, a packet of sugar-free chewing gum to aid saliva production, and 2 containers for urine collection. The

participants were advised not to consume any alcohol, carry out any scheduled exercise, or have any sexual activity within the 24-h period before the sample collection. Information sheets on collection and storage of saliva and urine samples were given to all participants as previously published [7]. The samples were then stored at -20°C and mean daily cortisol and cortisone levels were determined by taking an average of the three saliva samples and total urine excretion by sensitive and specific ELISA methods [7]. Height (cm), weight (kg), and systolic and diastolic blood pressure were measured, and to increase the reliability and validity, 3 readings of BP were done after participants had been at rest for 10 min. The second and third readings were considered for inclusion in the study following the protocol used by Jackson et al., 2007 and has been shown to reduce 'white coat' hypertension effects [32].

Right carotid femoral PWV (pulse wave velocity) was evaluated with a validated device (VicorderTM; Bristol, UK) [33]. PWV is a simple and non-invasive method which has been previously shown to provide accurate and reproducible results [34]. PWV measures pulse transit time and the distance travelled by the pulse between the two recording sites.

$$\text{PWV} = \text{distance (m)} / \text{transit time (s)}$$

Three readings of PWV were estimated with 1-min intervals between each measurement; the mean of all three readings was then calculated. The measurements were taken at the baseline and on the last day of the intervention period and care was exercised to ensure that both measurements sessions were carried out in the same manner to ensure reliability of the results. All raw data were kept in a protected file in the researcher's computer at QMU. There were no dropouts, and all 30 recruited subjects successfully completed the study.

2.3. Data Analysis and Statistics

The 2-d diet diaries were analyzed with the WinDiet 2015 program (RGU, Aberdeen, UK) to assess the dietary intake of vitamin D as well as total energy intake. Salivary cortisol and cortisone levels were analyzed using Indirect Competitive Enzyme-Linked Immuno Sorbent Assay (ELISA), with some modifications [35,36]. Validation studies has shown that this method to be very accurate and reliable. Data was analyzed using the Microsoft Excel program and the statistical package for social sciences (SPSS, version 2021, IBM, Armonk, NY, USA). A Paired-samples *t*-test was applied to compare the baseline and intervention BP, PWV, BMI, and salivary cortisol level. A *p* value of ≤ 0.05 was considered significant.

3. Results

3.1. Sample Characteristics and Diet Intake

Out of 20 recruited subjects for the Vitamin D group, 8 were males and 12 females with mean $\pm$ SD age of 27.7 ± 9.7 years. For the placebo, 10 matched volunteers were recruited. Upon analysis all participants had normal baseline salivary cortisol level [37]. Both systolic and diastolic blood pressure (SBP, DBP) were within normotensive ranges for all the subjects. Moreover, all the participants' PWV values were within reference ranges established for their age and BP [38]. All participants were within healthy BMI ranges. None of the subjects was a smoker. Two participants were abstainers whilst the rest of the group reported light alcohol consumption. Most of the subjects had at least a moderate exercise level, with only 4 reporting low levels of exercise. However, participants were asked to withdraw from drinking and exercise 24 h before measurements and collecting saliva samples (See Table 2).

There were no dropouts. Compliance purely relied on the participants' word and was regarded as high as most of participants reported taking supplements regularly. None of the participants went for a sunny holiday during the study. Moreover, as the experiment was carried out in the term time (March–April), participated students were carrying out their normal academic activities. The mean energy intake was not found to differ significantly between baseline and intervention period. However, the observed slightly low energy

intake for both women and men raise a possibility of under-reporting. The mean intake of vitamin D₃ after introducing 20 µg (800 IU) supplement was significantly higher ($p < 0.0001$) (See Table 3).

Table 2. Subjects' baseline characteristics.

Variable	Vit D (Mean ± SD)	Placebo (Mean ± SD)
Males/females	8/12	4/6
Age	27.5 ± 9.6	26.9 ± 8.8
Weight (kg)	66.5 ± 13.1	67.1 ± 12.5
BMI	24.1 ± 3.3	24.5 ± 2.9
SBP (mm Hg)	121.7 ± 8.1	123.7 ± 7.8
DBP (mm Hg)	71.4 ± 5.48	71.6 ± 5.5
PWV (m/s)	6.5 ± 0.8	6.5 ± 0.7

Table 3. Mean total energy and vitamin D₃ intake at baseline and intervention.

	Baseline (Mean ± SD)	Intervention (Mean ± SD)	Difference	<i>p</i> Value
Total energy intake (kcal/d)	1417.1 ± 301.6	1352.6 ± 256.5	65.4	0.28
Fat (g)	52.3 ± 17.9	49.1 ± 18.0	3.2	0.66
Protein (g)	48.2 ± 14.8	45.3 ± 12.0	2.9	0.53
Carbohydrates (g)	189.1 ± 44.1	182.9 ± 25.1	−6.2	0.07
Vitamin D ₃ (µg/d)	1.93 ± 1.11	21.99 ± 1.06	20.06	<0.0001

3.2. Blood Pressure

All the analyzed data sets were tested for normal distribution. Subjects 3 and 7 were removed from analysis of blood pressure as they reported being particularly stressed on the day of post-intervention measurements, which was also clearly reflected by the value of obtained readings. The series of paired-samples *t*-test were carried out to analyze the impact of vitamin D on BP in 18 eligible subjects. As demonstrated in Table 4, significant decrease in SBP by 5.3 ± 6.46 mmHg ($p = 0.032$) and DBP by 3.3 ± 4.46 mmHg ($p = 0.002$) was observed following 2 w of the supplementation. It was not possible to assess the power of the effect in males and females separately due to the low number of subjects in each group (see Table 4). No statistical differences in mean SBP and DBP readings between baseline and placebo were found in those taking the placebo.

Table 4. Mean difference in BMI, systolic and diastolic blood pressure, and pulse wave velocity (PWV).

Vitamin D3 Arm	Baseline (Mean ± SD)	Intervention (Mean ± SD)	Difference (Mean ± SD)	<i>p</i> -Value
Systolic (mmHg)	121.7 ± 8.1	116.4 ± 7.2	5.3 ± 6.46	0.032
Diastolic (mmHg)	71.4 ± 5.48	68.1 ± 6.17	3.3 ± 4.46	0.002
PWV (m/s)	6.51 ± 0.8	6.03 ± 0.6	0.48 ± 0.31	0.007
BMI (Kg/m ²)	24.1 ± 3.3	23.81 ± 3.23	0.29 ± 0.12	0.161
Placebo arm				
Systolic (mmHg)	123.7 ± 7.8	122.9 ± 8.1	0.8 ± 0.76	0.432
Diastolic (mmHg)	71.6 ± 5.5	71.3 ± 6.1	0.3 ± 0.46	0.752
PWV (m/s)	6.5 ± 0.7	6.46 ± 0.9	0.04 ± 0.4	0.542
BMI (Kg/m ²)	24.5 ± 2.9	24.35 ± 3.1	0.15 ± 0.3	0.338

3.3. BMI and Pulse Wave Velocity

Small but not significant decrease in mean body weight as BMI was observed (0.29 kg); ($p = 0.161$). The overall paired-samples *t*-test showed a modest but significant drop of mean PWV by 0.48 ± 0.31 m/s ($p = 0.007$) following vitamin D intake. However, there was no significant changes observed in BMI and PWV between basal and intervention in those taking the placebo (see Table 4).

3.4. Cortisol and Cortisol/Cortisone Ratio

Table 5 shows the slight increase in mean overall salivary cortisol levels from baseline to intervention but was not statistically no significant ($p = 0.556$). Salivary cortisol and cortisone levels showed clearly a typical circadian rhythm as expected indicating the healthy condition of participants. Interestingly, there was a significant but small increase in salivary cortisone at all collection times and the overall salivary cortisone increased from 5.33 ± 2.6 to 6.98 ± 3.3 nmole ($p = 0.042$). Salivary cortisol/cortisone ratio was reduced from 0.952 ± 0.54 to 0.784 ± 0.68 , $p = 0.028$. Urinary free cortisol/cortisone ratio was also reduced (1.71 ± 0.71 to 1.22 ± 0.62 , $p = 0.012$). No statistical differences in mean cortisol or cortisone levels between baseline and placebo were found at all collection times after analysis with paired-samples t -test; $p = 0.130$, $p = 0.806$, $p = 0.98$, respectively (data were not shown). Also, the changes in salivary cortisol/cortisone ratio and urinary free cortisol/cortisone ratio were not statistically significant in those taking the placebo.

Table 5. Salivary cortisol and cortisone levels in the vitamin D group at day 0 and day 14 (mean $\pm$ SD nmole) following vitamin D intake.

Pomegranate Group		Cortisol (ng/mL)	Sig.	Cortisone (ng/mL)	Sig.
		Mean $\pm$ SD	p Value	Mean $\pm$ SD	p Value
Morning	Day 0	6.17 ± 1.7		7.5 ± 2.8	
	Day 14	6.71 ± 2.1	0.321	8.72 ± 2.9	0.05
Noon	Day 0	4.11 ± 1.5		4.13 ± 1.4	
	Day 14	4.26 ± 1.7	0.568	6.26 ± 2.9	0.044
Evening	Day 0	2.89 ± 1.8		3.74 ± 1.3	
	Day 14	3.32 ± 1.5	0.508	5.28 ± 2.8	0.048
Overall	Day 0	4.54 ± 1.5		5.33 ± 2.6	
Overall	Day 14	4.76 ± 1.6	0.556	6.98 ± 3.3	0.042

4. Discussion

This study observed significant drops in SBP and DBP by 5.3 and 3.3 mmHg, respectively. This is in accordance with previous investigations into the effect of vitamin D supplementation [39]. Interestingly, while the intervention period applied in these studies was at least 8 w long, this study showed that 2 w of vitamin D supplementation can be enough to induce a significant drop in BP. It can be argued that such a fast effect could be attributed to the participants' lower mean age, in contrast to the earlier mentioned studies where participants were recruited from the elderly population. It has been shown that the older people experience a drop in intestinal absorption of cholecalciferol as well as a decrease in activity of renal 1α -hydroxylase enzyme, along with the gradual impairment of renal functions [40]. In addition, the effectiveness of vitamin D supplementation on different body organs could be attenuate with age. Other studies, however, reported no effect on BP [41]. This disagreement could be explained by the low dose of vitamin D applied in those studies, 400 IU and 200 IU (10 and 5 μ g/d), respectively. Several plausible explanations for the regulation of BP by vitamin D have been suggested; the observed drop of BP might likely be mediated by the down-regulating effect of vitamin D on renin synthesis and the renin–angiotensin–aldosterone system [42]. Moreover, the effect could be attributed to the suppressing effect of vitamin D of PTH, which has been shown to be positively correlated with SBP in normotensive subjects. In addition, BP could be partially reduced by the inhibition of 11β HSD type 1 enzyme activity indicated by the reduction of salivary and urine cortisol/cortisone ratio [43,44]. Despite being very modest, the observed decrease in BP has the potential of improving CV risk. It has been reported that, in the general population, a drop of DBP by as little as 2 mmHg could result in reduction of hypertension prevalence by 17% and the risk of stroke and CHD by 15%

and 6%, respectively [45]. It may therefore be argued that in hypertensive subjects the BP lowering effect of vitamin D might have been more profound. Moreover, the current study did not control for sodium intake differences which could have acted as a confounding factor. Vitamin D supplementation produced a significant decrease in right carotid femoral PWV of 0.48 m/s, which is considered a 'golden standard' parameter of assessing arterial compliance in the central vasculature. This finding agrees with a previous study [13] who reported that vitamin D₃ supplementation in healthy black youths resulted in a decrease of PWV by 0.8 m/s. The observed improvement in arterial compliance could be due to the reported anti-inflammatory effect of vitamin D [17] or could also be attributable to the role of vitamin D in calcium metabolism and its beneficial effect on insulin sensitivity and glucose metabolism, as reviewed by Razzaque [46] and Richart Li et al. [47]. In addition, it might be partly mediated by the observed drop in blood pressure since arterial compliance highly depends on blood pressure values [39]. The changes of vitamin D supplementation in arterial compliance and BP could offer the means to produce beneficial alterations in the arterial functions as well as the cardiovascular system in general. Epidemiological evidence suggested an inverse relationship between low vitamin D status and obesity and studies reported significant weight loss following vitamin D supplementation. However, our findings showed no significant change in weight or BMI following two of vitamin D intake. This might be due to the dose used was not enough to observe significant effect on BMI as we have not estimated the blood level of vitamin D achieved in our subjects coupled with the known low bioavailability of vitamin D supplements. In addition, the duration of our study could have been too short for any detectable change in weight to occur, and other studies have also reported no effect of weight loss [48].

Despite the slight increase in cortisol concentration observed after vitamin D₃ supplementation, it was not significant but the increase in salivary cortisone concentrations was significant and, consequently, produced a reduction in the cortisol/cortisone ratio which indicates an inhibition of 11 β HSD type 1 enzyme activity. This has also been substantiated by the reduction of urinary cortisol/cortisone ratio and might partially explain the observed reduction in BP following vitamin D intake [7]. It was reassuring to observe the clear typical circadian rhythm profile of salivary cortisol and cortisone levels in participants' samples which indicated good compliance, as far as saliva collection. It would be interesting to investigate if longer administration could result in significant alteration of the hormone levels. Such a study would be justified in the view of previously reported findings showing that longer supplementation with vitamin D is necessary to raise the level of 25-OH D₃ [49] depending on the extent of deficiency as well as the dose and chemical formula of vitamin D given. It may take up to 3 m to raise the blood concentration of 25-OH D to a satisfactory level [50]. Knowing the importance of glucocorticoids in cardiovascular health, further studies should also analyze the effect of vitamin D on the cortisol/cortisone ratio that can assess the activity of the enzyme 11 Beta Hydroxysteroid dehydrogenase (11B-HSD) [50,51]. Two isozymes of 11B-HSD exist that catalyze the interconversion of cortisone (inactive) and cortisol (active), thus always controlling their activity.

The main strength of this study is the introduction of 20 μ g/d vitamin D without any other supplements such as calcium allowed determination of the effect of vitamin D alone on CVD risk factors. Furthermore, the analysis of participants' food intake served as some measure of control regarding extra sources of vitamin D. A sunlight exposure questionnaire was not applied in this study [13], as it has been shown to be an unreliable method of estimating amount of synthesized vitamin D and whole-body vitamin D status [52]. It is important to acknowledge certain limitations; restricted time available for carrying out this study, number of participants, short intervention period, and limited resources that did not allow measurement of participants baseline and post intervention vitamin D status. This would have greatly assisted the data analysis as it would have shown to what level the subjects were deficient before entering the study, and to what degree supplementation raised plasma 25-OH D. It would also have been useful to include 20 subjects in the placebo arm of the study, however, due to time constraints this was not possible. Using saliva as a

means of determining systemic cortisol and cortisone levels is a well-established, reliable method that helps minimize the level of stress resulting from participating in the study [53]. However, introducing the perceived stress level questionnaire would have been beneficial.

5. Conclusions

Vitamin D₃ supplementation decreases both diastolic and systolic BP and improves arterial compliance but does not alter BMI or salivary cortisol levels. However, there was a reduction of salivary and urine free cortisol/cortisone ratio indicating an inhibition of 11 β HSD type 1 enzyme activity. The results suggest that vitamin D₃ could have the potential to reduce the risk of hypertension and cardiovascular diseases in young healthy adults. Further studies with larger number of participants for longer duration are warranted with controlled conditions to test the reproducibility of the obtained results in this study.

Author Contributions: Conceptualization and supervision, E.A.S.A.-D.; methodology and formal analysis, E.A.S.A.-D. and M.N.A.H.; writing and revision, M.N.A.H. and E.A.S.A.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Queen Margaret University (code Honors/07004211/VitaminD/BSc-NUT/DNBS/QMU).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Study data are available from the authors upon request.

Acknowledgments: The authors would like to thank Joanna Kita of the Queen Margaret University for her technical support and the University of Edinburgh for the estimation of steroid hormones. We are also grateful for all the volunteers who participated in this research study.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BP	Blood Pressure
BMI	Body Mass Index
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure;
ELISA	Enzyme-Linked Immuno Sorbent Assay
25-OH D	25-hydroxyvitamin D
1.25-(OH) ₂ D	1.25-dihydroxy-vitamin D
PTH	Parathyroid Hormone
NO	Nitric Oxide
PWV	Pulse Wave Velocity
SBP	Systolic Blood Pressure
SD	Standard Deviation

References

1. Davies, M.R.; Hruska, K.A. Pathophysiological mechanisms of vascular calcification in end-stage renal disease. *Kidney Int.* **2001**, *60*, 472–479. [[CrossRef](#)] [[PubMed](#)]
2. Pilz, S.; Dobnig, H.; Fischer, J.E.; Wellnitz, B.; Seelhorst, U.; Boehm, B.; März, W. Low vitamin D levels predict stroke in patients referred to coronary angiography. *Stroke* **2008**, *39*, 2611–2613. [[CrossRef](#)] [[PubMed](#)]
3. Giovannucci, E.; Rimm, E.B.; Liu, Y.; Hollis, B.W. 25-hydroxyvitamin D and risk of myocardial infarction in men: A prospective study. *Arch. Intern. Med.* **2008**, *168*, 1174–1180. [[CrossRef](#)] [[PubMed](#)]
4. Pilz, S.; Tomaschitz, A.; Ritz, E.; Drechsler, C.; Zittermann, A.; Dekker, J.M.; März, W. Vitamin D status and arterial hypertension: A systematic review. *Nat. Rev. Cardiol.* **2009**, *6*, 621–630. [[CrossRef](#)]
5. Ginde, A.A.; Scragg, R.; Schwartz, R.S.; Camargo, C.A. Prospective study of serum 25-hydroxyvitamin D level, cardiovascular disease mortality, and all-cause mortality in older US adults. *J. Am. Geriatr. Soc.* **2009**, *57*, 1595–1603. [[CrossRef](#)]
6. Holick, M.F. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin. Proc.* **2006**, *81*, 353–373. [[CrossRef](#)]

7. Al-Dujaili, E.A.; Munir, N.; Iniesta, R.R. Effect of vitamin D supplementation on cardiovascular disease risk factors and exercise performance in healthy participants: A randomized placebo-controlled preliminary study. *Ther. Adv. Endocrinol. Metab.* **2016**, *7*, 153–165. [\[CrossRef\]](#)
8. Zittermann, A.; Gummert, J.F. Nonclassical vitamin D actions. *Nutrients* **2010**, *2*, 408–425. [\[CrossRef\]](#)
9. Binkley, N.; Ramamurthy, R.; Krueger, D. Low vitamin D status: Definition, prevalence, consequences, and correction. *Endocrinol. Metab. Clin. N. Am.* **2010**, *39*, 287–301. [\[CrossRef\]](#)
10. Dawson-Hughes, B.; Heaney, R.P.; Holick, M.F.; Lips, P.; Meunier, P.J.; Vieth, R. Estimates of optimal vitamin D status. *Osteoporos. Int.* **2005**, *16*, 713–716. [\[CrossRef\]](#)
11. Alam, M.S.; Czajkowsky, D.M.; Islam, M.A.; Rahman, M.A. The role of vitamin D in reducing SARS-CoV-2 infection: An update. *Int. Immunopharmacol.* **2021**, *97*, 107686. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Laurent, S.; Boutouyrie, P.; Asmar, R.; Gautier, I.; Laloux, B.; Guize, L. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* **2001**, *37*, 1236–1241. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Dong, Y.; Stallmann-Jorgensen, I.S.; Pollock, N.K.; Harris, R.A.; Keeton, D.; Huang, Y.; Li, K.; Bassali, R.; Guo, D.; Thomas, J.; et al. A 16-w randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 4584–4591. [\[CrossRef\]](#)
14. Mancia, G.; De Backer, G.; Dominiczak, A.; Cifkova, R.; Fagard, R.; Germano, G.; Grassi, G.; Heagerty, A.M. 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). *J. Hypertens.* **2007**, *25*, 1105–1187. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Avolio, A. Arterial stiffness. *Pulse* **2013**, *1*, 14–28. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Blacher, J.; Asmar, R.; Djane, S.; London, G.M.; Safar, M.E. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* **1999**, *33*, 1111–1117. [\[CrossRef\]](#)
17. Sugden, J.; Davies, J.; Witham, M.; Morris, A.D.; Struthers, A.D. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. *Diabet. Med.* **2008**, *25*, 320–325. [\[CrossRef\]](#)
18. Van den Berghe, G.; Van Roosbroeck, D.; Vanhove, P.; Wouters, P.J.; De Pourcq, L.; Bouillon, R. Bone turnover in prolonged critical illness: Effect of vitamin D. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 4623–4632. [\[CrossRef\]](#)
19. Cannata-Andía, J.B.; Rodríguez-García, M.; Carrillo-López, N.; Diaz-Lopez, B. Vascular calcifications: Pathogenesis, management, and impact on clinical outcomes. *J. Am. Soc. Nephrol.* **2006**, *17* (Suppl. 3), S267–S273. [\[CrossRef\]](#)
20. Scragg, R.; Sowers, M.; Bell, C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am. J. Hypertens.* **2007**, *20*, 713–719. [\[CrossRef\]](#)
21. Kota, S.K.; Kota, S.K.; Jammula, S.; Tripathy, P.R.; Panda, S.; Modi, K.D. Renin–angiotensin system activity in vitamin D deficient, obese individuals with hypertension: An urban Indian study. *Indian J. Endocrinol. Metab.* **2011**, *15* (Suppl. 4), S395–S401. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Snijder, M.B.; van Dam, R.M.; Visser, M.; Seidill, J.C. Adiposity in relation to vitamin D status and parathyroid hormone levels: A population-based study in older men and women. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 4119–4123. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Querfeld, U.; Hoffmann, M.M.; Klaus, G.; Eifinger, F.; Ackerschott, M.; Michalk, D.; Kern, P.A. Antagonistic effects of vitamin D and parathyroid hormone on lipoprotein lipase in cultured adipocytes. *J. Am. Soc. Nephrol.* **1999**, *10*, 2158–2164. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Connell, J.M.; Davies, E. The new biology of aldosterone. *J. Endocrinol.* **2005**, *186*, 1–20. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Hammer, F.; Stewart, P.M. Cortisol metabolism in hypertension. *Best Pract. Res. Clin. Endocrinol. Metab.* **2006**, *20*, 337–353. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Whitworth, J.A.; Williamson, P.M.; Mangos, G.; Kelly, J.J. Cardiovascular consequences of cortisol excess. *Vasc. Health Risk Manag.* **2005**, *1*, 291. [\[CrossRef\]](#)
27. Stewart, J.; Manmathan, G.; Wilkinson, P. Primary prevention of cardiovascular disease: A review of contemporary guidance and literature. *JRSM Cardiovasc. Dis.* **2017**, *6*, 2048004016687211. [\[CrossRef\]](#)
28. Mancia, G.; Fagard, R.; Narkiewicz, K.; Rendon, J.; Zanchetti, A.; Böhm, M.; Christiaens, T.; Cifkova, R.; De Backer, G.; Dominiczak, A.; et al. 2013 ESH/ESC practice guidelines for the management of arterial hypertension: ESH-ESC 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.* **2013**, *34*, 2159–2219. [\[CrossRef\]](#)
29. Walker, B.R.; Best, R.; Shackleton, C.H.; Padfield, P.L.; Edwards, C.R. Increased vasoconstrictor sensitivity to glucocorticoids in essential hypertension. *Hypertension* **1996**, *27*, 190–196. [\[CrossRef\]](#)
30. Geer, E.B.; Islam, J.; Buettner, C. Mechanisms of glucocorticoid-induced insulin resistance: Focus on adipose tissue function and lipid metabolism. *Endocrinol. Metab. Clin. N. Am.* **2014**, *43*, 75–102. [\[CrossRef\]](#)
31. Morton, N.M.; Seckl, J.R. 11 β -hydroxysteroid dehydrogenase type 1 and obesity. *Front. Horm. Res.* **2008**, *36*, 146–164. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Jackson, L.V.; Thalange, N.K.; Cole, T.J. Blood pressure centiles for Great Britain. *Arch. Dis. Child.* **2007**, *92*, 298–303. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Hickson, S.S.; Butlin, M.; Broad, J.; Avolio, A.P.; Wilkinson, I.B.; McEniery, C.M. Validity and repeatability of the Vicorder apparatus: A comparison with the SphygmoCor device. *Hypertens. Res.* **2009**, *32*, 1079–1085. [\[CrossRef\]](#) [\[PubMed\]](#)

34. Laurent, S.; Cockcroft, J.; Van Bortel, L.; Boutouyrie, P.; Giannattasio, C.; Hayoz, D.; Pannier, B.; Vlachopoulos, C.; Wilkinson, I.; Struijker-Boudier, H. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. *Eur. Heart J.* **2006**, *27*, 2588–2605. [[CrossRef](#)]
35. Al-Dujaili, E.A. Development and validation of a simple and direct ELISA method for the determination of conjugated (glucuronide) and non-conjugated testosterone excretion in urine. *Clin. Chim. Acta* **2006**, *364*, 172–179. [[CrossRef](#)]
36. Al-Dujaili, E.A.; Baghdadi, H.H.; Howie, F.; Mason, J.I. Validation and application of a highly specific and sensitive ELISA for the estimation of cortisone in saliva, urine and in vitro cell-culture media by using a novel antibody. *Steroids* **2012**, *77*, 703–709. [[CrossRef](#)] [[PubMed](#)]
37. Aardal, E.; Holm, A.-C. Cortisol in saliva-reference ranges and relation to cortisol in serum. *Eur. J. Clin. Chem. Clin. Biochem.* **1995**, *33*, 927–932. [[CrossRef](#)]
38. Boutouyrie, P.; Vermeersch, S. Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur. Heart J.* **2010**, *31*, 2338–2350. [[CrossRef](#)]
39. Pfeifer, M.; Begerow, B.; Minne, H.W.; Nachtigall, D.; Hansen, C. Effects of a short-term vitamin D3 and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 1633–1637. [[CrossRef](#)]
40. Gallagher, J.; Riggs, B.L.; Eisman, J.; Hamstra, A.; Arnaud, S.B.; DeLuca, H.F. Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients: Effect of age and dietary calcium. *J. Clin. Investig.* **1979**, *64*, 729–736. [[CrossRef](#)]
41. Margolis, R.N.; Christakos, S. The nuclear receptor superfamily of steroid hormones and vitamin D gene regulation: An update. *Ann. N. Y. Acad. Sci.* **2010**, *1192*, 208–214. [[CrossRef](#)] [[PubMed](#)]
42. Li, Y.C. Vitamin D regulation of the renin-angiotensin system. *J. Cell Biochem.* **2003**, *88*, 327–331. [[CrossRef](#)] [[PubMed](#)]
43. Al-Dujaili, E.A.S.; Good, G.; Tsang, C. Consumption of Pomegranate Juice Attenuates Exercise-Induced Oxidative Stress, Blood Pressure and Urinary Cortisol/Cortisone Ratio in Human Adults. *EC Nutr.* **2016**, *4*, 982–995.
44. Palermo, M.; Shackleton, C.H.; Mantero, F.; Stewart, P.M. Urinary free cortisone and the assessment of 11 β -hydroxysteroid dehydrogenase activity in man. *Clin. Endocrinol.* **1996**, *45*, 605–611. [[CrossRef](#)] [[PubMed](#)]
45. Avenell, A.; Cook, J.A.; MacLennan, G.S.; MacPherson, G. Vitamin D supplementation and type 2 diabetes: A substudy of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). *Age Ageing* **2009**, *38*, 606–609. [[CrossRef](#)] [[PubMed](#)]
46. Razzaque, M.S. The dualistic role of vitamin D in vascular calcifications. *Kidney Int.* **2011**, *79*, 708–714. [[CrossRef](#)] [[PubMed](#)]
47. Richart, T.; Li, Y.; Staessen, J.A. Renal versus extrarenal activation of vitamin D in relation to atherosclerosis, arterial stiffening, and hypertension. *Am. J. Hypertens.* **2007**, *20*, 1007–1015. [[CrossRef](#)]
48. Zittermann, A.; Frisch, S.; Berthold, H.K.; Gotting, C.; Kuhn, J.; Kleesiek, K.; Stehle, P.; Koertke, H.; Koerfer, R. Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. *Am. J. Clin. Nutr.* **2009**, *89*, 1321–1327. [[CrossRef](#)]
49. Romero-Ortuno, R.; Cogan, L.; Browne, J.; Healy, M.; Casey, M.C.; Cunningham, C.; Walsh, J.B.; Kenny, R.A. Seasonal variation of serum vitamin D and the effect of vitamin D supplementation in Irish community-dwelling older people. *Age Ageing* **2011**, *40*, 168–174. [[CrossRef](#)]
50. Al-Dujaili, E.; Giudice, V.; Fyfe, L. Effects of vitamin D supplementation on blood pressure, glucocorticoids and cardiovascular risk markers in healthy subjects. *Endocr. Abstr. Biosci.* **2013**, *31*, 184. [[CrossRef](#)]
51. Quinkler, M.; Stewart, P.M. Hypertension and the cortisol-cortisone shuttle. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 2384–2392. [[CrossRef](#)] [[PubMed](#)]
52. Brock, K.; Huang, W.-Y.; Fraser, D.; Ke, L.; Tseng, M.; Stolzenberg-Solomon, R.; Peters, U.; Ahn, J.; Purdue, M.; Mason, R.S.; et al. Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 462–466. [[CrossRef](#)] [[PubMed](#)]
53. Aardal-Eriksson, E.; Karlberg, B.E.; Holm, A.-C. Salivary cortisol-an alternative to serum cortisol determinations in dynamic function tests. *Clin. Chem. Lab. Med.* **1998**, *36*, 215–222. [[CrossRef](#)] [[PubMed](#)]