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

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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. To investigate effect of short-term supplementation with vitamin D_3 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. 20 healthy, normotensive participants were instructed to consume 20 µg/day of vitamin D_3 for two weeks, and 10 volunteers received a placebo. BP, PWV, BMI and salivary cortisol level were assessed at baseline and after 2 weeks' time. Vitamin D and total energy intakes were also evaluated. Results: After 2 weeks 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 D_3 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 D_3 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

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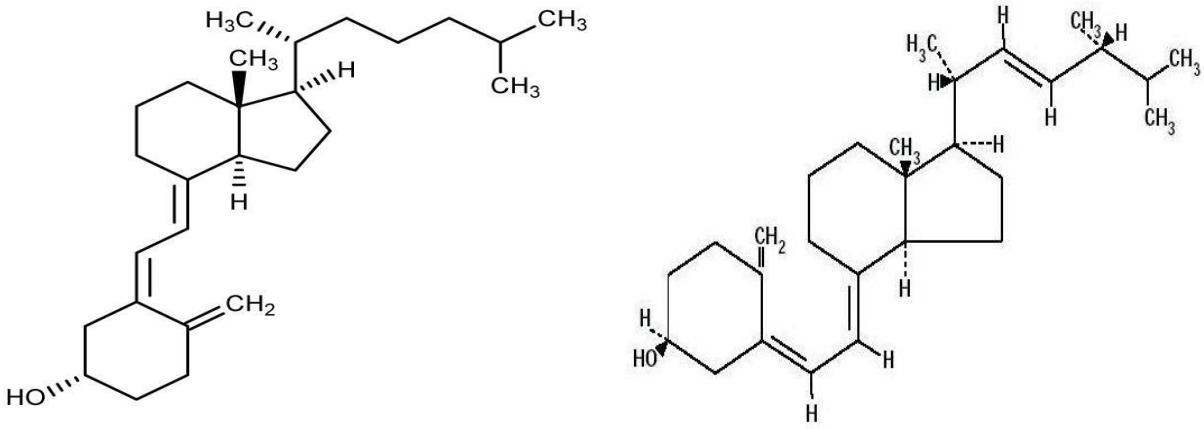
1. Introduction

Vitamin D has been established to have multiple functions 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 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 the sunlight since cutaneous synthesis of vitamin D_3 (Figure 1) upon sufficient exposure to ultraviolet-B (UVB) radiation could provide about 80% of body vitamin D [8]. Plasma vitamin D_3 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 the infection with COVID SARS-CoV-2 [11].



(**A**) (**B**)

Figure 1. (**A**) Structure of vitamin D_3 (Cholecalciferol), (**B**) Structure of 25-hydroxyvitamin D (vitamin D_3).

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 [16] 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 3mmHg 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 [10,24]. Glucocorticoids play an important role in maintaining extracellular fluid volume and normal BP [25].

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 [26]. 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 [26,27]. 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 [28]. Hypertension is among the leading causes of cardiovascular morbidity and mortality [29]. Cardiovascular event as consequences of increased cortisol secretion and circulating levels in patients with essential hypertension [30], glucose intolerance and insulin resistance have been reported [31]. It was also established that intracellular glucocorticoid reactivation was elevated in adipose tissue of humans, and 11β hydroxysteroid dehydrogenase (11β-HSD) is the principal enzyme responsible for regulating glucocorticoid metabolism [32]. 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 days with the total intervention period of 14 days over where participants were required to consume 20 μg/day of vitamin D_3 (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. Thirty volunteers (18 females and 12 males) agreed to take part in the study and breastfeeding. 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	5 days wash out period for all volunteers Completing 2-days diet diary
1 day before starting supplementation	Collection of three saliva samples and 24-h urine sample (Baseline data)
Week 1: Supplementation starts (Vitamin D3 or placebo)	First meeting (Day1); measure BP, PWV, weight and height.
Week 2: Supplementation continues (Vitamin D3 or placebo).	Completing 2-days diet diary
1 day 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 two-day 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-days 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 one day before the onset of the supplementation and on the 13th day 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 [33].

Right carotid femoral PWV (pulse wave velocity) was evaluated with a validated device (Vicorder™; Bristol, UK) [34]. PWV is a simple and non-invasive method which has been previously shown to provide accurate and reproducible results [35]. PWV measures pulse transit time and the distance travelled by the pulse between the two recording sites.

$$\text{PWV} = \text{distance (meters)}/\text{transit time (seconds)}.$$

Three readings of PWV were estimated with one-minute 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-day diet diaries were analyzed with the WinDiet 2015 program 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 [36,37]. 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). 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 ± 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 [38]. 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 [39]. 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).

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

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 underreporting. The mean intake of vitamin D_3 after introducing 20 μg (800 IU) supplement was significantly higher ($p < 0.0001$) (See Table 3).

Table 3. Mean total energy and vitamin D_3 intake at baseline and intervention.

	Baseline (Mean ± SD)	Intervention (Mean ± SD)	Difference	*p* Value
Total energy intake (kcal/day)	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/day)	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 & 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 weeks 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)	*p*-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 (0.29kg); ($p = 0.161$). 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 ± SD nmole) following vitamin D intake.

Pomegranate Group		Cortisol (ng/mL)	Sig.	Cortisone (ng/mL)	Sig.
		Mean ± SD	*p* Value	Mean ± 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 [40]. Interestingly, while the intervention period applied in these studies was at least eight weeks long, this study showed that two weeks 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 [41]. 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 [42]. This disagreement could be explained by the low dose of vitamin D applied in those studies, 400 IU and 200 IU (10 and 5 μg/day) 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 renin-angiotensin-aldosterone system [43]. 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 11βHSD type 1 enzyme activity indicated by the reduction of salivary and urine cortisol/cortisone ratio [44,45]. 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 2mmHg could result in reduction of hypertension prevalence by 17% and the risk of stroke and CHD by 15% and 6%, respectively [46]. 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 [47] study who reported that vitamin D_3 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 [18], 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 [48] and Richart Li et al. [49]. In addition, it might be partly mediated by the observed drop in blood pressure since arterial compliance highly depends on blood pressure values [50]. 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 weeks 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 [51].

Despite the slight increase in cortisol concentration observed after vitamin D_3 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 11βHSD 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 D_3 [52] depending on the extent of deficiency as well as the dose and chemical formula of vitamin D given. It may take up to 3 months to raise the blood concentration of 25-OH D to a satisfactory level [52]. 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) [53,54]. 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 μg/day 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 [47], as it has been shown to be an unreliable method of estimating amount of synthesized vitamin D and whole-body vitamin D status [55]. 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 [56]. However, introducing the perceived stress level questionnaire would have been beneficial.

5. Conclusions

Vitamin D_3 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 D_3 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.

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Conflicts of Interest: The authors declare no conflicts 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)2D	1.25-dihydroxy-vitamin D
PTH	Parathyroid Hormone
NO	Nitric Oxide
PWV	Pulse Wave Velocity
SBP	Systolic Blood Pressure
SD	Standard Deviation

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