

Review Article

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Vitamin D & endothelial function

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There is increasing interest in the extra-skeletal roles of vitamin D for health and well-being. Poor vitamin D status has been associated with obesity, cardiovascular disease, type 2 diabetes and mental health. Endothelial dysfunction may underscore insulin resistance and hence predispose to both cardiovascular disease (CVD) and type 2 diabetes. The objective of this review was to gain an appreciation of the recent causative evidence linking vitamin D and endothelial function. The PubMed database was searched from 2009 to date. Key words used were vitamin D, supplementation, systemic inflammation, endothelium, endothelial dysfunction and humans. Selected articles were restricted to the English language and to randomized control trials (RCTs) of vitamin D supplementation with direct measures of endothelial function. Final inclusion was based on a quality rating ≥ 3 , based on the Jadad score. Ten RCTs met these criteria and were summarized for their outcomes. Only two studies showed an improvement in flow mediated dilatation with vitamin D. Three other studies reported decreases in C-reactive protein, platelet activation inhibitor-1, tissue plasminogen activator or B type natriuretic peptide. Recent evidence from good quality RCTs did not support a beneficial effect of vitamin D on vascular reactivity. Future intervention studies may need to target a higher vitamin D status and longer duration to determine whether the vitamin has a regulatory role in endothelial function.

Key words Endothelial function - flow mediated dilatation - inflammation - obesity - supplementation - vitamin D

Vitamin D and health

Like many parts of the world including Australia, India faces the burden of obesity with a significant percentage (around 30-65%) of adult urban Indians diagnosed as overweight or obese or with abdominal obesity¹. Interestingly both India and Australia have abundant milk supplies and plentiful sunshine, yet large sections of their populations have lower than recommended dietary intakes of calcium (Ca)² and low vitamin D status³⁻⁵. Calcium and vitamin D have

many potential roles in human physiology but are accepted mainly for their influence on bone health⁶. The evidence base that associates calcium intake and vitamin D status with obesity, cardiovascular disease, type 2 diabetes and more recently cognitive effects is growing. It ranges from the cellular to animal to human clinical and epidemiological investigations⁷⁻¹⁰. However, the balance of evidence that tilts either one or both nutrients towards an extra-skeletal health effect is yet to be reached. In this review we questioned whether

vitamin D status was causally linked to endothelial function. We present an overview of vascular function and commonly used methods to measure vascular dysfunction. All randomised controlled trials conducted in the recent past that have supplemented vitamin D have been collated to determine its putative effects on vascular function.

The vascular endothelium is a monolayer at the interface between blood and tissue. This pivotal role allows endothelial cells to detect and react to blood-borne signals and changes in haemodynamic forces. The vasodilatory impact of three endothelial cell (EC) products, nitric oxide (NO), endothelium-derived hyperpolarising factor (EDHF) and prostacyclin (PGI₂), on the underlying smooth muscle cells is countered by the vasoconstrictor EC factor, endothelin-1 in the regulation of vascular tone¹¹. In response to various stimuli including shear stress at the endothelial cell surface, NO can also diffuse towards the lumen and prevent both monocyte and platelet adhesion¹¹. Thus NO's role reaches beyond vasodilation to encompass protection from inflammation and thrombosis after vascular injury. These roles are challenged by risk factors associated with cardiovascular disease.

Basal vasodilator tone is primarily controlled by the continual endothelium-dependent production of NO, however, the smooth muscle cells may not respond to this signal¹². While activation of endothelial nitric oxide synthase (eNOS) is central to endothelial-dependent vasodilation, mechanisms supporting endothelial-independent vasodilation include the stimulation of phospholipase A₂ activity¹³. In order to distinguish between EC and smooth muscle cell dysfunction, both endothelium dependant and independent systems need to be assessed¹¹.

Measurement of endothelial function

Endothelium function is usually measured by assessing either coronary arteries or peripheral arteries for vascular reactivity. The initial technique for evaluation of endothelial dysfunction was based on invasive procedures in which artery catheterization was required to assess endothelial dependent vasodilation¹⁴. Alternative non-invasive techniques have been developed that are more practicable than conventional methods. Peripheral artery studies usually focus on a phenomenon called flow-mediated dilation (FMD)¹⁵. FMD occurs when endothelial cells release NO in response to a shear stress^{15,16}. Built on this principle, evaluation of FMD was developed using ultrasound

imaging^{15,17} that captured the change in the diameter of a peripheral artery (typically brachial artery)¹⁷. The measurement of FMD is considered the gold standard in measuring EF and is usually accomplished in response to shear stress; acetylcholine infusion; salbutamol inhalation (reflecting the endothelium dependent pathway); or in response to sub-lingual glyceryl trinitrate (reflecting the endothelium independent pathway)¹⁷. Due to the high level of technical skill required in procuring and analysing ultrasound images, the use of other simpler techniques has gained popularity and credibility. Arterial applanation tonometry uses a sensitive probe applied to carotid and femoral arteries in the same subject to determine characteristics of the transmitted wave form¹⁸. Augmentation index (AI_x, ratio between the pulse pressure at the second systolic peak and the pulse pressure at the first systolic peak), is a derived variable that reflects endothelial function. Another validated system employs finger photoplethysmography to produce a digital volume pulse analysis (DVP)¹⁸. The calculated parameters from this analysis are stiffness index (SI), a measure of large artery stiffness, and reflective index (RI) that signifies small artery vascular tone. Vasodilation leads to a smaller RI while vasoconstriction results in a rise in its value. Hence, the analysis of DVP is relatively simple and the results are strongly correlated with AI_x and central pulse wave velocity^{17,18}. These non-invasive techniques have been used in conjunction with the appropriate pharmacological agent to measure both endothelium dependent and independent pathways of EF. Based on meal based stimuli, we demonstrated that the acute ingestion of calcium and vitamin D as part of a breakfast meal resulted in dose dependant changes in RI of Indian men based on a DVP analysis system¹⁹.

Vitamin D, systemic and vascular inflammation

The link between systemic inflammation and vascular-specific inflammation from endothelial activation is well established²⁰. There are numerous vascular markers of endothelial damage and their role in the pathophysiology of endothelial dysfunction is excellently covered elsewhere¹¹. Hence, factors associated with thrombus formation and control, like plasma level of von Willebrand factor or plasminogen activator inhibitor (PAI-1) are a reflection of endothelial dysfunction¹⁷. Secondly, increment in the inflammatory markers such as C-reactive protein (CRP), cellular adhesion molecules (CAMs), vascular adhesion molecules, and P- or E-selectin have also been used to detect endothelial dysfunction¹⁷. Table I briefly

Table I. Some commonly used circulating biomarkers in studies of endothelial function

Biomarker	Stands for	The role in body	The relationship with endothelial function
D-dimer	A fibrin degradation product of cross-linked fibrin	Indicates the occurrence of thrombin generation and plasmin generation in the blood	Inverse ²¹
sICAM-1	soluble intercellular adhesion molecule type 1	Helps leukocytes migrate across endothelial cells in the inflammatory state.	Inverse ²²
sVCAM-1	soluble vascular cell adhesion molecule-1	Assists lymphocytes, monocytes, eosinophil and basophils to adhere to vascular endothelium.	Inverse ²²
MPO	myeloperoxidase	Enzyme produced by the neutrophils; converts nitrite to nitrate; acts as a bactericidal agent, regulates the availability of nitric oxide in the blood; is associated with many chronic diseases.	Inverse ²³
P-selectin	P-selectin	Is an adhesion molecule for leukocytes on the surface of the endothelium.	Inverse ²⁴
E-selectin	E-selectin	Is an adhesion molecule for leukocytes in endothelial cells.	Inverse ²²
IL-6	interleukin 6	IL-6 triggers the production of collagenases and prostaglandins which reduce the pain threshold. Also stimulates T and B cells in their immune mechanisms.	Inverse ²⁵
IL-1 β	interleukin- 1 beta	IL-6 beta is able to induce fever, anorexia and hypotension. May also control mycobacterial proliferation in the macrophage.	Inverse ²⁶
IL-12	interleukin -12	Improves the functioning of T-helper 1 while reducing T-helper 2, increases the number of T-helper 1 and natural killer (NK) cells, stimulation of T and NK cell cytotoxic activity, initiation of macrophages and anti-angiogenic.	Inverse ²⁷
Leptin	leptin	Besides its role in energy balance, leptin plays an important role in immunity, inflammation and haematopoiesis. It improves the production of IL-2 and IFN- γ ; modulates cytokines production from monocytes/macrophages.	Inverse ²⁸
HGF	hepatocyte growth factor	Regulates growth and morphogenesis of many cells in the body, including endothelial cells. Inhibits production of cytokines; enhances endothelial integrity and vascular barrier function.	Inverse ²⁹
hsCRP	high-sensitive C-reactive protein	Has a defence role in the body through clearance of pathogens and dead cells.	Inverse ²⁵
TNF- α	tumour necrosis factor-alpha	Regulates the immune system by reducing the infectious, immune, toxic, traumatic and ischaemic stimuli. Also improves inflammation through leukocyte adhesion, trans-endothelial migration and vascular leak.	Inverse ³⁰

hs-CRP, high sensitive-C-reactive protein; TNF- α , tumour necrosis Factor alpha; IL-12, interleukin 12; IFN- γ , interferon gamma; IL-6, interleukin 6; sICAM, soluble intercellular adhesion molecules; sVCAM, soluble vascular cell adhesion molecule; MPO, myeloperoxidase; HGF, hepatocyte growth factor

describes some commonly used biomarkers of both systemic and endothelial inflammation²¹⁻³⁰.

The active vitamin D hormone, 1,25(OH) $2D_3$, can be produced in endothelial cells through activity of a specific endothelial α hydroxylase on circulating 25(OH) D_3 ³¹. There is now an abundance of data that demonstrate the beneficial effects of 1,25(OH) $2D_3$ on mediators of inflammation through the modulation of

macrophage/monocytes and T and B lymphocytes. It also affects the differentiation of active CD4+ T-cells, enhances the inhibitory function of T-cells and promotes differentiation of monocyte into mature macrophages. Overall, a role in antibacterial and antiviral activities seems proven³². The logical extension of such observations would be that the correction of vitamin D deficiency or insufficiency must have

Table II. Vitamin D supplementation and endothelial function: summary of randomized controlled trials

Study reference number	Study location	Study details	Study quality score ¹	Vitamin D status achieved (nmol/l)	Study outcomes			Comments
					Endothelial function	Endothelial inflammation	Systemic inflammation	
35	Hong Kong	Sample: N= 100 T2DM patients Dose: 5000 IU D3/day or placebo Duration: 12 wk	5	Baseline: 52.75 ± 21.5 End: 86.8	No difference in FMD, or brachial-ankle PWV	No change in endothelial progenitor cells	No significant change in hs-CRP, oxidative stress markers	No differences in LDL-C, HDL-C or HbA _{1c}
36	USA	Sample: N= 114 postmenopausal women, aged 60-70 yr Dose: 2500 IU/day D3 or placebo Duration: 16 wk	5	Baseline: 80 ± 26.3 End: + 39 ± 23.3	No difference in FMD, PWV or Aix		No significant difference in CRP	
37	USA	Sample: N =57 African-American men and women, aged 19-50 yr Dose: 60,000 IU D3 per month (~2000 IU/d) or placebo Duration: 16 wk	4	Baseline: 34.3 ± 2.2 End: 100.9 ± 6.6	Significant improvement in FMD			No change in PTH, serum calcium or urinary calcium: creatinine ratio
38	USA	Sample: N= 90 CAD patients Dose: 50,000 IU D2 per week or placebo Duration: 12 wk	4	Baseline: 85.75 ± 5.5 End: 100 ± 45	No difference in RH-PAT score	No difference in sICAM, sVCAM, or E-selectin	No difference in IL-12, IFN-γ, hs-CRP or IL-6	
39	Switzerland	Sample: N= 62 subjects with peripheral artery disease Dose: Single dose of 100,000 IU D3 or placebo Duration: 4 wk	5	Baseline: 40.8 ± 16.8 End: 60.75 ± 15.5	No difference in endothelial function or arterial stiffness		No significant change in CRP, D-dimer, PAI-1	Short duration, low power
40	UK	Sample: 50 South Asian women living in UK Dose: 100,000 IU oral vitamin D3 or placebo Duration: 8 wk	5	Baseline: 27 ±13 End: +16 by wk 4 and +10 nmol/l by wk 8	No difference in FMD	Platelet activation inhibitor-1 and tissue plasminogen activator levels fell significantly in the vitamin D group relative compared with placebo	No significant change in markers of inflammation	No significant change in insulin resistance

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Study reference number	Study location	Study details	Study quality score ¹	Vitamin D status achieved (nmol/l)	Study outcomes			Comments
					Endothelial Function	Endothelial inflammation	Systemic inflammation	
41	UK	Sample: N=61 T2DM with <100 nmol/l status Dose: 3 groups: Single dose of 100,000 IU or 200,000 IU or placebo Duration: 16 wk	5	100,000 IU group: Baseline: 41 ± 14 End: 63 ± 20 200,000 IU group Baseline: 48 ± 21 End: 79 ± 31	No difference in FMD	Improvement in B type natriuretic peptide	BP was reduced in the vitamin D groups. Insulin resistance was similar	
42	UK	Sample: N= 75 patient with a history of myocardial infarction Dose: 100,000 IU of oral vitamin D3 (at baseline, 2 months and 4 months) or placebo Duration: Three doses at baseline, 2 months and 4 months	5	Baseline: 49 ± 20 End: +7 after 2 months; +13 after 6 months	No difference in endothelial function as measured by peripheral artery tonometry	No differences in TNF- α , E-selectin, or vWF	Significant reduction in CRP	
43	UK	Sample: N= 58 patients with history of stroke and vitamin D <75 nmol/l Dose: 100,000 IU oral vitamin D2 or placebo. Duration: 16 wk	4	Baseline: 38.7± (17.6) End: 54 ± 15 in 8 wk	Significant improvement in FMD at 8 wk but not 16 wk		No significant change in diastolic blood pressure	
44	UK	Sample: N= 159 aged > 70 yr & vitamin D level < 75 nmol/l Dose: 100,000 IU D3/3 months over one year or placebo Duration: 52 wk	5	Baseline: 45 nmol/l End: +20	No difference in FMD, and arterial stiffness	No significant change in CRP or HOMA	No differences in cholesterol, glucose and blood pressure	

T2DM, type two diabetes mellitus; FMD, follow mediated dilatation; hs-CRP, high sensitive-C-reactive protein; HOMA, homeostasis model assessment; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycated haemoglobin; PWV, pulse wave velocity; IU, international unit; TNF- α , tumour necrosis factor alpha; CRP, C-reactive protein; IL-12, interleukin 12; IFN- γ , interferon gamma; IL-6, interleukin 6; A1c, augmentation index; RH-PAT score, reactive hyperemia peripheral arterial tonometry score; sVCAM, soluble intercellular adhesion molecules; sVCAM, soluble vascular cell adhesion molecule; PTH, parathyroid hormone; vWF, Von Willebrand Factor; PAI-1, plasminogen activator inhibitor-1

some effect on endothelial function, possibly through abrogation of the inflammatory response, both systemic and endothelial. Recent outcomes from observational studies extend this point since hypovitaminosis D was directly associated with the extent of coronary artery disease determined by angiography³³.

The PubMed database was searched from 2009 to date. Key words used were vitamin D, supplementation, systemic inflammation, endothelium, endothelial dysfunction and humans. Selected articles were restricted to the English language and randomized controlled trials of vitamin D supplementation with some physical measures of endothelial function. All resultant studies were finally graded for their quality based on the score of Jadad *et al*³⁴ and only those 10 studies that met criteria of a good score (≥ 3) were included³⁵⁻⁴⁴ (Table II).

It was perhaps surprising to find that the RCTs in this area did not support a role for the vitamin on endothelial function, with only two trials of eight showing an improvement. Moreover, of the many biomarkers of inflammation and endothelial activation reported, only three studies showed some improvement in either C-reactive protein, platelet activation inhibitor-1, tissue plasminogen activator or B type natriuretic peptide (Table II).

We restricted our search to one major database over the last five years. Perhaps a more extensive search strategy over a longer time frame was needed. While there were many methods for determining EF, the majority in this review used FMD which is regarded as the gold standard. Hence methodology may not be the issue here. The current value for adequate vitamin D status is 50 nmol/l and this is essentially meant to cover bone health. However, there are well argued views that even for bone health a value ≥ 75 nmol/l is essential^{10,45,46}. It is possible that the target value may be much higher for non-skeletal endpoints. We have opined that the precise status achieved as well as the duration over which the target value is maintained, may be crucial to some extra-skeletal effects^{7,47}. In the trials reviewed here (Table II), half the number had achieved a value between 85-100 nmol/l though one started from a baseline of 50 nmol/l and two from ~ 80 nmol/l. Duration of these trials was < 16 wk, with only one lasting a year. It was not clear from these publications, for how long the achieved status had been maintained (Table II). These two facets may prove to be critical, as indicated by a RCT in South Asian women living in New Zealand. The authors of this study found a

significant change in insulin resistance, only in those participants who achieved a value of 80 nmol/l at 12 wk and maintained that value until 24 wk⁴⁸. While data like these are scarce, they provide the impetus for future trials to aim for specific 25(OH)D₃ levels and to maintain them over a defined period. Merely correcting vitamin inadequacy or deficiency may not be sufficient for extra-skeletal effects.

Conclusions

In this overview of vitamin D and endothelial function, it is found that the available evidence base does not support a role for the vitamin. Prospective studies could involve dose response trials that target a range of status values and maintain that target value for at least six months. In this regard, multicentre trials are a potential way forward to make such desirable outcomes applicable to the ethnic mix of their population, or across the world.

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