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 obesity\(^1\). 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 status\(^3-5\). Calcium and vitamin D have many potential roles in human physiology but are accepted mainly for their influence on bone health\(^6\). 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 investigations\(^7-10\). 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 tone\(^1\). 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 adhesion\(^1\). 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 signal\(^1\). While activation of endothelial nitric oxide synthase (eNOS) is central to endothelial-dependent vasodilation, mechanisms supporting endothelium-independent vasodilation include the stimulation of phospholipaseA\(_2\) activity\(^1\). In order to distinguish between EC and smooth muscle cell dysfunction, both endothelium dependant and independent systems need to be assessed\(^1\).

**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 vasodilation\(^1\). 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)\(^1\). FMD occurs when endothelial cells release NO in response to a shear stress\(^1\). Built on this principle, evaluation of FMD was developed using ultrasound imaging\(^1\) that captured the change in the diameter of a peripheral artery (typically brachial artery)\(^1\). 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)\(^1\). 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 form\(^1\). Augmentation index (AI\(_\text{x}\), 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 plethysmography to produce a digital volume pulse analysis (DVP)\(^1\). 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\(_\text{x}\) and central pulse wave velocity\(^1\). 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 dependent changes in RI of Indian men based on a DVP analysis system\(^1\).

**Vitamin D, systemic and vascular inflammation**

The link between systemic inflammation and vascular-specific inflammation from endothelial activation is well established\(^1\). There are numerous vascular markers of endothelial damage and their role in the pathophysiology of endothelial dysfunction is excellently covered elsewhere\(^1\). 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 dysfunction\(^1\). 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 dysfunction\(^1\). Table I briefly
Table I. Some commonly used circulating biomarkers in studies of endothelial function

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Stands for</th>
<th>The role in body</th>
<th>The relationship with endothelial function</th>
</tr>
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<tbody>
<tr>
<td>D-dimer</td>
<td>A fibrin degradation product of cross-linked fibrin</td>
<td>Indicates the occurrence of thrombin generation and plasmin generation in the blood</td>
<td>Inverse\textsuperscript{21}</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>soluble intercellular adhesion molecule type 1</td>
<td>Helps leukocytes migrate across endothelial cells in the inflammatory state.</td>
<td>Inverse\textsuperscript{22}</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>soluble vascular cell adhesion molecule-1</td>
<td>Assists lymphocytes, monocytes, eosinophil and basophils to adhere to vascular endothelium.</td>
<td>Inverse\textsuperscript{22}</td>
</tr>
<tr>
<td>MPO</td>
<td>myeloperoxidase</td>
<td>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.</td>
<td>Inverse\textsuperscript{23}</td>
</tr>
<tr>
<td>P-selectin</td>
<td>P-selectin</td>
<td>Is an adhesion molecule for leukocytes on the surface of the endothelium.</td>
<td>Inverse\textsuperscript{24}</td>
</tr>
<tr>
<td>E-selectin</td>
<td>E-selectin</td>
<td>Is an adhesion molecule for leukocytes in endothelial cells.</td>
<td>Inverse\textsuperscript{24}</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin 6</td>
<td>IL-6 triggers the production of collagenases and prostaglandins which reduce the pain threshold. Also stimulates T and B cells in their immune mechanisms.</td>
<td>Inverse\textsuperscript{25}</td>
</tr>
<tr>
<td>IL-1(\beta)</td>
<td>interleukin-1 beta</td>
<td>IL-6 beta is able to induce fever, anorexia and hypotension. May also control mycobacterial proliferation in the macrophage.</td>
<td>Inverse\textsuperscript{26}</td>
</tr>
<tr>
<td>IL-12</td>
<td>interleukin -12</td>
<td>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.</td>
<td>Inverse\textsuperscript{27}</td>
</tr>
<tr>
<td>Leptin</td>
<td>leptin</td>
<td>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-(\gamma), modulates cytokines production from monocytes/macrophages.</td>
<td>Inverse\textsuperscript{28}</td>
</tr>
<tr>
<td>HGF</td>
<td>hepatocyte growth factor</td>
<td>Regulates growth and morphogenesis of many cells in the body, including endothelial cells. Inhibits production of cytokines; enhances endothelial integrity and vascular barrier function.</td>
<td>Inverse\textsuperscript{29}</td>
</tr>
<tr>
<td>hsCRP</td>
<td>high-sensitive C-reactive protein</td>
<td>Has a defence role in the body through clearance of pathogens and dead cells.</td>
<td>Inverse\textsuperscript{30}</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>tumour necrosis factor-alpha</td>
<td>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.</td>
<td>Inverse\textsuperscript{30}</td>
</tr>
</tbody>
</table>

hs-CRP, high sensitive-C-reactive protein; TNF-\(\alpha\), tumour necrosis Factor alpha; IL-12, interleukin 12; IFN-\(\gamma\), 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 inflammation\textsuperscript{21-30}. The active vitamin D hormone, 1,25(OH)\textsubscript{2}D\textsubscript{3}, can be produced in endothelial cells through activity of a specific endothelial \(\alpha\) hydroxylation on circulating 25(OH)D\textsubscript{3}\textsuperscript{31}. There is now an abundance of data that demonstrate the beneficial effects of 1,25(OH)\textsubscript{2}D\textsubscript{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 proven\textsuperscript{32}. The logical extension of such observations would be that the correction of vitamin D deficiency or insufficiency must have
<table>
<thead>
<tr>
<th>Study reference number</th>
<th>Study location</th>
<th>Study details</th>
<th>Study quality score</th>
<th>Vitamin D status achieved (nmol/l)</th>
<th>Study outcomes</th>
<th>Systemic inflammation</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 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 significant change in hs-CRP, oxidative stress markers | No differences in LDL-C, HDL-C or HbA1c |
| 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 |  |
<table>
<thead>
<tr>
<th>Study reference number</th>
<th>Study location</th>
<th>Study details</th>
<th>Study quality score&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Vitamin D status achieved (nmol/l)</th>
<th>Study outcomes</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 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  |
| 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  |

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; Aix, augmentation index; RH-PAT score, reactive hyperemia peripheral arterial tonometry score; sICAM, 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 angiography.\textsuperscript{33}

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.\textsuperscript{34} and only those 10 studies that met criteria of a good score (\( \geq 3 \)) were included\textsuperscript{35-44} (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 \( \geq 75 \) nmol/l is essential\textsuperscript{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.\textsuperscript{3,47} 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 \( \sim 80 \) 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 wk.\textsuperscript{48} While data like these are scarce, they provide the impetus for future trials to aim for specific 25(OH)D\textsubscript{3} 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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