Review Article

Indian J Med Res 140, October 2014, pp 483-490

Vitamin D & endothelial function

A. Alyami, M.J. Soares, J.L. Sherriff & J.C. Mamo

Directorate of Nutrition, Dietetics & Food Technology, School of Public Health, Curtin University, Perth, Australia

Received June 17, 2014

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[11]. 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[11]. 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[12]. While activation of endothelial nitric oxide synthase (eNOS) is central to endothelial-dependent vasodilation, mechanisms supporting endothelial-independent vasodilation include the stimulation of phospholipaseA_2 activity[13]. In order to distinguish between EC and smooth muscle cell dysfunction, both endothelium dependant and independent systems need to be assessed[11].

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[14]. 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 sheer 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 form[18]. 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 photo-plethysmography 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 system[19].

Vitamin D, systemic and vascular inflammation

The link between systemic inflammation and vascular-specific inflammation from endothelial activation is well established[20]. There are numerous vascular markers of endothelial damage and their role in the pathophysiology of endothelial dysfunction is excellently covered elsewhere[11]. 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[17]. 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[17]. 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-1β	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 inflammation[21-30].

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 proven[32]. 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 $HbA1_c$
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

Contd...

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; 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[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*[34] and only those 10 studies that met criteria of a good score (≥3) were included[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 ≥ 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 trails 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[48]. While data like these are scarce, they provide the impetus for future trials to aim for specific $25(OH)D_3$ 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.

Acknowledgment

The study was supported by the School of Public Health, Curtin University. The first author (AA) is a recipient of a PhD scholarship from the Saudi Ministry of Health.

References

1. Misra A, Chowbey P, Makkar B, Vikram N, Wasir J, Chadha D, *et al*. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. *J Assoc Physcians India* 2009; *57* : 163-70.
2. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PV, Sarma KV, *et al*. High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. *Am J Clin Nutr* 2007; *85* : 1062-7.
3. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. *Am J Clin Nutr* 2005; *81* : 1060-4.
4. Goswami R, Marwaha RK, Gupta N, Tandon N, Sreenivas V, Tomar N, *et al*. Prevalence of vitamin D deficiency and its relationship with thyroid autoimmunity in Asian Indians: a community-based survey. *Br J Nutr* 2009; *102* : 382-6.
5. Nowson CA, McGrath JJ, Ebeling PR, Haikerwal A, Daly RM, Sanders KM, *et al*. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust* 2012; *196* : 686-7.
6. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, *et al*. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011; *96* : 53-8.

7. Soares MJ, Pathak K, Calton EK. Calcium and vitamin D in the regulation of energy balance: where do we stand? *Int J Mol Sci* 2014; *15* : 4938-45.
8. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008; *87* : 1080S-6S.
9. Peterlik M, Cross H. Vitamin D and calcium insufficiency-related chronic diseases: molecular and cellular pathophysiology. *Eur J Clin Nutr* 2009; *63* : 1377-86.
10. Heaney RP. Vitamin D in health and disease. *Clin J Am Soc Nephrol* 2008; *3* : 1535-41.
11. Szmitko PE, Wang C-H, Weisel RD, de Almeida JR, Anderson TJ, Verma S. New markers of inflammation and endothelial cell activation part I. *Circulation* 2003; *108* : 1917-23.
12. Sandoo A, van Zanten JJV, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. *Open Cardiovasc Med J* 2010; *4* : 302.
13. Johns DG, Charpie JR, Webb RC. Is ceramide signaling a target for vascular therapeutic intervention? *Curr Pharma Design* 1998; *4* : 481-8.
14. Pasimeni G, Ribaudo MC, Capoccia D, Rossi F, Bertone C, Leonetti F, *et al*. Non-invasive evaluation of endothelial dysfunction in uncomplicated obesity: Relationship with insulin resistance. *Microvasc Res* 2006; *71* : 115-20.
15. Tousoulis D, Antoniades C, Stefanadis C. Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart* 2005; *91* : 553-8.
16. Smith S, Greenland P, Grundy S. American Heart Association Conference Proceedings, Prevention Conference V: Beyond secondary prevention: identifying the high-risk patient for primary prevention. Executive summary. *Circulation* 2000; *10* : E12-6.
17. Barac A, Campia U, Panza JA. Methods for evaluating endothelial function in humans. *Hypertenion* 2007; *49* : 748-60.
18. Woodman R, Watts G. Measurement and application of arterial stiffness in clinical research: focus on new methodologies and diabetes mellitus. *Med Sci Monitor* 2003; *9* : RA81-9.
19. Soares MJ, Kuriyan R, Kurpad AV. Calcium and vitamin D modulate postprandial vascular function: A pilot dose–response study. *Diab Met Syn: Clin Res Rev* 2010; *4* : 128-31.
20. Huang AL, Vita JA. Effects of systemic inflammation on endothelium-dependent vasodilation. *Trends Cardiovas Med* 2006; *16* : 15-20.
21. Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ. Hemostatic function and progressing ischemic stroke D-dimer predicts early clinical progression. *Stroke* 2004; *35* : 1421-5.
22. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *JAMA* 2004; *291* : 1978-86.
23. Zhang R, Brennan M-L, Fu X, Aviles RJ, Pearce GL, Penn MS, *et al*. Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA* 2001; *286* : 2136-42.
24. Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. *Circulation* 2001; *103* : 491-5.
25. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001; *286* : 327-34.
26. Reinhart K, Bayer O, Brunkhorst F, Meisner M. Markers of endothelial damage in organ dysfunction and sepsis. *Crit Care Med* 2002; *30* : S302-S12.
27. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* 1999; *17* : 189-220.
28. Ren J. Leptin and hyperleptinemia-from friend to foe for cardiovascular function. *J Endocrinol* 2004; *181* : 1-10.
29. Yasuda S, Goto Y, Baba T, Satoh T, Sumida H, Miyazaki S, *et al*. Enhanced secretion of cardiac hepatocyte growth factor from an infarct region is associated with less severe ventricular enlargement and improved cardiac function. *J Am Coll Cardiol* 2000; *36* : 115-21.
30. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, *et al*. Role of TNF-alpha in vascular dysfunction. *Clin Sci* 2009; *116* : 219-30.
31. Zehnder D, Bland R, Chana RS, Wheeler DC, Howie AJ, Williams MC, *et al*. Synthesis of 1, 25-dihydroxyvitamin D3 by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. *J Am Soc Nephr* 2002; *13* : 621-9.
32. Lai Y-H, Fang T-C. The pleiotropic effect of Vitamin D. *ISRN Nephr* 2013; Article ID898125 : 6 pages. Available from: *http://dx.doi.org/10.5402/2013/898125*, accessed March 28, 2014.
33. Verdoia M, Schaffer A, Sartori C, Barbieri L, Cassetti E, Marino P, *et al*. Vitamin D deficiency is independently associated with the extent of coronary artery disease. *Eur J Clin Invest* 2014; *44* : 634-42.
34. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, *et al*. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Contr Clin Trials* 1996; *17* : 1-12.
35. Yiu YF, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, *et al*. Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. *Atherosclerosis* 2013; *227* : 140-6.
36. Gepner AD, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH. A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. *PLoS One* 2012; *7* : e36617.
37. Harris RA, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, *et al*. Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *Am J Hypertens* 2011; *24* : 557-62.
38. Sokol SI, Srinivas V, Crandall JP, Kim M, Tellides G, Lebastchi AH, *et al*. The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease. *Vasc Med* 2012; *17* : 394-404.
39. Stricker H, Tosi Bianda F, Guidicelli-Nicolosi S, Limoni C, Colucci G. Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral arterial disease: a randomised controlled pilot study. *Eur J Vasc Endovasc Surg* 2012; *44* : 307-12.

40. Witham MD, Adams F, Kabir G, Kennedy G, Belch JJ, Khan F. Effect of short-term vitamin D supplementation on markers of vascular health in South Asian women living in the UK--a randomised controlled trial. *Atherosclerosis* 2013; *230* : 293-9.

41. Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. *Diabetoliga* 2010; *53* : 2112-9.

42. Witham MD, Dove FJ, Khan F, Lang CC, Belch JJ, Struthers AD. Effects of vitamin D supplementation on markers of vascular function after myocardial infarction--a randomised controlled trial. *Int J Cardiol* 2013; *167* : 745-9.

43. Witham MD, Dove FJ, Sugden JA, Doney AS, Struthers AD. The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. *Nutr Metab Cardiovasc Dis* 2012; *22* : 864-70.

44. Witham MD, Price RJ, Struthers AD, Donnan PT, Messow CM, Ford I, *et al*. Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic hypertension: the VitDISH randomized controlled trial. *JAMA Intern Med* 2013; *173* : 1672-9.

45. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos Int* 2005; *16* : 713-6.

46. Vieth R. What is the optimal vitamin D status for health? *Prog Biophys Mol Bio* 2006; *92* : 26-32.

47. Pathak K, Soares M, Calton E, Zhao Y, Hallett J. Vitamin D supplementation and body weight status: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev* 2014; *15* : 528-37.

48. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient-a randomised, placebo-controlled trial. *Br J Nutr* 2010; *103* : 549-55.

Reprint requests: Dr Mario J. Soares, Associate Professor, Directorate of Nutrition Dietetics & Food Technology, School of Public Health, Curtin University, Bentley Campus, Kent Street, Perth Western Australia 6845, Australia
e-mail: m.soares@curtin.edu.au