Association between vitamin D status and markers of vascular health in patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME)

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Low circulating 25 hydroxyvitamin D (25OHD) levels have been associated with increased blood pressure, impaired vascular health and an increased risk of cardiovascular events [1]. We have previously shown that patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) have vascular dysfunction [2,3], which is related to increased levels of low grade inflammation and oxidative stress [2]. Vitamin D may affect the cardiovascular system through multiple pathways — via influence on the inflammatory process [1] and oxidative stress [4], by effects on cardiac myocyte hypertrophy, vascular stiffness and calcification, and by direct effects on endothelial function [1]. The purpose of this pilot investigation in CFS/ME patients was to explore the association between serum 25-hydroxyvitamin D (25(OH)D) and markers of cardiovascular disease risk, including endothelial function, arterial stiffness, low grade inflammation and oxidative stress.

Participants were recruited from a local register of CFS/ME patients and fulfilled the Centres for Disease Control (CDC) classification for CFS [5]. The local medical ethics committee approved the study (Ref 07/S1402/65) and all volunteers gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Serum 25(OH)D, CRP and TNFα were measured using commercially available ELISA kits (IDS Ltd. UK, Kalon Biological Ltd., UK and R&D Systems, UK, respectively). Plasma isoprostanes were measured by gas chromatography–mass spectrometry [2]. Total, HDL and LDL cholesterol levels were measured on a Cobas Bio centrifugal analyser using products from Roche.

We assessed forearm microvascular function, as described previously [6], by measuring skin vascular responses to iontophoresis of 1% acetylcholine (ACh; Sigma-Aldrich Co. Ltd.) and sodium nitroprusside (SNP; David Bull Laboratories). Skin perfusion was measured using a laser Doppler imager (moorLDI, Moor Instruments, Axminster, UK). Two baseline scans of skin perfusion were taken before the iontophoresis protocol was administered. The peak response to ACh and SNP was recorded.

An index of arterial stiffness was assessed non-invasively using the SphygmoCor pulse waveform analysis system (Scanmed Medical Instruments, Moreton-in-Marsh, UK). We measured the augmentation index (AIx) [7], normalised for a heart rate of 75 beats/min, and aortic pulse wave velocity (PWV) by sequentially recording gated ECG carotid and radial waveforms.

Descriptive statistics for baseline characteristics were calculated. All variables were normally distributed save for CRP and TNFα, which were log-transformed prior to further analysis. Pearson's correlation coefficients were calculated to analyse the relationship between 25(OH) results and each marker of vascular risk. Linear regression analysis was used to adjust for factors known to affect both 25(OH)D levels and vascular risk, i.e. age, sex, smoking and body mass index.

Forty one participants (19–63 years old) were included in the study and were assessed between 1st November 2009 and 31st March 2010. The mean length of illness was 9.7 years (SD 5.7 years) and all patients were of white European descent. None had a history of renal disease. Seventeen patients were on more than one medication. Eight patients were on low dose amitriptyline taken at night for sleeping problems, 7 were on thyroxine, 13 patients were on paracetamol based drugs, 2 were on β-blockers, and 5 were on benzodiazepine derivatives. Table 1 displays the details of the patients and data for markers of vascular function, inflammation and oxidative stress. Levels of 25(OH)D ranged from 7 to 108 nmol/l. We found significant correlations between 25(OH)D levels and markers of inflammation, oxidative stress, endothelial function and arterial stiffness as shown in Table 2. Levels of 25(OH)D correlated significantly with age ($r = 0.40$, $P = 0.01$), but not with gender ($r = 0.05$, $P = 0.69$) or BMI ($r = 0.03$, $P = 0.85$).

We found significant correlations between 25(OH)D levels and markers of cardiovascular risk, inflammation and oxidative stress in this group of patients with CFS/ME. Our results thus extend findings in other populations to patients with CFS/ME, who have recently been shown to have impaired vascular function [2,3].

The relationship between impaired vascular function, low 25(OH)D levels and symptoms in CFS/ME is likely to be complex. It is possible that microvascular dysfunction might contribute to impaired muscle function in the syndrome, but vitamin D is also known to have direct effects on neuromuscular function [8]. Conversely, the low levels of physical activity that are a hallmark of CFS/ME are likely to lead to

Table 1
Characteristics of CFS/ME patients (n = 41) and data for markers of vascular function, inflammation and oxidative stress.

Age (years)	50 (11)
Body mass index (kg m^{-2})	26.1 (4.5)
No. males (%)	17 (41)
No. smokers (%)	7 (17)
25-Hydroxyvitamin D (nmol/l)	45 (31)
Total cholesterol (mmol/l)	5.5 (1.2)
HDL cholesterol (mmol/l)	1.3 (0.4)
C-reactive protein (μg/ml)	1.3 (0.4–2.4)
TNFα (pg/ml)	2.0 (1.5–2.7)
Isoprostanes (pg/ml)	462 (244)
Brachial systolic blood pressure (mm Hg)	125 (19)
Brachial diastolic blood pressure (mm Hg)	80 (12)
Heart rate (beats/min)	72 (13)
Augmentation index (%)	13.7 (9.8)
Pulse wave velocity (m/s)	8.5 (1.0)
Baseline perfusion before acetylcholine (AU)	15.1 (10.0)
Acetylcholine peak perfusion (AU)	103 (45)

HDL: High density lipoprotein. TNF: Tumour necrosis factor.
Values are means (standard deviation), except for CRP and TNFα which are presented as median (interquartile range).

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Table 2
Association between 25(OH)D levels (nmol/l) and markers of lipids, inflammation, oxidative stress, endothelial function and arterial stiffness.

	R	P	Exp (B)	P
Isoprostanes (pg/ml)	−0.44	0.009	−0.46	0.013
Log TNFα (pg/ml)	−0.39	0.02	−0.38	0.04
Log C-reactive protein (μg/ml)	−0.10	0.43	−0.11	0.42
Total cholesterol (mmol/l)	−0.13	0.31	−0.06	0.68
LDL cholesterol (mmol/l)	−0.36	0.06	−0.29	0.23
HDL cholesterol (mmol/l)	0.01	0.92	−0.24	0.10
Brachial systolic blood pressure (mm Hg)	−0.13	0.31	−0.22	0.16
Brachial diastolic blood pressure (mm Hg)	−0.34	0.009	−0.40	0.006
Carotid-radial pulse wave velocity (m/s)	−0.55	0.008	−0.28	0.09
Augmentation index (%)	0.19	0.13	0.14	0.24
ACh peak perfusion (AU)	0.30	0.07	0.22	0.28

R by Pearsons's correlation coefficient. Exp (B) by linear regression, adjusted for age, sex, BMI and smoking.
HDL: High density lipoprotein. LDL: Low density lipoprotein. TNF: Tumour necrosis factor. ACh: Acetylcholine.

impaired vascular function. Low activity levels are also likely to lead to reduced outdoor activity and consequent lower sun exposure, which in turn would lead to low 25(OH)D levels. The role of inflammation is likely to be important; inflammation may mediate development of both muscle weakness and vascular dysfunction [9]; although vitamin D supplementation may be able to reduce inflammation [10], acute inflammation may itself lower 25(OH)D levels [11]. Thus demonstrating or disproving a causal role for 25(OH)D in the development of impaired vascular function in CFS/ME is not possible using observational studies.

Strengths of our study include the use of a range of markers of vascular risk, as well as measures of oxidative stress and inflammation. The population was carefully phenotyped for CFS/ME according to standard CFS classification, and the sample size was large enough to show convincing associations between 25OHD levels and vascular risk markers. Weaknesses of the study include the cross-sectional design, which does not permit an attempt to dissect out causal relationships, and the fact that all participants were of Caucasian extraction, living in a single geographical area, which limits the generalisability of our results. It is not known whether living at high latitude is a risk factor for developing CFS; such studies would complement this work by relating ultraviolet radiation (a key step in vitamin D synthesis) to the risk of CFS. We cannot exclude the influence of over-the-counter supplemental vitamin D ingestion. However, as such supplements would change 25(OH)D levels, then this supplement use would not invalidate our findings.

These findings provide a rationale for proceeding to randomised controlled trials to examine the effects of vitamin D supplementation on cardiovascular disease risk in patients with CFS/ME. Intervention trials in other patient groups have given mixed results, but improvements in blood pressure [1], endothelial function [12] and in novel markers of vascular risk [13] have been noted in previous trials. If vitamin D supplementation is shown to have a beneficial effect on cardiovascular disease risk, it could prove to be a relatively simple, effective way of contributing to reducing cardiovascular disease burden in CFS/ME patients.

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