

## Vitamin D Deficiency Is Associated With Inflammation in Older Irish Adults

E. Laird, H. McNulty, M. Ward, L. Hoey, E. McSorley, J. M. W. Wallace,† E. Carson, A. M. Molloy, M. Healy, M. C. Casey, C. Cunningham, and J. J. Strain

Institute of Molecular Medicine (E.L., A.M.M.), School of Medicine, Trinity College, Dublin, Ireland; Northern Ireland Centre for Food and Health (H.M., M.W., L.H., E.M., J.M.W.W., E.C., J.J.S.), University of Ulster, Coleraine, Londonderry BT52 1SA, Northern Ireland; Department of Clinical Biochemistry (M.H.), St James's Hospital, Dublin, Ireland; and The Mercers Institute for Research on Ageing (M.C.C., C.C.), St James's Hospital, Dublin, Ireland

**Context:** Inadequate vitamin D status is common within elderly populations and may be implicated in the etiology of autoimmune disease and inflammation. Few studies have investigated the relationship between vitamin D status and age-related immune dysfunction in humans.

**Objective:** The aim of this study was to investigate the association between vitamin D status and immune markers of inflammation in a large sample of older adults.

**Design, Setting, and Participants:** An observational investigation of 957 Irish adults (>60 years of age) recruited in Northern Ireland (55°N latitude) as part of the Trinity Ulster Department of Agriculture aging cohort study.

**Main Outcome Measure:** We measured serum 25-hydroxyvitamin D (25(OH)D) by liquid chromatography tandem mass spectrometry and serum cytokines IL-6, TNF- $\alpha$ , IL-10, and C-reactive protein (CRP) by ELISA.

**Results:** Concentrations of IL-6, CRP, and the ratios of IL-6 to IL-10 and CRP to IL-10 were significantly higher in individuals with deficient (<25 nmol/L) serum 25(OH)D compared with those with sufficient (>75 nmol/L) status after adjustment for age, sex, and body mass index ( $P < .05$ ). Vitamin D status was a significant predictor of the IL-6 to IL-10 cytokine ratio, and those participants defined as deficient were significantly more likely to have an IL-6 to IL-10 ratio >2:1 compared with those defined as sufficient.

**Conclusions:** This study demonstrated significant associations between low vitamin D status and markers of inflammation (including the ratio of IL-6 to IL-10) within elderly adults. These findings suggest that an adequate vitamin D status may be required for optimal immune function, particularly within the older adult population. (*J Clin Endocrinol Metab* 99: 1807–1815, 2014)

Vitamin D is traditionally viewed as a key modulator of bone metabolism via its classical role in calcium regulation, but recent evidence suggests a more diverse role of the vitamin, including effects on immune regulation (1, 2). The primary source of vitamin D is endogenous synthesis after skin exposure to UVB sunlight (2). Synthesis is followed by a 2-step hydroxylation process, first in the liver

to 25-hydroxyvitamin D (25(OH)D) and then in the kidney to the active metabolite 1,25-dihydroxyvitamin D (1,25(OH)D), which exerts its influence via the vitamin D receptor (2). Expression of the vitamin D receptor has been identified on a variety of cells of the immune system including macrophages, T lymphocytes, dendritic cells, and monocytes (3). Furthermore, 1,25(OH)D has been ob-

ISSN Print 0021-972X ISSN Online 1945-7197  
Printed in U.S.A.

Copyright © 2014 by the Endocrine Society

Received September 16, 2013. Accepted January 22, 2014.

First Published Online February 25, 2014

† Deceased, February 7, 2012.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; 1,25(OH)D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; MS, multiple sclerosis; TH-1, T-helper cell 1; TUDA, Trinity Ulster Department of Agriculture.

served to be an influential immune modulator of both the adaptive and innate immune system through its ability to alter cytokine secretion (4, 5) and to act as a regulator of cell signaling pathways via its effect on toll-like receptor expression and function (4, 6).

Owing to its importance in immune regulation, ensuring sufficient vitamin D status in humans may help to optimize immune function; yet over 1 billion individuals worldwide are estimated to have deficient vitamin D status (7, 8). This inadequacy of vitamin D is a consequence of few rich dietary sources being available on the one hand and poor endogenous synthesis of vitamin D at latitudes with low sunshine exposure on the other (6). Insufficient vitamin D status may lead to impaired immune function with an increased vulnerability to a proinflammatory state (9). For example, the seasonal occurrence of upper respiratory tract infections and influenza has been associated with the nadir of vitamin D status in many far latitude countries (10). Furthermore, an increased incidence of autoimmune disorders has been reported in regions with low levels of UV radiation leading to suggestions that the reduced ability to synthesize *sc* vitamin D may be a contributing factor in this association (9, 11).

Numerous *in vitro* studies have reported that vitamin D can significantly decrease concentrations of the inflammatory cytokines TNF- $\alpha$  and IL-6 and increase secretion of the anti-inflammatory cytokine IL-10 (12). Evidence from both *in vitro* and *in vivo* studies suggests that vitamin D shifts the immune response from a T-helper cell 1 (TH-1) (inflammatory) to a TH-2 (anti-inflammatory) profile (13, 14). However, both observational and vitamin D intervention studies in humans have reported conflicting results, with some reporting decreased concentrations of inflammatory cytokines (15–18), whereas others have observed no effect (19–23).

Older adults are at an increased risk of both insufficient and deficient vitamin D status (2, 7, 8). Few studies, however, have fully investigated the association of vitamin D status with immune markers of inflammation within an exclusively elderly population cohort. Therefore, the aim of this study was to investigate the association between vitamin D status and immune markers of inflammation in a sample of well-characterized elderly adults.

## Subjects and Methods

This observational investigation was conducted as part of the Trinity Ulster Department of Agriculture (TUDA) aging cohort study, a large observational study of elderly Irish adults. The TUDA study was designed to investigate nutritional factors and gene-nutrient interactions in the development of chronic diseases of aging in noninstitutionalized adults  $\geq 60$  years of age. TUDA

study participants were recruited either from hospital clinics or the community as 3 subcohorts to focus on osteoporosis, cognitive dysfunction, or hypertension. The participants reported in this analysis were recruited between 2009 and 2011 from general practitioner practices in the Western and Northern Health and Social Care Trusts, Northern Ireland, to the hypertensive subcohort (ie, on the basis of a diagnosis of hypertension at the time of recruitment). Apart from having a diagnosis of hypertension, this subcohort was considered generally healthy; all participants were living independently and were recruited in the community, although relevant medical details and drug use were recorded as for all TUDA study participants. Of the 2000 volunteers recruited into the hypertensive cohort, samples from 998 participants were randomly selected for this study by an independent researcher and stratified by age, sex, and season: winter (December through February), spring (March through May), summer (June through August), and autumn (September through November) (Figure 1). Exclusion criteria included those not born in or who had at least 1 parent not born on the Island of Ireland or those previously diagnosed with dementia. Subjects were also excluded if they were receiving glucocorticoid treatment or cytokine modulator treatment (including abatacept, adalimumab, anakinra, belimumab, certolizumab pegol, etanercept, golimumab, infliximab, rituximab, and tocilizumab); 41 participants were identified as receiving glucocorticoid, and none were receiving cytokine modulator treatment. For the data analysis, 957 subjects were available. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and ethical approval was granted by the Office for Research Ethics Northern Ireland (ORECNI Ref. 08/NIR03/113). All participants provided written informed consent.

## Clinical parameters

### *Lifestyle and anthropometric information*

Data associated with lifestyle factors were obtained by questionnaire and included sex, age, ethnicity, and medical history. Anthropometric measurements included height to the nearest 0.01 m (using a wall-mounted stadiometer from Seca Ltd), weight to the nearest 0.01 kg (using electronic scales from Brosch Direct Ltd), and waist and hip circumference to the nearest 0.1 cm (using a flexible tape measure from Seca Ltd). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared.

### *Biochemical analysis*

A nonfasting blood sample (50 mL) was collected by venipuncture into an evacuated clotting tube (Sarstedt) by a trained phlebotomist. Samples were kept chilled and centrifuged (3000 rpm for 15 minutes) within 3 hours of collection. Serum aliquots were labeled and stored at  $-80^{\circ}\text{C}$  until required for analysis. Stored samples were accessed for vitamin D analysis, and total serum 25(OH)D ( $\text{D}_2 + \text{D}_3$ ) concentrations were quantified by a fully validated method (Chromsystems Instruments and Chemicals GmbH; MassChrom 25-OH-Vitamin D3/D2, catalog item 62000) using liquid chromatography-tandem mass spectrometry (API 4000; AB SCIEX) and batch analyzed in the Biochemistry Department of St James's Hospital, Dublin, Ireland. The quality and accuracy of the method was continuously monitored using internal quality controls and by participation in the Vitamin D External Quality Assessment Scheme and use of the Na-

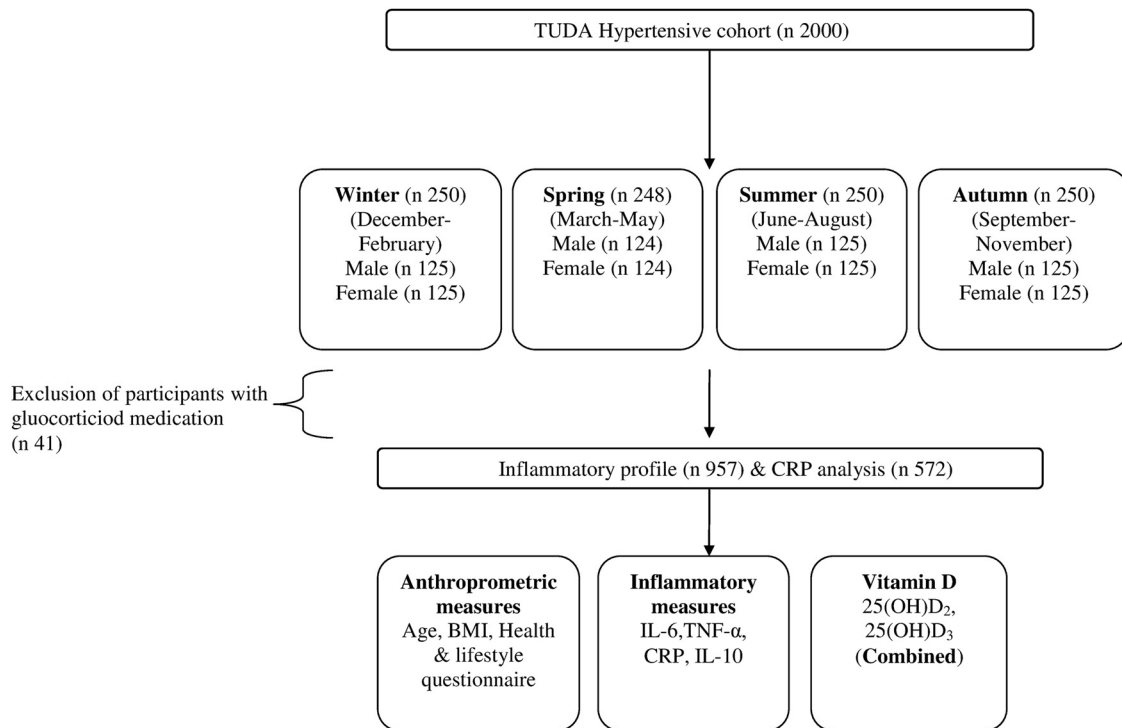


Figure 1. Study design.

tional Institute of Standards and Technology 972 vitamin D standard reference material. The respective inter- and intra-assay coefficients of variation were 5.7% and 4.5%. For the purposes of this study and corresponding with previously published immune cutoffs, vitamin D deficiency, insufficiency, and sufficiency were defined as <25, 25 to 75, and >75 nmol/L, respectively (24).

For the inflammatory marker analysis, serum C-reactive protein (CRP) concentrations (n = 572) were measured using high-sensitivity ELISA CRP kits (Roche Diagnostics, UK). Serum concentrations of proinflammatory markers TNF- $\alpha$  and IL-6 and the anti-inflammatory marker IL-10 (n = 957) were measured and batch analyzed using high-sensitivity ELISA kits (R&D Systems) and a Triturus ELISA analyzer (Grifols). Respective inter- and intra-assay coefficients of variation were 6.3% and 7.0% (CRP), 8.4% and 5.3% (TNF- $\alpha$ ), 7.7% and 7.4% (IL-6), and 11.3% and 7.8% (IL-10). Kits were stored at 4°C, and all reagents were prepared according to the manufacturer's instructions.

### Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences version 19.0 (SPSS UK Ltd). Data were assessed for normality by means of the Kolmogorov-Smirnov test and Q-Q plots and data were log-transformed or square rooted for normalization purposes. Data are expressed as means and SDs, with the median (25th and 75th interquartile ranges) used for nonnormal variables. Where appropriate, an independent-sample *t* test or a one-way ANOVA and analysis of covariance (adjusting for age and BMI) was applied to normally distributed and transformed data to determine statistical significance ( $P \leq .05$ ). A partial correlation adjusting for age, sex, BMI, smoking, and presence of inflammatory conditions (rheumatoid arthritis, ulcerative colitis, and type 1 diabetes) was ap-

plied to ascertain statistical significance between inflammatory markers/ratios and 25(OH)D concentration.

The immune profiles of TH-1/TH-2 were calculated by dividing the proinflammatory cytokine/CRP concentrations by the anti-inflammatory cytokine IL-10 (this ratio has been previously documented to be more informative regarding inflammatory balance compared with solely relying on the absolute cytokine/inflammatory marker concentration) (25, 26). The subdivided ratios included <1:1 (anti-inflammatory), 1:1 to 2:1 (neutral), and >2:1 (proinflammatory). A multinomial logistic regression analysis was performed to determine the predictors of cytokine ratio with age, sex, BMI, smoking, and vitamin D status included in the model. All observed *P* values  $\leq .05$  were considered significant.

### Results

Analysis of immune markers was completed in 957 participants (481 men and 476 women), and the baseline characteristics of this sample are presented in Table 1. Males had a significantly higher waist to hip ratio than females ( $P < .001$ ), whereas females had significantly lower 25(OH)D concentrations ( $P < .05$ ). No significant difference in proinflammatory markers was observed between the sexes. The concentration of IL-10, however, was significantly higher in males compared with females ( $P = .05$ ). A seasonal variation in 25(OH)D concentration was observed, with the highest median concentration in summer (55.3 nmol/L, SD 28.0) compared with the lowest in spring (38.6 nmol/L, SD 21.3) (not shown). Furthermore,

**Table 1.** Baseline Characteristics of Study Participants<sup>a</sup>

	Total Sample, n = 957	Males, n = 481	Females, n = 476	P Value
Age, y	70.5 (65.9–74.9)	70.6 (65.8–74.2)	70.2 (66.0–75.4)	.378
BMI, kg/m <sup>2</sup>	28.8 (26.2–32.5)	28.7 (26.5–31.7)	29.0 (25.9–33.6)	.308
Waist/hip ratio	0.93 (0.88–0.98)	0.97 (0.93–1.02)	0.89 (0.84–0.94)	<.001
% Smokers (n)	11.7 (81)	12.3 (41)	11.2 (40)	.652
% Autoimmune disease (n) <sup>b</sup>	12.2 (117)	11.0 (53)	13.4 (64)	.252
% Rheumatoid arthritis (n)	11.5 (110)	11.0 (51)	12.4 (59)	.385
% Ulcerative colitis (n)	0.5 (5)	0.4 (2)	0.6 (3)	.645
% Celiac disease (n)	0.2 (2)	0	0.4 (2)	.155
Vitamin D concentration				
25(OH)D, nmol/L	43.9 (29.3–62.2)	45.8 (31.8–63.9)	41.3 (26.8–61.2)	.008
% Vitamin D supplements (n)	36.9 (352)	33.4 (160)	40.3 (192)	.026
Inflammatory markers				
TNF- $\alpha$ , pg/mL	1.30 (0.98–1.95)	1.30 (1.00–1.97)	1.30 (0.97–1.92)	.624
IL-6, pg/mL	1.79 (1.12–3.12)	1.85 (1.09–3.21)	1.72 (1.13–3.00)	.596
CRP, mg/L <sup>c</sup>	2.00 (1.00–6.00)	2.00 (1.00–6.00)	2.00 (1.00–6.65)	.716
IL-10, pg/mL	1.59 (1.08–2.54)	1.70 (1.18–2.62)	1.50 (0.98–2.43)	.081

<sup>a</sup> Values are median (interquartile range) or percentage (n). Differences between genders were assessed using an independent *t* test on transformed data where applicable. Categorical variables were assessed by using  $\chi$ -square analysis. *P* < .05 was considered significant.

<sup>b</sup> Diagnosed with rheumatoid arthritis, ulcerative colitis or coeliac disease.

<sup>c</sup> CRP data were available for 572 participants: 272 males and 300 females.

a significant seasonal variation was also observed for IL-10, with the highest median concentration in summer (1.81 pg/mL, SD 7.25) compared with spring (1.27 pg/mL, SD 6.90). No effect of seasonality was observed for the markers IL-6, CRP, or TNF- $\alpha$ .

The immune markers were categorized by vitamin D status (Table 2). Those with sufficient vitamin D status (25(OH)D >75 nmol/L) were older (*P* = .048) and leaner (*P* < .001) compared with those deemed to be insufficient or deficient. As vitamin D status increased, a significant decrease was also observed in the concentration of IL-6,

CRP, and the ratios of IL6 to IL-10 and CRP to IL-10 after adjustment for age, sex, and BMI (*P* < .05). The percentage of participants within each inflammatory marker ratio (<1:1, 1:1–2:1, and >2:1) was quantified according to vitamin D status (Figure 2). The vitamin D-sufficient group had a significantly lower number of individuals with an IL-6 to IL-10 cytokine ratio >2:1 compared with those within the insufficient and deficient groups (*P* = .029 and *P* < .001, respectively).

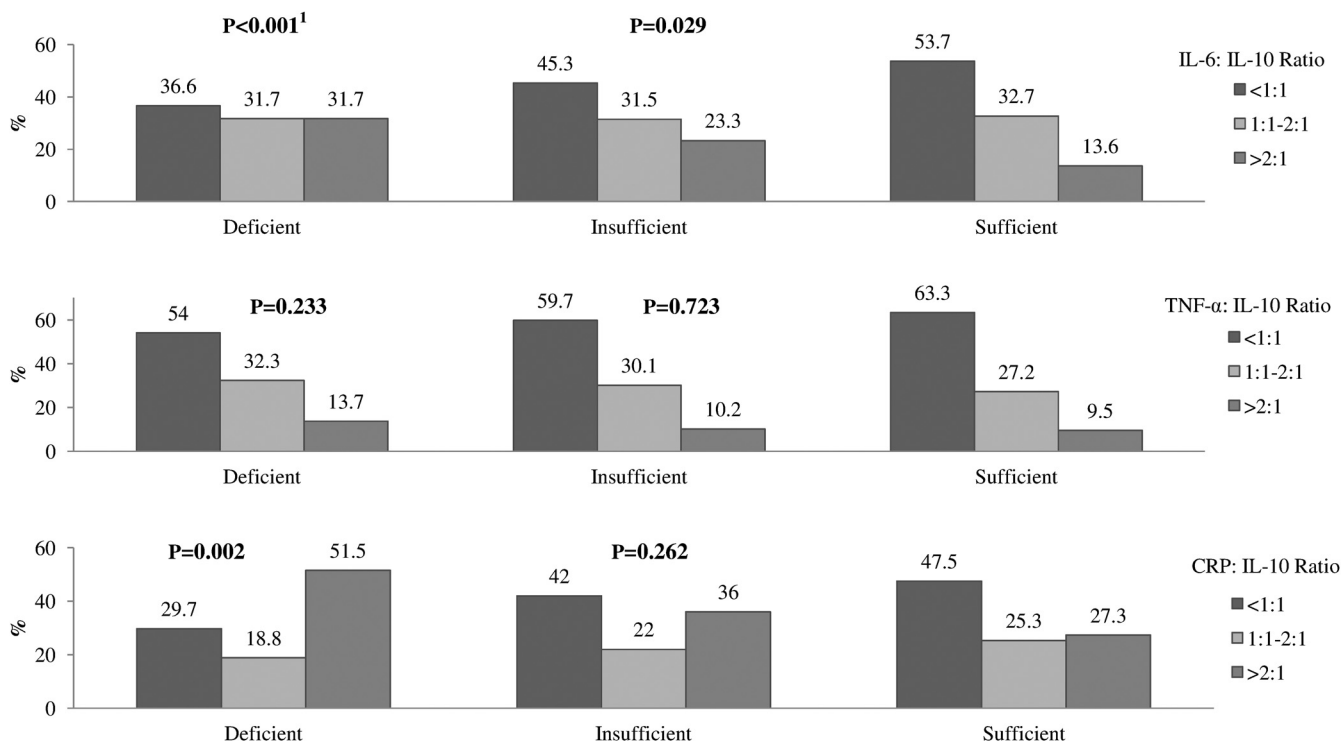
A significant negative correlation was observed between serum 25(OH)D and IL-6, CRP, and the IL-6 to

**Table 2.** Immune Profile of Participants Categorized by Vitamin D Status<sup>a</sup>

	Deficient (<25 nmol/L), n = 162	Insufficient (25–75 nmol/L), n = 648	Sufficient (>75 nmol/L), n = 147	P
General characteristics				
Age, y	70.5 (65.1–75.9) <sup>a,b</sup>	70.0 (65.9–74.1) <sup>a</sup>	72.1 (66.9–76.5) <sup>b</sup>	
% Male	38.9	53.7	47.6	
BMI, kg/m <sup>2</sup>	29.9 (26.8–34.6) <sup>a</sup>	29.3 (26.6–32.8) <sup>a</sup>	26.9 (24.6–30.1) <sup>b</sup>	
Waist/hip ratio, cm	0.94 (0.89–0.99) <sup>a,b</sup>	0.94 (0.88–0.98) <sup>a</sup>	0.90 (0.86–0.96) <sup>b</sup>	
25(OH)D, nmol/L	18.2 (14.4–21.8) <sup>a</sup>	44.2 (33.9–55.8) <sup>b</sup>	90.9 (82.0–102.0) <sup>c</sup>	
Inflammatory markers				
TNF- $\alpha$ , pg/mL	1.52 (1.10–2.04)	1.28 (0.97–1.95)	1.17 (0.95–1.77)	.081
IL-6, pg/mL	2.29 (1.37–4.11) <sup>a</sup>	1.71 (1.11–3.10) <sup>b</sup>	1.45 (0.94–2.39) <sup>c</sup>	<.001
IL-10, pg/mL	1.57 (1.00–2.64) <sup>a</sup>	1.59 (1.11–2.53) <sup>a</sup>	1.72 (1.10–2.44) <sup>a</sup>	.719
CRP, mg/L	4.20 (1.35–8.00) <sup>a</sup>	2.00 (1.00–6.00) <sup>b</sup>	2.00 (1.00–4.00) <sup>c</sup>	<.001
Cytokine ratios				
TNF- $\alpha$ /IL-10	0.88 (0.55–1.47)	0.82 (0.52–1.25)	0.70 (0.45–1.21)	.150
IL-6/IL-10	1.45 (0.71–2.58) <sup>a</sup>	1.10 (0.59–1.98) <sup>a</sup>	0.88 (0.40–1.53) <sup>b</sup>	.008
CRP/IL-10	2.10 (0.78–5.03) <sup>a</sup>	1.28 (0.58–3.57) <sup>a,b</sup>	1.11, (0.51–2.19) <sup>b</sup>	.045

<sup>a</sup> Values are median (interquartile range) unless indicated otherwise. Differences between groups were assessed using ANOVA with Tukey's post hoc test on transformed data. *P* values were calculated by one-factor analysis of covariance adjusted for age, sex, and BMI. CRP and CRP to IL-10 ratio data were available for 572 participants: deficient (*n* = 101); insufficient (*n* = 372); Sufficient (*n* = 99).

<sup>a–c</sup> Values in the same row with different superscript letters are significantly different, *P* < .05.



**Figure 2.** Vitamin D status as a determinant of cytokine ratio group. <sup>1</sup>*P* value assessed by Pearson  $\chi^2$  with sufficient status as reference category; percentage is of participant number.

IL-10 ratio ( $P < .001$ ). A negative correlation was also observed between serum 25(OH)D and the TNF- $\alpha$  to IL-10 and CRP to IL-10 ratio ( $P < .05$ ) after adjustment for age, sex, BMI, smoking, and presence of inflammatory conditions (Table 3). No significant correlation was observed between IL-10, TNF- $\alpha$ , and 25(OH)D concentrations. Significant predictors of cytokine/inflammatory marker ratios are outlined in Table 4. Participants who were vitamin D-deficient ( $<25$  nmol/L) were significantly more likely to have an IL-6 to IL-10 ratio  $>2:1$  than those with sufficient status ( $>75$  nmol/L), after adjustment for identified copredictors (age, sex, BMI, and smoking) ( $P = .031$ ). Additional factors such as smoking and BMI were also significant predictors of an IL-6 to IL-10 ratio  $>2:1$

( $P < .001$ ). To account for any potential confounding by the presence of inflammatory conditions, the data were reanalyzed excluding participants with a diagnosed inflammatory disease ( $n = 130$ ). The removal of these participants did not significantly alter the outcome (data not shown).

## Discussion

This study observed significant negative associations between vitamin D and the inflammatory markers IL-6 and CRP. Furthermore, a deficient vitamin D status ( $<25$  nmol/L) was associated with a more proinflammatory

**Table 3.** Partial Correlations of Vitamin D and Cytokines<sup>a</sup>

	25(OH)D	IL-10	IL-6	TNF- $\alpha$	CRP	IL-6/IL-10 Ratio	TNF- $\alpha$ /IL-10 Ratio
25(OH)D	1						
IL-10	+0.026	1					
IL-6	-0.199 <sup>b</sup>	+0.068	1				
TNF- $\alpha$	-0.100	+0.214 <sup>b</sup>	+0.396 <sup>b</sup>	1			
CRP	-0.222 <sup>b</sup>	+0.103 <sup>c</sup>	+0.284 <sup>b</sup>	+0.260 <sup>b</sup>	1		
IL-6/IL-10 ratio	-0.154 <sup>b</sup>			+0.095 <sup>c</sup>	+0.108 <sup>c</sup>	1	
TNF- $\alpha$ /IL-10 ratio	-0.092 <sup>c</sup>		+0.210 <sup>b</sup>		+0.086		1
CRP/IL-10 ratio	-0.158 <sup>c</sup>		+0.228 <sup>b</sup>	+0.054			

<sup>a</sup> Adjusted for age, sex, BMI, smoking, and presence of inflammatory conditions.

<sup>b</sup>  $P < .001$ .

<sup>c</sup>  $P < .05$ .



**Table 4.** Vitamin D as a Predicator of the IL-6 to IL-10 Ratio<sup>a</sup>

Reference Category	Category	>2:1 Versus 1:1 (IL-6 to IL-10 ratio)			
		$\beta$ -Value	Odds Ratio	95% Confidence Interval	P Value
BMI >36 kg/m <sup>2</sup>	BMI 20–25 kg/m <sup>2</sup>	–2.07	0.126	0.05–0.30	<.001
	BMI 25–30 kg/m <sup>2</sup>	–1.51	0.220	0.11–0.41	<.001
	BMI 30–35 kg/m <sup>2</sup>	–0.70	0.493	0.26–0.92	.027
Female	Male sex	+0.03	1.034	0.68–1.56	.872
	>80 y	–0.56	0.570	0.26–1.24	.159
Smoker	Age 60–69 y	–0.43	0.648	0.29–1.40	.274
	Age 70–79 y	–1.12	0.326	0.18–0.59	<.001
>75 nmol/L	Nonsmoker	+0.87	2.406	1.08–5.33	.031
	Vitamin D <25 nmol/L	+0.44	1.567	0.77–3.15	.209
	Vitamin D 25–75 nmol/L				

<sup>a</sup> Reference category is IL-6 to IL-10 ratio 1:1 (multinomial logistic regression).

profile (as determined by the IL-6 to IL-10 ratio) compared with individuals with an insufficient or sufficient status. Importantly, this study illustrates that 25(OH)D concentration could be a possible determinant of the IL-6 to IL-10 ratio, with those deficient in vitamin D 3 times more likely to have an IL-6 to IL-10 ratio >2:1 compared with those with sufficiency after adjustment for covariate predictors. These findings suggest that insufficient vitamin D status may have the potential to affect the inflammatory response particularly within the older adult population. It is important to note, however, that the data are observational and such associations do not necessarily indicate a causal relationship.

The significant negative correlations of vitamin D with IL-6 and CRP concentrations within this large study are consistent with previous *in vitro* (4, 14) and *ex vivo* findings (27, 28). Results from published *in vivo* studies, however, have been less clear. Previously, Peterson and Hefernan (29) reported that serum 25(OH)D status was inversely related to TNF- $\alpha$  concentration in 69 healthy women (25–82 years), whereas only a weak association between IL-6 and vitamin D status was observed in 1381 healthy participants from the Framingham Offspring observational study (30). Importantly, however, the latter study included both middle-aged to elderly adults (median age 59, range 35–89, years) and did not investigate associations separately for older participants (>60 years of age). The results of vitamin D supplementation studies have also been ambiguous. Inanir et al (17) reported a significant reduction in serum IL-6 and TNF- $\alpha$  concentrations in 70 postmenopausal women in response to calcitriol supplementation, whereas Schleithoff et al (18) reported that supplementation with vitamin D<sub>3</sub> stabilized TNF- $\alpha$  concentration in 123 patients with congestive heart failure. However, a number of other intervention studies found no significant effect of vitamin D supplementation on inflammatory markers (19–23). A possible explanation for this inconsistency may be that the signif-

icant associations shown here and elsewhere (15–18) are generally observed in populations diagnosed with some form of chronic disease, whereas most studies that reported no significant associations generally involved healthy populations, although this is not always the case (19, 21–23). Conversely, this could indicate that inflammation itself may cause low 25(OH)D concentrations. To date, however, only one study, which involved a postoperative population, has observed 25(OH)D to decrease after an inflammatory insult (31). No direct mechanism was suggested for this observation, and the authors suggested that the association might have been through the influence of other variables including a dilution effect of postoperative fluids and loss of vitamin D binding proteins. Until further studies are undertaken, it is unknown whether inflammation itself can result in significantly lower 25(OH)D concentrations.

Notably, within the current study, no association of vitamin D status with IL-10 concentration was observed, a finding that is inconsistent with evidence from *in vitro* studies that have reported significant increases in IL-10 concentration and receptor expression in response to vitamin D (32). However, IL-10 was the only inflammatory marker to display a significant seasonal variation, with the lowest concentrations observed in spring (1.27 pg/mL) compared with summer where concentrations were highest (1.81 pg/mL), and thereby displaying a similar pattern to the widely reported seasonal variation in vitamin D concentrations (2). Although the seasonal variation in IL-10 concentration could be explained by the reported seasonal increase of pollen levels (33), it may also have been to some extent affected by vitamin D status indirectly. For example, vitamin D-mediated adiponectin secretion has been previously associated with an increased IL-10 concentration (32, 34). Zittermann et al (35) observed that low vitamin D status in winter was associated with low cord blood concentrations of IL-10, whereas Stewart et al (36) reported that multiple sclerosis (MS)

patients had an excess of IL-10 in the summer period. However, not all studies have reported a significant modulatory effect of vitamin D status on IL-10 concentration (21, 23), but these studies were conducted in relatively healthy populations and with smaller sample sizes and thus may have failed to observe significant effects. In relation to the other inflammatory makers and seasonality, no association was observed, although the markers were correlated with 25(OH)D concentrations. This lack of association with seasonality is not unusual because other biochemical parameters that are strongly associated with vitamin D do not have a corresponding seasonal rhythm (such as PTH). This lack of association might be owing to other variables (separately or combined) having a much larger effect and overshadowing the effect (if any) of seasonality, making it difficult to ascertain. Currently, few studies exist in the literature that have investigated inflammatory measures and seasonality and the variables that may affect these factors.

Notably, for the first time in a large study, we observed that IL-6 and CRP concentrations were significantly lower in individuals with sufficient compared with deficient vitamin D status after adjustment for potential confounders. This observed association of vitamin D with inflammatory markers could (if confirmed from randomized controlled trials) have potential health benefits, particularly for those with autoimmune or inflammatory disease given that inflammatory markers are often raised in conditions such as MS, Crohn's disease, and rheumatoid arthritis (37–39). For example, previous studies have reported that lower circulating inflammatory cytokine concentrations have been associated with decreased disease severity in individuals with MS, ulcerative colitis, and arthritis (40–42).

Sufficient vitamin D status was also associated with a less inflammatory IL-6 to IL-10 ratio. This potential influence of vitamin D to shift immune response to a TH-2 anti-inflammatory profile has not only the obvious benefits for those with autoimmune/inflammatory conditions, but it may also have benefits for the wider population (43–45). Taniguchi et al (46) reported that an increase in the IL-6 to IL-10 ratio was associated with a poorer outcome in patients with systemic inflammatory response syndrome, whereas Kilic et al (47) reported that the IL-6 to IL-10 ratio was the most important predictor for non-ST elevation in acute coronary syndrome. Furthermore, an impaired IL-6 to IL-10 ratio has also been associated with acute myocardial infarction (48). Given the associations of the IL-6 to IL-10 ratio with cardiovascular disease, it is not surprising, therefore, that a significant association of vitamin D with inflammation was observed in the current hypertensive population.

To achieve the 75 nmol/L concentration of 25(OH)D (shown within the current study to be sufficient in terms of immune function and confirming what others have proposed) (2, 24, 27), a dietary intake of 41.1  $\mu\text{g}/\text{d}$  of vitamin D would be required to maintain this concentration throughout the year in populations with poor UVB sunlight exposure (49). Recent dietary intake studies indicate, however, that actual dietary intake of vitamin D across Northern latitude populations (with mean daily intake within the Irish population of just 3.9  $\mu\text{g}/\text{d}$ ) (50–52) is significantly below recommendations aimed at meeting even the lower Institute of Medicine vitamin D cutoffs based on bone health (53). There is, therefore, an urgent need for further studies to ascertain with certainty the vitamin D concentration that is optimal for immune function and the potential implications of increasing dietary recommendations for vitamin D intake within the population.

One of the major strengths of this study was the study size, because it is the largest observational study ( $n = 957$ ) conducted to date within an exclusively older adult population to investigate the association between vitamin D status and immune function using liquid chromatography tandem mass spectrometry, now considered the gold standard of 25(OH)D measurement (54). These findings, therefore, contribute significantly to the large body of evidence supporting a role for vitamin D in inflammatory conditions, albeit the authors acknowledge that low vitamin D could be the result of inflammation associated with the pathogenesis and progression of disease rather than the disease itself (55).

In conclusion, to our knowledge, this is the first study to demonstrate an association between vitamin D status, markers of inflammation, and the IL-6 to IL-10 ratio exclusively within independently living, older adults (>60 years of age). The findings provide evidence that vitamin D deficiency is significantly associated with a more pronounced proinflammatory status and that a higher vitamin D status (>75 nmol/L) may be required to optimize immune function toward an anti-inflammatory profile.

## Acknowledgments

We are grateful to the volunteers who participated in this research.

Address all correspondence and requests for reprints to: Professor Mary Ward, School of Biomedical Sciences, University of Ulster Cromore Road, Coleraine, Londonderry BT52 1SA, Northern Ireland, UK. E-mail: mw.ward@ulster.ac.uk.

This research was funded by the Northern Ireland Department for Employment and Learning (DEL), Cross-Border Research and Development Programme: "Strengthening the all-

Island Research Base” and the Irish Department of Agriculture, Food & the Marine and Health Research Board, Food Institutional Research Measure (FIRM) initiative.

Disclosure Summary: The authors have nothing to disclose.

## References

- Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D<sub>3</sub> analogs as immunomodulatory agents. *Trends Mol Med*. 2002; 8:174–179.
- Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc*. 2006;81:353–373.
- Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D<sub>3</sub> receptor in the immune system. *Arch Biochem Biophys*. 2000;374:334–338.
- van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D<sub>3</sub>: basic concepts. *J Steroid Biochem Mol Biol*. 2005;97: 93–101.
- Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab*. 2008;4:80–90.
- Zhang Y, Leung DY, Richers BN, et al. Vitamin D inhibits monocyte/macrophage pro-inflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol*. 2012;188:2127–2135.
- Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*. 2008;87:1080S–1086S.
- van Schoor NM, Lips P. Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab*. 2011;25(4):671–680.
- Ponsonby AL, McMichael A, Van-Der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology*. 2002;181–182:71–78.
- Ginde AA, Mansbach JM, Camargo CA. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med*. 2009;169:384–390.
- Staples JA, Ponsonby AL, Lim LL, McMichael AJ. Ecologic analysis of some immune-related disorders, including type 1 diabetes, in Australia: latitude, regional ultraviolet radiation, and disease prevalence. *Environ Health Perspect*. 2003;111:518–523.
- DeLuca HF, Cantorna MT. Vitamin D: its role and uses in immunology. *FASEB J*. 2001;15:2579–2585.
- Smolders J, Damoiseaux J, Menheere P, Hupperts R. Vitamin D as an immune modulator in multiple sclerosis, a review. *J Neuroimmunol*. 2008;194:7–17.
- Correale J, Ysraaelit MC, Gaitán MI. Immunomodulatory effects of vitamin D in multiple sclerosis. *Brain*. 2009;132:1146–1160.
- Türk U, Akbulut M, Yildiz A, et al. Comparative effect of oral pulse and intravenous calcitriol treatment in hemodialysis patients: the effect on serum IL-1 and IL-6 levels and bone mineral density. *Nephron*. 2002;90:188–194.
- Borazan A, Ustün H, Cefle A, Sekitmez N, Yilmaz A. Comparative efficacy of oral and intravenous calcitriol treatment in haemodialysis patients: effects on serum biochemistry and cytokine levels. *J Int Med Res*. 2003;31:489–496.
- Inanir A, Ozoran K, Tutkak H, Mermerci B. The effects of calcitriol therapy on serum interleukin-1, interleukin-6 and tumour necrosis factor- $\alpha$  concentrations in post-menopausal patients with osteoporosis. *J Int Med Res*. 2004;32:570–582.
- Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr*. 2006;83:754–759.
- Gannagé-Yared MH, Azoury M, Mansour I, Baddoura R, Halaby G, Naaman R. Effects of a short-term calcium and vitamin D treatment on serum cytokines, bone markers, insulin and lipid concentrations in healthy post-menopausal women. *J Endocrinol Invest*. 2003;26:748–753.
- Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. *J Neuroimmunol*. 2003;134:128–132.
- Jorde R, Sneve M, Torjesen PA, Figenschau Y, Gøransson LG, Omdal R. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. *Cytokine*. 2010;50:175–180.
- Yusupov E, Li-Ng M, Pollack S, Yeh JK, Mikhail M, Aloia JF. Vitamin D and serum cytokines in a randomized clinical trial. *Int J Endocrinol*. 2010;pii:305054.
- Barnes MS, Horigan G, Cashman KD, et al. Maintenance of wintertime vitamin D status with cholecalciferol supplementation is not associated with alterations in serum cytokine concentrations among apparently healthy younger or older adults. *J Nut*. 2011;141:476–481.
- Soubierbielle JC, Body JJ, Lappe JM, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice. *Autoimmun Rev*. 2010;9: 709–715.
- Dodoo D, Omer FM, Todd J, Akanmori BD, Koram KA, Riley EM. Absolute levels and ratios of proinflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to *Plasmodium falciparum* malaria. *J Infect Dis*. 2002;185:971–979.
- Cox SE, Arthur P, Kirkwood BR, Yeboah-Antwi K, Riley EM. Vitamin A supplementation increases ratios of proinflammatory to anti-inflammatory cytokine responses in pregnancy and lactation. *Clin Exp Immunol*. 2006;144:392–400.
- Müller K, Haahr PM, Diamant M, Rieneck K, Kharazmi A, Bendtzen K. 1,25-Dihydroxyvitamin D<sub>3</sub> inhibits cytokine production by human blood monocytes at the post-transcriptional level. *Cytokine*. 1992;4:506–512.
- Liu N, Nguyen L, Chun RF, et al. Altered endocrine and autocrine metabolism of vitamin D in a mouse model of gastrointestinal inflammation. *Endocrinology*. 2008;149:4799–4808.
- Peterson CA, Heffernan ME. Serum Tumor necrosis factor- $\alpha$  concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. *J Inflamm (Lond)*. 2008;5:10.
- Shea MK, Booth SL, Massaro JM, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. *Am J Epidemiol*. 2008;167:313–320.
- Reid D, Toole BJ, Knox S, et al. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. *Am J Clin Nutr*. 2011;93:1006–1011.
- Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O’Garra A. 1 $\alpha$ ,25-Dihydroxyvitamin D<sub>3</sub> has a direct effect on naive CD4<sup>+</sup> T cells to enhance the development of Th2 cells. *J Immunol*. 2001; 167:4974–4980.
- Nouri-Aria KT, Wachholz PA, Francis JN, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol*. 2004;172:3252–3259.
- Vaidya A, Williams JS, Forman JP. The independent association between 25-hydroxyvitamin D and adiponectin and its relation with BMI in two large cohorts: the NHS and the HPFS. *Obesity*. 2012; 20:186–191.
- Zittermann A, Dembinski J, Stehle P. Low vitamin D status is associated with low cord blood levels of the immunosuppressive cytokine interleukin-10. *Pediatr Allergy Immunol*. 2004;15:242–246.
- Stewart N, Taylor B, Ponsonby AL, et al. The effect of season on cytokine expression in multiple sclerosis and healthy subjects. *J Neuroimmunol*. 2007;188:181–186.
- Plevy SE, Landers CJ, Prehn J, et al. A role for TNF- $\alpha$  and mucosal T helper-1 cytokines in the pathogenesis of Crohn’s disease. *J Immunol*. 1997;159:6276–6282.
- Imitola J, Chitnis T, Khoury SJ. Cytokines in multiple sclerosis: from bench to bedside. *Pharmacol Ther*. 2005;106:163–177.



39. O'Shea JJ, Ma A, Lipsky P. Cytokines and autoimmunity. *Immunology*. 2002;2:37–45.
40. Raab Y, Hällgren R, Gerdin B. Enhanced intestinal synthesis of interleukin-6 is related to the disease severity and activity in ulcerative colitis. *Digestion*. 1994;55:44–49.
41. Giovannoni G, Miller DH, Losseff NA, et al. Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis. *J Neurol*. 2001;248:487–495.
42. Spîrchez M, Samaşca G, Iancu M, Bolba C, Miu N. Relation of interleukin-6, TNF- $\alpha$  and interleukin-1 $\alpha$  with disease activity and severity in juvenile idiopathic arthritis patients. *Clin Lab*. 2012;58:253–260.
43. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*. 2000;148:209–214.
44. Ding C, Parameswaran V, Udayan R, Burgess J, Jones G. Circulating levels of inflammatory markers predicts change in bone mineral density and resorption in older adults: a longitudinal study. *J Clin Endocrinol Metab*. 2008;93:1952–1958.
45. Gorelick PB. Role of inflammation in cognitive impairment: results of observational epidemiological studies and clinical trials. *Ann N Y Acad Sci*. 2010;1207:155–162.
46. Taniguchi T, Koido Y, Aiboshi J, Yamashita T, Suzaki S, Kurokawa A. Change in the ratio of interleukin-6 to interleukin-10 predicts a poor outcome in patients with systemic inflammatory response syndrome. *Crit Care Med*. 1999;27:1262–1264.
47. Kilic T, Ural D, Ural E, et al. Relation between proinflammatory to anti-inflammatory cytokine ratios and long-term prognosis in patients with non-ST elevation acute coronary syndrome. *Heart*. 2006;92:1041–1046.
48. Biswas S, Ghoshal PK, Mandal SC, Mandal N. Relation of anti- to pro-inflammatory cytokine ratios with acute myocardial infarction. *Korean J Intern Med*. 2010;25:44–50.
49. Cashman KD, Hill TR, Lucey AJ, et al. Estimation of the dietary requirement for vitamin D in healthy adults. *Am J Clin Nutr*. 2008;88:1535–1542.
50. Bailey RL, Dodd KW, Goldman JA, et al. Estimation of total usual calcium and vitamin D intakes in the United States. *J Nutr*. 2010;140:817–822.
51. Sette S, Le Donne C, Piccinelli R, Arcella D, Turrini A, Leclercq C. The third Italian National Food Consumption Survey, INRAN-SCAI 2005-06—part 1: nutrient intakes in Italy. *NMCD*. 2011;21:922–932.
52. Irish Universities Nutrition Alliance. National Adult Nutrition Survey. Dublin, Ireland: Irish Universities Nutrition Alliance; 2011.
53. Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academies Press; 2010.
54. De la Hunty A, Wallace AM, Gibson S, Viljakainen H, Lamberg-Alkardt C, Ashwell M. UK Food Standards Agency Workshop Consensus Report: the choice of method for measuring 25-hydroxyvitamin D to estimate D status for the UK National Diet and Nutrition Survey. *Br J Nutr*. 2010;104:612–619.
55. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol*. 2014;2:76–89.



Improve your clinical knowledge with the all  
new ***Pediatric ESAP™ 2013.***

[www.endoselfassessment.org](http://www.endoselfassessment.org)

