Sede Amministrativa: Università degli Studi di Padova

Dipartimento di Scienze Cardiologiche, Toraciche e Vascolari

DOTTORATO DI RICERCA IN SCIENZE MEDICHE, CLINICHE E SPERIMENTALI

INDIRIZZO: Scienze Geriatriche ed Ematologiche

CICLO: XXV

Vitain D, Physical Performance and Neuropsychological Functioning in Elderly Subjects:
The Pro.V.A Study

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INTRODUZIONE

Nelle ultime due decadi, è cresciuto l'interesse nei confronti dei ruoli extra-scheletrici della Vitamina D. Esperimenti in vitro avrebbero dimostrato la presenza di recettori per la vitamina D nel tessuto muscolare, documentando l'attività dei metaboliti attivi della vitamina sulla sintesi ex novo di proteine e sull'incremento di fibre muscolari di tipo II. Recettori della vitamina D (VDR) sembrano essere diffusi anche nel tessuto cerebrale e la forma biologicamente attiva della vitamina D (1,25OHD3) ha mostrato effetti neuroprotettivi compresa la clearance delle placche di amiloide. Diversi trials clinici sugli anziani avrebbero evidenziato come bassi livelli sierici di 25-idrossivitamina D (25OHD) si associno non solo a scarse performance motorie, ma anche a deterioramento cognitivo e aumentato rischio di depressione. Pertanto l'associazione tra vitamina D, performance fisica, funzioni cognitive e sintomi depressivi in età avanzata è diventato nell'ultima decade un argomento di grande interesse, con ampi e importanti risvolti clinici.

Lo scopo dello studio è stato quello di verificare in un ampia popolazione di soggetti in età geriatrica, l'associazione tra stato della vitamina D, performance fisica e funzioni neuropsichiche.

OBIETTIVI SPECIFICI DELLO STUDIO

Sono stati presi in considerazioni i seguenti obiettivi:

- valutare la relazione tra i livelli sierici di 25OHD e diversi test di performance fisica, validati in ambito geriatrico, in grado di esplorare le capacità di equilibrio, la velocità di camminata, la capacità di coordinazione, la forza degli arti superiori e inferiori, e la capacità aerobica. Identificare livelli sierici di 25OHD “ottimali” ai fini della performance motoria in età avanzata.
- esplorare l'associazione a lungo termine tra i livelli sierici di 25OHD e il rischio di declino cognitivo a 4 anni.

- esplorare l'associazione tra i livelli sierici di 25OHD e il rischio di sviluppare sintomi depressivi a 4 anni.

METODI

I dati per questa analisi provengono dal Progetto Veneto Anziani (Pro.V.A.). Il campione di studio è costituito da uomini e donne di età superiore a 65 anni, residenti in comunità, i cui dati sulla performance fisica, sulla valutazione cognitiva e psicologica erano completi.

Valutazione delle performance motorie:

Le performance fisiche sono state valutate sulla base dei seguenti test standardizzati:[11]

- Tandem test, **TT** (capacità di equilibrio statico): ai partecipanti è stato chiesto di mantenere l'equilibrio in tre diverse posizioni: una posizione side-by-side, una posizione semi tandem, e una posizione full-tandem. E' stato registrato il tempo espresso in secondi in cui i pazienti hanno mantenuto le suddette posizioni;
- 5 timed chair stands, **TCS** (coordinazione e la forza): ai partecipanti è stato chiesto di alzarsi e sedersi dalla sedia per 5 volte di seguito il più rapidamente possibile, con le mani incrociate sul petto: è stato registrato il tempo, in secondi, necessario per completare il test;
- Gait speed, **GS**: è stata registrata la migliore performance realizzata in due passeggiate a passo normale, lungo un corridoio di 4 metri, registrando la velocità massima in metri al secondo. Ai partecipanti era permesso usare bastoni o deambulatori;
- 6-minute walking test, **6mw** (capacità aerobica): ai partecipanti è stato chiesto di camminare alla loro andatura normale per 6 minuti, la distanza percorsa è stata registrata in metri;
- Handgrip and quadriceps strength: la forza di prensione della mano, in kg, è stata misurata utilizzando un dinamometro portatile JAMAR (BK-7498, Fred Sammons, Inc.). La forza muscolare (in Newton) degli estensori del ginocchio (quadricipite) e dei flessori dell'anca (ileopsoas) è stata determinata utilizzando un dinamometro manuale Nicholas (BK-7454, Fred Sammons, Inc.).

Valutazione delle funzioni neuropsicologiche:

Le funzioni cognitive sono state valutate al basale e al follow-up, con la somministrazione del Mini Mental State Examination a 30 items, secondo Folstein. Questo test è lo strumento neuropsicologico più diffuso e validato per misurare la funzione cognitiva in ambiente geriatrico. La diagnosi di demenza è stata effettuata da geriatri e psicologi esperti nel deterioramento cognitivo in base ai criteri stabiliti nel *Diagnostic and Statistical Manual of Mental Disorders* (quarta edizione). La depressione è stata valutata utilizzando la Geriatric Depression Scale (GDS), e un punteggio ≥ 11 è stato considerato indicativo di sintomatologia depressiva.

Analisi statistica

Tutte le analisi statistiche sono state effettuate utilizzando il programma SAS rel. 9.13 (Cary NC: SAS Institute), con significatività statistica $p<0.05$. Le caratteristiche dei partecipanti sono state analizzate utilizzando le medie (± deviazione standard) per le variabili continue e le percentuali per le variabili categoriali. Medie e proporzioni delle variabili analizzate sono state ottenute per quintili/terzili di distribuzione sesso-specifici dei valori sierici al basale di 25OHD o per le seguenti classi cliniche: deficienza (25OHD <50 nmol/L), insufficienza (25OHD compresa tra 50 e 75 nmol/L), sufficienza (25OHD >75 nmol/L). Le differenze tra le variabili categoriali sono state esaminate con il test del Chi-quadro, mentre per le variabili continue è stata condotta l'analisi della varianza (ANOVA), verificando il trend lineare tra livelli crescenti di 25OHD. Sono stati utilizzati modelli di regressione (GLM) per testare l'associazione indipendente tra i livelli di 25OHD e i test di perfomance motoria e neuropsichici. Fattori notoriamente associati ai livelli 25OHD e/o alla funzionalità fisica/cognitiva sono stati inseriti nei modelli di analisi come variabili confondenti. Al fine di individuare i livelli di 25OHD che si associano al migliore livello di performance fisica nell'anziano, abbiamo condotto un'analisi loess aggiustata per le variabili confondenti, per ciascun test di performance fisica. Sono stati utilizzati modelli di regressione logistica multivariata per determinare la relazione tra livelli sierici di 25OHD e il declino cognitivo clinicamente sensibile, definito come una riduzione del punteggio di MMSE di 3 o più punti al follow-up. Una analisi di regressione mediante random-effects è stata utilizzata per analizzare l'associazione tra livelli sierici di 25OHD e la variazione media del punteggio ottenuto al MMSE nel periodo di follow-up. Sono stati ottenuti infine modelli di regressione di Cox per verificare il potere predittivo dei livelli di 25OHD sull'insorgenza di sintomi depressivi a 4 anni.

RISULTATI

Inizialmente la popolazione dello studio consisteva di 2694 anziani, tutti residenti in comunità al momento dell'arruolamento: 1597 donne di età media 75,6 aa ± 7,5 aa (range 65-98 aa) e 1097 uomini, di età media 76,2 aa ± 7,8 aa (range 65-99 aa). La concentrazione sierica media di 25OHD era 65,0 nmol / L (± 41,3, range: 2,5-329) nelle donne, e 101,9 nmol / L (± 62,4, range: 2,5-441) negli uomini. Una carenza di vitamina D (25OHD livello <50 nmol / L), era presente nel 40% delle donne e nel 20% degli uomini, mentre una severa ipovitaminosi (25OHD <25 nmol / L) è stata identificata nel 13,5% delle donne e il 5,9% degli uomini.

Tra i 2694 anziani reclutati al basale, 1904 hanno completato al follow-up la valutazione cognitiva mediante MMSE e sono stati inclusi nell'analisi per il declino cognitivo (media [DS] di follow-up, 4.4 [1,1] anni), mentre 1675 soggetti (363 M / 1039 F, età 65-99 anni) ha completato la valutazione mediante GDS e sono stati inclusi nelle analisi per il rischio di depressione.

Associazione tra i livelli sierici di 25OHD e i test di performance motoria

L'analisi di regressione lineare, aggiustata solo per l'età dei partecipanti, ha evidenziato una associazione significativa tra i livelli di 25OHD e il test della sedia (TCS) (p <.0001 nelle donne; 0,03 negli uomini), la velocità di camminata (GS) e il test del cammino di 6 minuti (6mW) (p <.0001, in entrambi i sessi), nonchè per la forza di prensione (p <0,0009 delle donne; <0,0001 negli uomini). Dopo l’aggiustamento per i fattori confondenti, un significativo trend lineare rimaneva evidente per la prova 6mW in entrambi i sessi (p = 0,0002 nelle donne; <.0001 negli uomini), per il test della sedia (TCS) solo nelle donne (p = .004), e per la velocità di camminata (p = 0,0006) e la forza di prensione (p = 0,03) negli uomini, come mostrato in **Tabella 1a** e **1b**. L'associazione tra le concentrazioni sieriche di 25OHD e i risultati ottenuti ai test di performance motoria è rappresentata graficamente nelle Figure A, B, C e D, rispettivamente per il test della sedia, la velocità di camminata, la distanza percorsa in 6 minuti (6mW) e forza di prensione. Il tempo necessario per completare il test della sedia diminuisce significativamente all'aumentare dei livelli sierici di

25OHD, solo nei soggetti di sesso femminile: il miglior tempo di esecuzione del test si osserva per valori di 25OHD sierica compresi tra 20 e 100 nmol/L. Negli uomini ma non nelle donne, la velocità di camminata aumenta significativamente all'aumentare dei livelli di 25OHD fino a valori di vitamina prossimi a 100 nmol/L. L'incremento più significativo nel test della camminata veloce si osserva per valori di 25OHD compresi tra 50 e 75 nmol/L. La forza di prensione nei soggetti di sesso maschile aumenta progressivamente con i livelli di vitamina D, così come la distanza percorsa al test del 6mW, che aumenta per valori crescenti di 25OHD in entrambi i sessi. Per tutti i test considerati i tempi di esecuzione e le performance migliori si osservano, in entrambi i sessi, per valori di 25OHD sierici prossimi a 100 nmol/L. Per valori di 25OHD superiori non si sono osservati ulteriori miglioramenti di performance.

Associazione tra livelli sierici di 25OHD, declino cognitivo e rischio di depressione.

All'analisi logistica, aggiustando solo per il punteggio ottenuto al MMSE eseguito al basale, i partecipanti con deficit di vitamina D (25OHD < 50 nmol/L) o con insufficienza (25OHD compresa tra 50 e 75nmol/L) presentavano una probabilità maggiore rispetto a quelli con normali valori di 25OHD di avere un declino cognitivo clinicamente significativo a 4 anni come evidenziato in **Tabella 2**. Tale associazione risultava significativa, dopo il controllo per le variabili confondenti, solamente nei soggetti con carenza di vitamina D, mentre non era più significativa nei soggetti con insufficienza di 25OHD. I partecipanti con ipovitaminosi D avevano circa il 40% in più di probabilità rispetto ai soggetti con normali livelli di 25OHD di presentare un declino cognitivo clinicamente significativo al follow-up di 4 anni. Restringendo il campione ai partecipanti che non erano affetti da demenza al momento del reclutamento, l'associazione tra i livelli di 25OHD e declino cognitivo risultava ancor più evidente (**Tabella 3**), dal momento che non solo i soggetti con deficienza vitaminica ma anche quelli con valori di 25OHD tra 50 e 75 nmol/L risultavano essere a maggior rischio di declino cognitivo a 4 anni. All'analisi di regressione lineare aggiustata inizialmente solo per il punteggio del MMSE al basale **(Tabella 4)**, i soggetti con deficit di 25OHD presentavano una diminuzione al punteggio di MMSE di circa due volte quello osservato nei partecipanti con livelli di 25OHD normali. Aggiustando l'analisi per le variabili confondenti, i partecipanti con deficit di 25OHD peggioravano in media di 1.59 punti/annui al MMSE. La differenza di punteggio di MMSE tra basale e longitudinale aumenta linearmente al ridursi dei valori sierici di 25OHD. Lo stesso pattern di associazioni si è osservato quando si è ristretto il campione ai partecipanti che non erano dementi al basale, come evidenziato in **Tabella 4**.

In merito alla associazione tra livelli di vitamina D e rischio di depressione, il campione totale è stato suddiviso per terzili di distribuzione sesso-specifici di 25OHD sierica. Per i soggetti nei terzili più bassi di 25OHD, non si è osservato nessun incremento del rischio a 4 anni di sintomi depressivi, come evidenziato dall'analisi di regressione di Cox in **Tabella 5**.

CONCLUSIONI

Nel nostro campione di soggetti anziani italiani residenti in comunità abbiamo documentato una significativa associazione positiva tra le concentrazioni sieriche di 25OHD e i tests di performance comunemente utilizzati per la valutazione della motricità. Abbiamo inoltre evidenziato come in termini di outcomes muscoloscheletrici, livelli di vitamina D prossimi a 100 nmol/L sarebbero da considerarsi ottimali e quindi raccomandabili. Abbiamo inoltre evidenziato una associazione statisticamente significativa tra l'ipovitaminosi D intesa come livelli di 25OHD sierica inferiori a 50 nmol/L e e il rischio di declino cognitivo a 4 anni. Non solo, nei soggetti non affetti da impairment cognitivo, già per valori inferiori a 75 nmol/L si osserva un più rapido e significatico deterioramento a 4 anni. Pertanto valori superiori a 75 nmol/L sarebbero da considerarsi come raccomandabili per il loro potenziale effetto protettivo sul declino cognitivo. Infine non abbiamo osservato alcuna relazioni tra livelli di vitamina D e rischio di sviluppare sintomi depressivi a 4 anni.

Sulla base dei risultati dell'attività di ricerca e considerata l’alta prevalenza di ipovitaminosi D nella popolazione anziana dell'Italia settentrionale, riteniamo utile promuovere la supplementazione di vitamina D nei soggetti che invecchiano. Mantenere i livelli sierici di 25OHD il più vicino possibile a valori di 100 nmol/L sembrerebbe efficace nel preservare la performance fisica e cognitiva dell'anziano. Infine ulteriori studi trials clinici randomizzati sono necessari per ottenere unanime consenso sulla soglia di 25OHD necessaria per evitare sia problemi di sotto che di sovra trattamento.

TABELLE

Tabella 1a: Medie (Standard Error) stimate dei risultati ottenuti ai test di performance fisica, per concentrazione sierica di 25OHD nelle donne: the Pro.V.A Study.

DONNE	***25OHD quintiles (nmol/L)***					
	≤32	**>32 & ≤49**	**>49 & ≤68**	**>68 & ≤93**	**>93**	***p* for trend**
5 timed chair stands, sec						
Model 1	15.3 (0.5)	13.7 (0.4)	13.7 (0.4)	13.5 (0.4)	12.9 (0.4)	.001
Model 2	15.2 (0.5)	13.7 (0.4)	13.7 (0.4)	13.4 (0.4)	13.0 (0.4)	.004
Gait speed, m/s						
Model 1	0.64 (0.01)	0.64 (0.01)	0.64 (0.01)	0.65 (0.01)	0.66 (0.01)	.03
Model 2	0.64 (0.01)	0.64 (0.01)	0.65 (0.01)	0.66 (0.01)	0.66 (0.01)	.10
6-minute walking distance, m						
Model 1	276.1 (5.4)	300.6 (5.0)	303.2 (4.8)	309.4 (5.0)	309.7 (4.9)	<.0001
Model 2	280.3 (5.3)	301.8 (4.8)	304.5 (4.7)	309.9(4.8)	309.9 (4.7)	.0002
Handgrip strength, kg						
Model 1	22.3 (0.3)	23.3 (0.3)	25.5 (0.3)	23.9 (0.3)	23.0 (0.3)	.10
Model 2	22.5 (0.3)	23.3 (0.3)	23.6 (0.3)	24.0 (0.3)	22.9 (0.3)	.25

Tabella 1b. Medie (Standard Error) stimate dei risultati ottenuti ai test di performance fisica, per concentrazione sierica di 25OHD negli uomini: the Pro.V.A Study

MASCHI	***25OHD quintiles (nmol/L)***					
	≤ 53	**>53 & ≤79**	**>79 & ≤103**	**>103 & ≤143**	**>143**	***p* for trend**
Test della sedia, sec						
Modello 1	13.1 (0.5)	11.4 (0.5)	12.1 (0.4)	11.4 (0.4)	11.6 (0.4)	.05
Modello 2	13.0 (0.5)	11.4 (0.5)	12.0 (0.4)	11.5 (0.4)	11.6 (0.4)	.08
Velocità di camminata, m/s						
Modello 1	0.71 (0.01)	0.76 (0.01)	0.78 (0.01)	0.77 (0.01)	0.77 (0.01)	.0001
Modello 2	0.72 (0.01)	0.76 (0.01)	0.78 (0.01)	0.77 (0.01)	0.77 (0.01)	.0006
Distanza percorsa in 6 minuti, m						
Modello 1	330.3 (7.6)	362.1 (7.2)	361.9 (6.8)	379.0 (6.7)	378.6 (6.6)	<.0001
Modello 2	334.6 (7.4)	362.6 (6.9)	366.0 (6.6)	376.4 (6.5)	376.6 (6.3)	<.0001
Forza di Prensione, kg						
Modello 1	34.7 (0.6)	34.8 (0.5)	35.1 (0.5)	36.6 (0.5)	35.9 (0.5)	.01

Note: le medie aggiustate sono state ottenute mediante regressione lineare generalizzata (GLM). Il Modello 1 è aggiustato per età, BMI, abitudine al fumo, ativitàfisica, stagionalità, depressione, deterioramento cognitivo, filtrato glomerulare (GFR, secondo la formula MDRD). Il Modello 2 è aggiustato per le variabili nel Modello 1 plus comorbidità cardiovascolare, patologia osteoarticolare, BPCO, ipovisus.

FIGURE

Figure 1: Analisi grafica (Loess Plott) dell'associazione tra livelli sierici di 25OHD e il test della sedia (5 timed chiar stands, **A**), la velocità di camminata (gait speed, **B**), la distanza percorsa in sei minuti (6-minute walking distance, **C**), e la forza di prensione (handgrip strength, **D**) (linea sottile – per le donne, linea drappeggiata ♦♦ per gli uomini). Le curve sono aggiustate per le seguenti variabili: età, BMI, abitudine al fumo, atività fisica, stagionalità, depressione, deterioramento cognitivo, filtrato glomerulare (GFR, secondo la formula MDRD), comorbidità cardiovascolare, patologia osteoarticolare, BPCO, ipovisus.

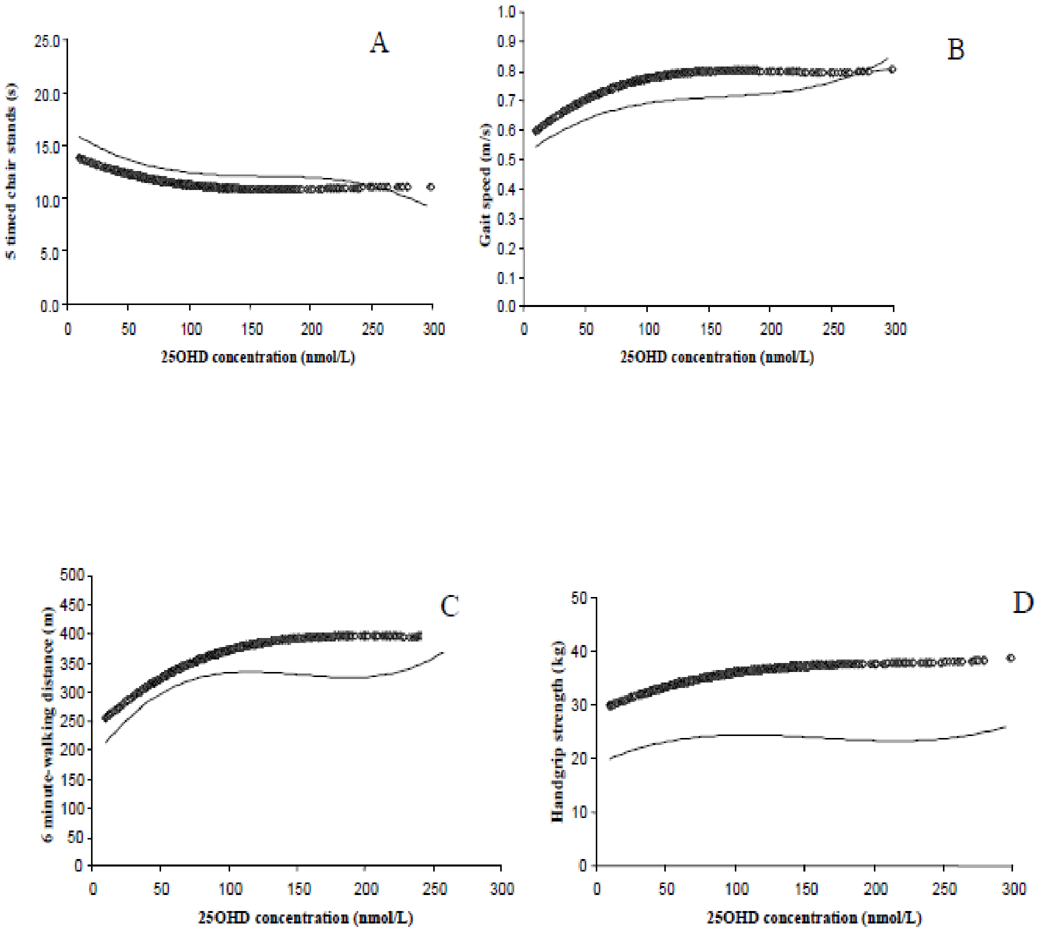

Tabella 2: Rischio Relativo (RR) di declino cognitivo clinicamente significativo a 4 anni (riduzione al punteggio di MMSE ≥ 3 punti) nei partecipanti dello studio Pro.V.A. in base a livelli basali di 25OHD.

	Serum 25OH vitamin D cut off levels, nmol/L				
	<50	***p-value***	**≥50 and < 75**	***p-value***	**≥75**
Modello 1	1.96(1.50-2.41)	<.0001	1.46(1.10-1.87)	.01	**1 [reference]**
Modello 2	1.37(1.23-1.78)	.05	1.17(0.89-1.55)	.24	**1 [reference]**

I dati sono presentati come Rischio Relativo e intervallo di confidenza 95%.
Modello 1: aggiustato per il punteggio di MMSE al basale.
Modello 2: aggiustato per il punteggio di MMSE al basale, plus: età, sesso, livello di educazione, BMI, stagione, attività fisica, dipendenza nelle ADL, sintomi depressivi, malattie cardiovascolari, diabete, BPCO e valori al basale di PTH .

Tabella 3: Rischio Relativo (RR) di declino cognitivo clinicamente significativo a 4 anni (riduzione al punteggio di MMSE ≥ 3 punti) nei partecipanti **NON AFFETTI DA DEMENZA** dello studio Pro.V.A. in base a livelli basali di 25OHD.

	Serum 25OH vitamin D cut off levels, nmol/L				
	<50	***p-value***	**≥50 and < 75**	***p-value***	**≥75**
Modello 1	2.11(1.5-2.7)	<.0001	1.59(1.1-2.0)	.01	**1 [reference]**
Modello 2	1.48(1.0-1.9)	.03	1.39(1.0-1.9)	.05	**1 [reference]**

I dati sono presentati come Rischio Relativo e intervallo di confidenza 95%.
Modello 1: aggiustato per il punteggio di MMSE al basale.
Modello 2: aggiustato per il punteggio di MMSE al basale, plus: età, sesso, livello di educazione, BMI, stagione, attività fisica, dipendenza nelle ADL, sintomi depressivi, malattie cardiovascolari, diabete, BPCO e valori al basale di PTH

Tabella 4: Variazioni medie per anno di follow-up, al punteggio di MMSE in tutti i partecipanti e nei soggetti non affetti da demenza al basale. I risultati sono presentati come Medie (Errore Standard) per ciascun livello di sierico di 25OHD

	All participants (n=1904)		***Non-demented participants (n=1724)***	
Serum 25OHD levels, nmol/L	**Modello 1**	**Modello 2**	**Modello 1**	**Modello 2**
≥75	-1.13(0.14)	-1.00(0.14)	-1.24 (0.14)	-1.48(0.14)
≥50 and < 75	-1.51(0.20)	-1.20(0.20)	-1.47(0.22)	-1.52(0.21)
<50	-2.24(0.19)	-1.59(0.20)	-1.97(0.21)	-1.58(0.21)
p-values for linear trend	<.0001	0.04	<.0001	0.05

Modello 1: aggiustato per il punteggio di MMSE al basale.
Modello 2: aggiustato per il punteggio di MMSE al basale, plus: età, sesso, livello di educazione, BMI, stagione, attività fisica, dipendenza nelle ADL, sintomi depressivi, malattie cardiovascolari, diabete, BPCO e valori al basale di PTH

Tabella 5: Rischio di sintomi depressivi a 4 anni nei partecipanti dello studio Pro.V.A.
I dati sono presentati come Hazard Ratio e Intervallo di Confidenza al 95% secondi i livelli sierici basali di 25OHD calcolati sui terzili di distribuzione sesso-specifici.

	Donne ***HR (95%CI)***	***p –value***	***Uomini*** ***HR (95%CI)***	***p –value***
Terzile 1 vs 3	0.80(0.51-1.27)	0.35	0.95(0.50-1.82)	0.89
Terzile 2 vs 3	1.00(0.65-1.53)	0.98	1.38(0.74-2.55)	0.30

Analisi aggiustata per: età, livello di educazione, BMI, stagione, attività fisica, performance motoria (punteggio ottenuto alla Short Physical Performance Battery) dipendenza nelle ADL, demenza, malattie cardiovascolari, diabete, BPCO e valori al basale di PTH.
Nelle Donne: terzile 1 ≤49 nmol/L, terzile 2 >49 and ≤ 81 nmol/L, terzile 3 >81 nmol/L;
Negli Uomini : terzile 1 ≤80 nmol/L, terzile 2 >80 and ≤125 nmol/L, terzile 3 >125 nmol/L;

PRIMA PARTE

Vitamin D and Physical Performance in Elderly Subjects: The Pro.V.A Study

INTRODUCTION

Aging people develop mobility impairment as a first step in the disablement process [1]. Given the rising numbers of elderly people, there is an increasing need to identify modifiable risk factors of mobility impairment in order to prevent or delay disability onset.

In the past two decades, it has become evident that the role of vitamin D extends beyond calcium homeostasis [2]. Experimental studies have revealed vitamin D receptors (VDR) in skeletal muscle [3,4], and vitamin D metabolites have been found to affect muscle metabolism by stimulating de novo protein synthesis, increasing the proportion of type II muscle fibers and improving muscle function [5-7]. Clinical studies on older people have shown that low serum levels of 25-hydroxyvitamin D (25OHD) correlate with a decrease in lower-extremity muscle strength and poorer performances in rising from a chair [8-11]. However, several other studies failed to show any association between vitamin D status and motor performance measures [12-15]. Controversial findings may result from differences in the number and type of performance tests considered, and an inadequate control over potential confounders that might impair physical performance. As an example, in several studies analyses were not stratified by gender, or they were only performed in women [10,11,16], introducing a significant bias since women perform less well than men, and they have lower vitamin D levels [17]. In addition, most of the studies supporting the association between vitamin D and physical performance limited their analysis to two or three tests, such as chair stands or gait tests [10,11,16-19], which are not representative of global muscle function. Another bias concerns the application of vitamin D cut-offs usually established to define the risk for osteoporosis, which are not expected to define mobility risk [10, 20]. Finally, several studies failed in controlling for confounders such as depression and cognitive impairments, two chronic conditions with a relevant impact on elderly people's performance [10, 11, 17, 19, 20]. The association between serum 25OHD concentrations and physical performance thus remains controversial and a consensus on which 25OHD levels are adequate for mobility function in older-aged people is still lacking.

The first aim of the present study was to ascertain the association between vitamin D status and mobility in a large sample of Italian older people by testing the relationship between their 25OHD serum levels and a large battery of physical performance tests, exploring balance, gait speed, coordination, upper and lower limb strength and aerobic capacity. The second aim was to identify an adequate serum 25OHD level for musculoskeletal functions in elderly men and women.

METHODS

Data Source and Subjects

Data for this analysis are from the *Progetto Veneto Anziani* (Pro.V.A.), an observational cohort study on the Italian population aged ≥65 yrs, living in two geographical areas in the North-East of Italy (Camposampiero and Rovigo). The study population included 3099 age- and sex-stratified Caucasian participants (1245 men and 1854 women), who were randomly selected between 1995 and 1997, using a multistage stratified method. Sampling procedures and data collection methods have been described elsewhere [21]. Participants were examined at the clinics by trained physicians and nurses. Disease status was determined by integrating information from physical examination and medical records review. Disability was defined as the inability to perform 1 or more of the activities of daily living (ADLs): bathing, dressing, eating, using the toilet, or transferring. Participants who lacked serum 25OHD values (n = 272), those in wheel chairs or unable to walk (n=89), or with leg and/or arm amputations (n=23), and cases of hyperparathyroidism - defined as serum calcium levels >10.5 mg/dl and parathyroid hormone (PTH) levels >55 ng/L (n=21) - were ruled out. The final sample consisted of 2694 subjects whose data on physical performance were complete. The local ethical committees of Padua University and of the Local Health Units (USSL) n. 15 and n. 18 of the Veneto Region approved the study protocol, and participants gave their written informed consent. Subjects unable to give their informed consent were not enrolled.

Physical performance measures

Physical performance measures were assessed using standardized performance tests [21]:

- Tandem test (static balance ability): participants were asked to maintain balance in three different positions: a side-by-side position, a semi tandem position, and a full-tandem position. The amount of time they succeeded in remaining so, in seconds, was recorded;
- 5 timed chair stands, TCS (coordination and strength): participants were asked to stand up and sit down 5 times as quickly as possible, with their hands folded across their chest; the time taken to complete the test, in seconds, was recorded;
- Gait speed: the best performance achieved in two walks at usual pace along a 4m corridor was recorded in meter/seconds. Participants were allowed to use canes or walkers;
- 6-minute walking test, 6mW (aerobic capacity): participants were asked to walk at their usual pace for 6 minutes, recording the distance they covered in meters [22];
- Handgrip and quadriceps strength: handgrip strength, in kg, was measured using a JAMAR hand-held dynamometer (BK-7498, Fred Sammons, Inc.). The best result obtained at two attempts with each hand was used for analyses. Knee extensor (quadriceps) and hip flexor

(iliopsoas) muscle strengths were determined using a Nicholas Manual dynamometer (BK-7454, Fred Sammons, Inc.). Quadriceps strength, in Newton, was determinate in the dominant leg [23].

Biochemical measurements

Venous blood samples were obtained after an overnight fast, centrifuged and stored at -80°C. Routine biochemical tests were performed at the city hospitals, whereas PTH and 25OHD tests were performed at the university laboratory of Padua. Serum 25OHD levels were measured by radioimmunoassay (RIA kit; DiaSorin). The intra-assay and interassay coefficients of variation for 25OHD were 8.1% and 10.2%, respectively. Serum intact PTH levels were measured using a two-site immunoradiometric assay kit (N-tact PTHSP; DiaSorin): the intra-assay and interassay coefficients of variation for PTH were 3.0% and 5.5%, respectively. Serum creatinine was measured using a standard creatinine Jaffe method (Roche Diagnostics, Germany) and glomerular filtration rate (GFR) was calculated with the MDRD formula. Serum albumin was measured using an agarose electrophoretic technique (Hydragel Protein(E) 15/30; Sebia, France).

Statistical analyses

Participants' characteristics were summarized using means (± standard deviations) for continuous variables and counts and percentages for categorical variables. Given the gender-related differences, all data analyses were stratified by sex. Means and proportions were calculated for sex-specific quintiles of the distribution of 25OHD serum levels. For continuous variables normal distributions were tested using the Shapiro Wilk test. Differences in categorical variables were examined using the C*hi*-square test. Age-adjusted *p* values for trends were calculated, checking the differences between means of covariates by quintiles of vitamin D status using analysis of variance (ANOVA). General linear models (GLM) were used to examine the independent association between 25OHD levels and performance tests. The association between performance and vitamin D status was tested considering 25OHD levels both as a continuous variable (per unit of 25OHD) and as a categorical variable (sex-specific quintiles). The presence of a nonlinear (quadratic) effect of 25OHD concentrations was examined but did not emerge, so only the linear associations were modeled. Known factors associated with 25OHD levels and/or physical functionality were examined for inclusion in the analyses and two multivariate models were obtained. Age, smoking habit (never/former vs current smoker), body mass index (BMI; calculated as the weight in kg/height in meters squared), physical activity (defined as ≥4 h/week in the previous month of at least moderate physical activity, e.g. brisk, walking, cycling, swimming, dancing, gardening or physical exercising), cognitive impairment (Indexed Mini-Mental State Examination score <0.8; [24]), depression (defined as a score ≥11 on the Geriatric Depression Scale [25]), season of the year

(November–February vs March–October) and GFR were added as confounders in the first model (Model 1). Among adjudicated diagnoses of cardiovascular diseases (CVD: coronary heart disease, congestive heart failure, cerebrovascular disease, peripheral artery disease, hypertension), diabetes, chronic obstructive pulmonary disease (COPD), osteoarthritis (including hand/knee/hip osteoarthrosis, hip fracture), neurodegenerative diseases (Parkinson, dementia), cancer, and visual impairments, those with a p-value <.10 at bivariate analysis (i.e. CVD, osteoarthritis, COPD and visual impairment) were considered as covariates and added into Model 2, including both confounders and covariates. Also variables that could act as intermediate factors of altered 25OHD levels- i.e. PTH concentrations and osteoporosis- were added to Model 2. To identify the best 25OHD levels for musculoskeletal functions in the elderly men and women, we conducted a loess analysis, where loess smoother is a form of locally weighted regression line using a weighted average of a set of data points at each part of the curve and is robust to outlying values. The procedure, applied to the whole data set in both genders, included the same variables as the multivariate models.

All analyses were performed using the SAS rel. 9.13 (Cary NC: SAS institute). All statistical tests were two-tailed and statistical significance was assumed for a p-value <.05.

RESULTS

Participants' characteristics

The sample consisted of 2694 community-dwelling elderly subjects (1597 F and 1097 M). They were not currently disabled in ADL$_s$. Their mean age (±SD) was 75.6 y (±7.5; range 65-98 y) in women, and 76.2 y (±7.8; range 65-99) in men. The mean serum 25OHD level was 65.0 nmol/L (±41.3; range: 2.5-329) in women, and 101.9 nmol/L (±62.4; range: 2.5-441) in men. Vitamin D deficiency, often defined as a serum 25OHD level <50 nmol/L [26], was present approximately in the 40% of the women and in the 20% of the men; whereas severe deficiency (25OHD <25 nmol/L; [26]) was identified in 13.5% of women and 5.9% of men.

The age-adjusted characteristics of the participants, classified by the quintiles of their 25OHD levels, are shown in Tables 1a and 1b, respectively for women and men. Participants in the lowest 25OHD quintile were significantly older than those with higher levels of 25OHD (*p* for trend <.0001). After adjusting for age, both male and female participants in the lowest quintiles were significantly less active, more depressed and more cognitively impaired than participants in the highest quintiles.

Association between 25OHD levels and performance measure

The age-adjusted mean physical performance measures by 25OHD quintiles are shown for women and men in Tables 2a and 2b, respectively. No differences in 25OHD quintiles emerged for tandem test performance and quadriceps strength in either gender, so these tests were excluded from further analyses. Significant linear associations were found for TCS test (*p for trend* <.0001 in women; .03 in men), gait speed and 6mW test (*p for trend* <.0001, in both genders), and for handgrip strength (*p for trend* <.0009 in women; <.0001 in men). The multivariate adjusted mean measures obtained for these performance tests are shown in Tables 3a and 3b, for women and men respectively. After controlling for confounders and covariates, a significant linear trend was still evident for the 6mW test in both genders (p=.0002 in women; <.0001 in men), for the TCS test in women (p=.004), and for gait speed (p=.0006) and handgrip strength (p=.03) in men. Further adjustment for PTH levels and osteoporosis slightly attenuated the associations between 25OHD levels and physical performance measures (details not shown).

When the associations between physical performance and vitamin D levels were examined using serum 25OHD levels as a continuous variable, a significant linear association emerged in the adjusted model for both genders only for the 6mW test: the β coefficient [*SE*] per unit of serum 25OHD was 0.15 [0.05] in women, p=.007; and 0.14 [0.05] in men, p=.004. Further adjustment for

PTH levels and osteoporosis did not affect the associations between 25OHD levels and this performance test.

Loess analyses for 25OHD levels and performance measures

The association between 25OHD concentrations and performance measures is shown for TCS, gait speed, 6mW distance and handgrip strength tests in Figure 1a,b,c, and d respectively. The time taken to complete the TCS test decreased significantly with increasing levels of 25OHD in women only and most of the improvements occurred between 20 to 100 nmol/L of 25OHD. In men but not in women, gait speed increased significantly for 25OHD levels up to 100 nmol/L, most of the improvement occurring at concentrations ranging from 50 to approximately 75 nmol/L. Handgrip strength improved in men with increasing level of vitamin D up to 100 nmol/L. The 6mW distance continued to increase up to 25OHD serum levels of 100 nmol/L in both genders. No further significant improvements in these four motor performances were seen for 25OHD levels >100 nmol/L, in both genders.

DISCUSSION

The association between vitamin D and performance tests exploring mobility impairments has become a clinically hot topic in the last two decades.

In our large, population-based sample of community-dwelling Italian elderly subjects, we found a significant positive association between 25OHD concentrations and 4 of the 6 performance tests habitually used to assess mobility impairment. The association was strong for TCS, gait speed, 6mW distance and handgrip strength. After controlling for several confounders, 25OHD levels were clearly associated with TCS results in women, but not in men, and with gait speed and handgrip strength only in men.

Such gender-related differences are probably explained by the significantly higher 25OHD levels seen in men, who were less likely to have vitamin D deficiency than women. This large sex-difference in 25OHD levels between genders is not so unusual, as reported in previous studies [17]. In the Longitudinal Aging Study Amsterdam (LASA), longer times to complete the TCS were only observed in participants with 25OHD < 50 nmol/L [18]. In our study, less than 20% of the men had 25OHD levels lower than 50 nmol/L, and this might explain why an association with TCS in this cohort was only seen in women.

A slower walking speed coinciding with 25OHD levels < 50 nmol/L has already been reported too [10,11,18]. In our study, gait speed increased with serum 25OHD levels in both genders in the unadjusted models, but after controlling for confounders, this association persisted in men but not in women. Unfortunately, we cannot compare our results with those of other cross-sectional studies because gender-specific analyses are often unavailable and gait speed is not always considered as a measure in its own right, but scored as part of the Short Physical Performance Battery [11,18,20].

The Rancho Bernard Study found vitamin D status associated with handgrip strength in elderly men, but not in older women [17], whereas in Zamboni et al [27], 25OHD < 40 nmol/ml correlated with arm strength in women, but not in men. Differences vis-à-vis other studies might be explained by the larger number of potential confounders for which our analyses were adjusted. Another, more likely explanation for all the sex-related differences found in our study is that men might have more preserved muscle strength than women of the same age, therefore women are already below strength and speed thresholds at which vitamin D might have a significant impact. Other potential mechanisms, such as differences in vitamin D receptor gene polymorphisms, might also be behind the sex-specific differences identified [28].

Despite the above mentioned differences in performance and serum 25OH levels, the “pattern” of the association between vitamin D concentrations and performance measures is similar in both genders, so that the lower the 25OHD level, the lower the observed performance.

Among all the performance measures considered, the 6mW distance was the motor test most strongly related to vitamin D status in both genders. Results in the 6mW test, regarded as a measure of aerobic capacity, have recently been correlated with cardiovascular risk [19,29], while the relationship between vitamin D and the cardiovascular system is still unclear. Ours is the first study to be performed on a large sample of elderly individuals, confirming the linear association between 25OHD levels and the 6mW distance in both genders, even after adjusting for numerous confounders. Serum 25OHD concentrations might therefore be able to predict the aerobic capacity of elderly subjects: the higher the vitamin levels, the higher the tolerance of exercise.

In our study, higher vitamin D serum levels are clearly associated with higher prevalence of regular physical activity and lower frequency of disease. In this sample of elderly subjects one of the most practiced physical activities was gardening (details not shown). We might suppose that this out-door activity might be related to higher sun-exposition, leading to higher serum levels of 25OHD. On the other hand, regular physical activity was stated by the healthiest elderly subjects, with the lowest prevalence of comorbidity. Given that the cross-sectional design of this study does not allow the knowledge of sequential events, vitamin D levels might be cautiously interpreted as a biomarker of good health status and good quality of life.

Our study did not confirm the association between vitamin D and static balance tests or quadriceps strength [16,30,31]. In a few cross-sectional studies, an impaired static balance was only found in women with serum 25OHD levels < 25nmol/L [10,18,32], while several other studies enrolling elderly subjects with higher vitamin D levels failed to demonstrate any association between 25OHD concentrations and the tandem test. Interventional studies also failed to show any significant improvement in static balance after vitamin D supplementation [16]. Similar discrepancies have been found for quadriceps strength, with which several studies failed to show any association with 25OHD levels [13-16]. The results from EPIDOS study confirmed that leg extensor strength was associated with age, sex and BMI, but not with 25OHD or PTH concentrations [13]. Given the above-mentioned published reports, our results confirm the lack of any association between vitamin D levels and quadriceps strength, highlighting the complexity of this poorly understood relationship.

In our study, loess analyses confirmed that 25OHD levels as close as 100 nmol/l are clearly associated in elderly people with a faster walking time, better performances in rising from a chair, higher upper limb strength, and greater aerobic capacity. Thresholds for adequate vitamin D have already been defined, but they are generally based on PTH levels, not on physical performance outcomes. One study drawn from the NHANES III data concluded that it was desirable to reach 25OHD concentrations of at least 40 nmol/L for optimal lower extremity function [11]. Some have suggested that older adults should be supplemented to maintain 25OHD levels at 70 nmol/L at least [18]. Therefore defining adequate 25OHD thresholds for both muscle skeletal and extra skeletal outcomes is more than a challenge. In our study, the identified vitamin D threshold for physical performance outcome was slightly higher than those previously reported and in contrast with Bischoff-Ferrari *et al* [11], we found no significant decline in performance among subjects with the highest of 25OHD levels, going against any hypothesis that higher vitamin D concentrations might be toxic to motor function. However for upper levels of serum 25OHD sparse data are available, particularly regarding long-term effects of chronically high concentrations, thus a margin of safety for public health recommendation is prudent. According to the last report of the Institute of Medicine on the tolerable upper vitamin D levels, serum 25OHD concentration above 125 nmol/L should raise concerns among clinicians about potential adverse effect, particularly on extra skeletal outcomes [33].

The present study has limitations. A participation bias probably attenuated the results, since the participants were probably the healthiest. This might explained why in our population-based sample vitamin D levels were significantly higher than those reported in previous studies on elderly Italian subjects [34,35]. Differences in methods used to measure 25OHD make it difficult to compare optimal levels with those observed in other studies [36], although methodological differences would not affect the linear association seen between 25OHD and physical function. Moreover the cross-sectional design of our study did not allow us to formulate hypothesis on the causality of the relationship between vitamin D and performance.

The main strengths of our study lie in its population-based design and large sample size, comprising a proportion of men and women representative of the general older population in northern Italy. A further strength relates to the large number of confounders and adjudicated diseases investigated, and the numerous performance tests conducted to explore different dimensions of mobility.

In conclusion, serum concentrations of 25OHD close to 100 nmol/L seem to be associated to greater benefit for musculoskeletal functions in our elderly community-dwelling subjects. Given the high prevalence of vitamin D insufficiency in the elderly population of northern Italy, ageing people should be given supplementation to keep their 25OHD levels as nearest as possible to this threshold, in order to preserve their physical performance. Besides additional researches are needed for consensus on 25OHD threshold in order to avoid problems of both under and overtreatment.

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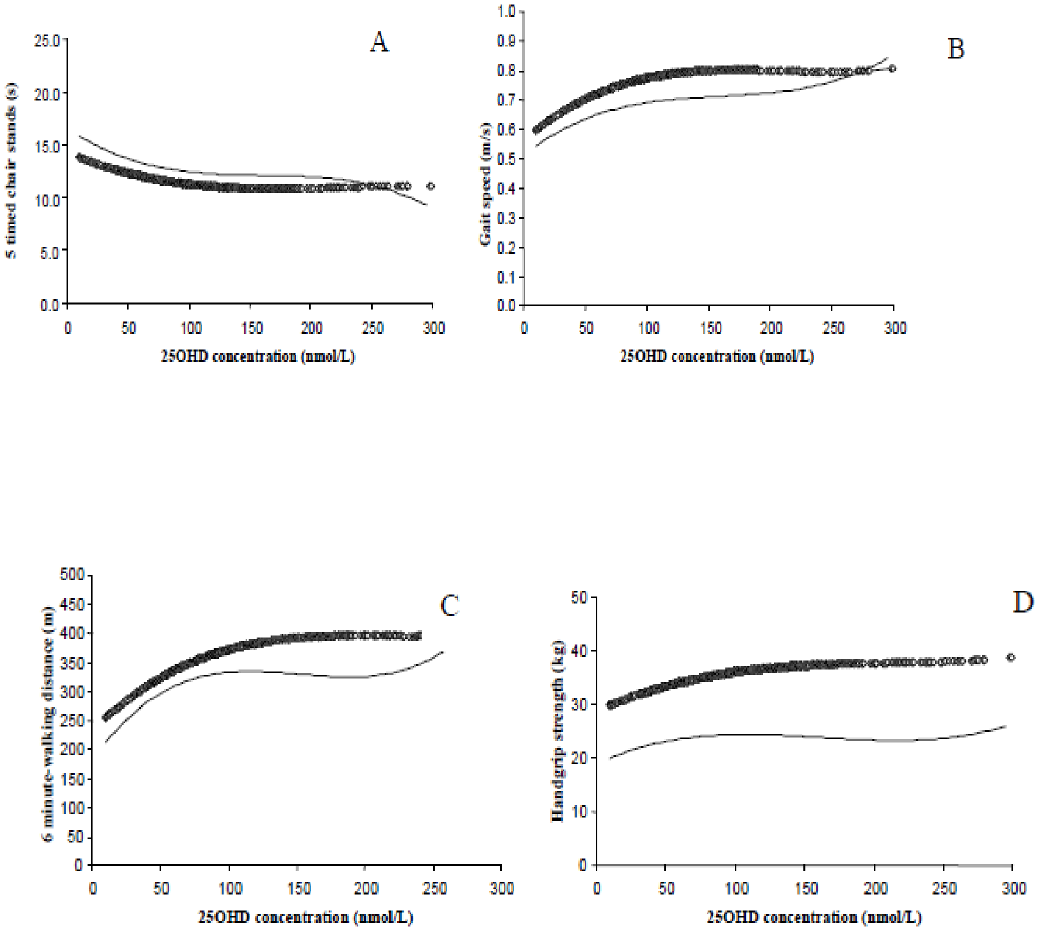
FIGURE LEGEND

Figure 1: Loess regression plots of 5 timed chair stands (**1a**), gait speed (**1b**), 6-minute walking distance (**1c**), and handgrip strength (**1d**) by 25-hydroxyvitamin D (25OHD) concentrations (**straight line** – for women; **diamond line** ◊◊ for men). Plots are adjusted for confounder variables (age, BMI, smoking habit, regular physical activity, season, depression, cognitive status, glomerular filtration rate [according to the MDRD formula]), plus the covariates cardiovascular diseases, osteoarticular disease, COPD, and visual impairment.

For conversion from nmol/L to ng/ml divide by 2.496



TABLES

Table 1a. Participants' characteristics by serum 25-hydroxyvitamin D (25OHD) quintiles in women: the PRO.V.A. Study. Numbers are mean values (and Standard Deviations) or percentage (%), as appropriate.

	25OHD quintiles (nmol/L)					
	≤32	**>32 & ≤49**	**>49 & ≤68**	**>68 & ≤93**	**>93**	***Age-adjusted***
	(n=339)	(n= 315)	(n= 322)	(n=304)	(n=317)	***p for trend***
Age (yrs)	79.9(7.5)	76.4(7.7)	75.2(6.9)	73.5(6.6)	72.4(6.0)	<.0001 (unadjusted)
BMI (kg/m^2)	27.9(5.3)	28.1(5.1)	28.0(4.5)	28.5(4.7)	27.7(4.5)	.03
Current smokers, %	6.8	4.4	7.4	6.2	4.4	.01
Regular activity,%	49.6	62.4	67.3	77.2	80.2	<.0001
Depression,%	50.5	41.0	37.5	33.8	37.6	.07
MMSE ratio	0.72(0.23)	0.77(0.17)	0.79(0.16)	0.82(0.14)	0.83(0.13)	.006
Cardiovascular diseases, %	31.6	21.7	16.5	14.9	12.0	.003
Neuro-degenerative diseases,%	18.3	9.2	6.8	4.6	3.5	.03
Osteoarticular diseases,%	78.6	73.5	72.4	63.5	65.2	.03
Any cancer,%	7.1	8.6	6.5	6.2	7.3	.97
Visual impairments,%	36.6	26.0	23.6	20.0	18.3	.05
Diabetes ,%	13.3	10.8	9.6	8.9	8.5	.10
COPD,%	7.1	7.9	3.4	4.3	2.8	.005*
Serum levels						
Calcium (mg/dl)	9.4(2.4)	9.4(0.4)	9.4(0.4)	9.7(5.7)	9.5(0.43)	.49
PTH (ng/L)	56.4(43.1)	44.6(20.6)	42.6(21.0)	39.4(18.9)	36.3(31.5)	<.0001
GFR (ml/min/1.73 m^2)	64.5(19.0)	65.9(20.2)	65.9(16.9)	67.9(17.3)	68.9(16.1)	.88
Albumin (g/dL)	4.22(0.40)	4.32(0.47)	4.33(0.36)	4.36(0.31)	4.38(0.35)	.005
25OHD (nmol/L)	20.4(7.8)	41.2(4.9)	58.5(5.3)	80.1(7.2)	128.5(37.8)	

* also adjusted for smoking habit; BMI (body mass index), MMSE (Mini-Mental State Examination), COPD (Chronic Obstructive Pulmonary Diseases), PTH (parathyroid hormone), GFR (glomerular filtration rate), 25OHD (serum 25-hydroxyvitamin D).

For conversion from nmol/L to ng/ml divide by 2.496

Table 1b. Participants' characteristics by serum 25-hydroxyvitamin D (25OHD) quintiles in men: the Pro.V.A. Study. Numbers are mean values (and Standard Deviations) or percentage (%), as appropriate.

	25OHD quintiles (nmol/L)					
	≤ 53 (n= 231)	**>53 & ≤79** (n= 212)	**>79 & ≤103** (n= 217)	**>103 & ≤143** (n= 219)	**>143** (n= 218)	***Age-adjusted p for trend***
Age (yrs)	80.6(8.0)	76.7(8.1)	75.9(7.44)	74.0(6.9)	73.5(6.4)	<.0001 (unadjusted)
BMI (kg/m^2)	26.2(4.4)	26.9(4.1)	26.7(3.7)	26.6(3.3)	27.1(3.5)	.83
Current smokers, %	24.2	20.3	20.7	26.5	22.5	.20
Regular activity, %	65	79.6	85.1	83.4	95.0	<.0001
Depression, %	35.4	22.7	20.2	19.7	17.4	.002
MMSE ratio	0.74(0.21)	0.80(0.18)	0.81(0.14)	0.84(0.12)	0.84(0.11)	.007
Cardiovascular diseases, %	38.7	28.4	28.1	23.8	22.6	.03
Neuro-degenerative diseases, %	20.4	10.3	1.8	3.6	2.3	<.0001
Osteoarticular diseases, %	49.8	49.5	46.0	37.4	36.7	.09
Any cancer ,%	13.0	10.0	6.4	6.8	6.4	.02
Visual impairment ,%	35.2	28.9	20.7	21.5	17.0	.1
Diabetes, %	10.4	7.6	8.8	7.8	4.6	.02
COPD,%	20.3	14.6	21.2	14.2	9.2	.13*
Serum levels						
Calcium (mg/dl)	9.7(2.3)	9.3(0.6)	9.4(0.4)	10.0(8.6)	9.4(0.7)	.95
PTH (ng/L)	48.3(32.8)	39.3(18.9)	39.5(18.9)	32.9(14.2)	29.4(16.5)	<.0001
GFR (ml/min/1.73 m^2)	70.2(20.3)	72.9(19.4)	74.7(19.5)	74.3(16.9)	78.4(18.5)	.16
Albumin (g/dL)	4.23(0.43)	4.34(0.36)	4.38(0.34)	4.37(0.32)	4.42(0.32)	.0003
25OHD (nmol/L)	34.3(14)	66.1(7.7)	91.7(7.1)	121.5(11.4)	198.8(56.5)	

* also adjusted for smoking habit; BMI (body mass index), MMSE (Mini-Mental State Examination), COPD (Chronic Obstructive Pulmonary Diseases), PTH (parathyroid hormone), GFR (glomerular filtration rate), 25OHD (serum 25-hydroxyvitamin D).

For conversion from nmol/L to ng/ml divide by 2.496

Table 2a. Observed physical performance measures (mean [SD]) by serum 25-hydroxyvitamin D (25OHD) quintiles in women: the Pro.V.A. Study.

	25OHD quintiles					
	≤32 (n=339)	**>32 & ≤49** (n= 315)	**>49 & ≤68** (n= 322)	**>68 & ≤93** (n=304)	**>93** (n=317)	***Age- adjusted p for trend***
Performance tests						
Side by side, sec	9.9(0.6)	9.9(0.4)	9.9(0.4)	9.9(0.6)	9.9(0.1)	.68
Semi-tandem, sec	9.2(2.0)	9.5(1.9)	9.6(1.5)	9.7(1.4)	9.9(0.8)	.07
Full tandem, sec	7.9(3.0)	8.4(2.7)	8.2(2.9)	8.3(2.9)	8.5(2.8)	.30
5 timed chair stands, sec	16.4(10.8)	14.0(6.3)	14.0(9.1)	13.1(5.2)	12.4(3.5)	<.0001
Gait speed, m/s	0.55(0.20)	0.61(0.21)	0.64(0.18)	0.67(0.18)	0.70(0.18)	<.0001
6-min walking distance, m	221.5(117.8)	283.5(109.9)	297.3(109.1)	321.4(96.8)	332.8(86.4)	<.0001
Handgrip strength, kg	20.0(5.8)	22.5(5.7)	23.1(6.0)	24.1(5.9)	23.9(5.6)	<.0009
Quadriceps strength						
Knee extension, N	18.7(9.0)	19.9(7.6)	22.1(20.2)	21.4(8.2)	21.1(19.1)	.39
Hip flexion, N	20.9(52.0)	18.2(7.11)	20.3(9.4)	20.8(8.2)	21.1(21.9)	.89

Table 2b. Observed physical performance measures (mean[SD]) by serum 25-hydroxyvitamin D (25OHD) in men: the Pro.V.A. Study

	25OHD quintiles (nmol/L)					
	≤ 53 (n= 231)	**>53 & ≤79** (n= 212)	**>79 & ≤103** (n= 217)	**>103 & ≤143** (n= 219)	**>143** (n= 218)	***Age-adjusted p for trend***
Performance tests						
Side by side, sec	9.9(0.6)	9.9(0.7)	10(0)	10(0)	9.9(0.6)	0.43
Semi-tandem, sec	9.6(1.4)	9.8(0.9)	9.6(1.5)	9.8(1.1)	9.8(1.1)	0.38
Full tandem, sec	8.4(3.0)	9.1(2.3)	9.0(2.3)	9.0(2.3)	9.4(1.8)	0.36
5 timed chair stands, sec	14.5(11.8)	12.0(4.4)	12.0(4.4)	12.0(9.0)	11.0(3.2)	0.03
Gait speed, m/s	0.63(0.21)	0.72(0.20)	0.76(0.19)	0.77(0.17)	0.80(0.17)	<0.0001
6-min walking distance, m	281.6(133.8)	345.6(119.0)	349.7(119.4)	384.1(96.3)	395.8(92.5)	<0.0001
Handgrip strength, kg	30.5(9.1)	33.8(8.7)	34.9(8.8)	37.2(8.2)	37.1(7.9)	<0.0001
Quadriceps strength						
Knee extension, N	24.3(21.5)	30.2(30.8)	28.8(24.9)	31.0(24.2)	30.4(17.8)	0.26
Hip flexion, N	24.9(24.8)	31.2(31.9)	26.7(10.1)	31.2(24.1)	29.7(11.0)	0.47

Table 3a. Adjusted estimates of physical performance mean values [mean (SE)] by serum 25-hydroxyvitamin D (25OHD) in women: the Pro.V.A Study

FEMALE	***25OHD quintiles (nmol/L)***					
	≤32	**>32 & ≤49**	**>49 & ≤68**	**>68 & ≤93**	**>93**	***p* for trend**
5 timed chair stands, sec						
Model 1	15.3 (0.5)	13.7 (0.4)	13.7 (0.4)	13.5 (0.4)	12.9 (0.4)	.001
Model 2	15.2 (0.5)	13.7 (0.4)	13.7 (0.4)	13.4 (0.4)	13.0 (0.4)	.004
Gait speed, m/s						
Model 1	0.64 (0.01)	0.64 (0.01)	0.64 (0.01)	0.65 (0.01)	0.66 (0.01)	.03
Model 2	0.64 (0.01)	0.64 (0.01)	0.65 (0.01)	0.66 (0.01)	0.66 (0.01)	.10
6-minute walking distance, m						
Model 1	276.1 (5.4)	300.6 (5.0)	303.2 (4.8)	309.4 (5.0)	309.7 (4.9)	<.0001
Model 2	280.3 (5.3)	301.8 (4.8)	304.5 (4.7)	309.9(4.8)	309.9 (4.7)	.0002
Handgrip strength, kg						
Model 1	22.3 (0.3)	23.3 (0.3)	25.5 (0.3)	23.9 (0.3)	23.0 (0.3)	.10

Notes: Adjusted mean values were obtained using the GLM procedure. Model 1 was adjusted for confounder variables (age, BMI, smoking habit, regular physical activity, season, depression, cognitive status, glomerular filtration rate (according to the MDRD formula). Model 2 was adjusted for the variables in Model 1 plus the covariates cardiovascular diseases, osteoarticular disease, COPD, and visual impairment;

SE = standard error.

Table 3b. Adjusted estimates of physical performance mean values [mean (SE)] by serum 25-hydroxyvitamin D (25OHD) in men: the Pro.V.A Study

MALE	***25OHD quintiles (nmol/L)***					
	≤ 53	**>53 & ≤79**	**>79 & ≤103**	**>103 & ≤143**	**>143**	***p* for trend**
5 timed chair stands, sec						
Model 1	13.1 (0.5)	11.4 (0.5)	12.1 (0.4)	11.4 (0.4)	11.6 (0.4)	.05
Model 2	13.0 (0.5)	11.4 (0.5)	12.0 (0.4)	11.5 (0.4)	11.6 (0.4)	.08
Gait speed, m/s						
Model 1	0.71 (0.01)	0.76 (0.01)	0.78 (0.01)	0.77 (0.01)	0.77 (0.01)	.0001
Model 2	0.72 (0.01)	0.76 (0.01)	0.78 (0.01)	0.77 (0.01)	0.77 (0.01)	.0006
6-minute walking distance, m						
Model 1	330.3 (7.6)	362.1 (7.2)	361.9 (6.8)	379.0 (6.7)	378.6 (6.6)	<.0001
Model 2	334.6 (7.4)	362.6 (6.9)	366.0 (6.6)	376.4 (6.5)	376.6 (6.3)	<.0001
Handgrip strength, kg						
Model 1	34.7 (0.6)	34.8 (0.5)	35.1 (0.5)	36.6 (0.5)	35.9 (0.5)	.01

Notes: Adjusted mean values were obtained using the GLM procedure. Model 1 was adjusted for confounder variables (age, BMI, smoking habit, regular physical activity, season, depression, cognitive status, glomerular filtration rate (according to the MDRD formula). Model 2 was adjusted for the variables in Model 1 plus the covariates cardiovascular diseases, osteoarticular disease, COPD, and visual impairment;

SE = standard error.

SECONDA PARTE

Vitamin D and Cognitive Decline in Elderly Subjects: The Pro.V.A Study

INTRODUCTION

Dementia and cognitive impairment have an increasing burden on public health and society. Therefore identify early markers and modifiable risk factors of development of such disorders is more than a challenge. Recent insights suggest that vitamin D, mostly known for its effects on calcium and bone metabolism, may be important for preserving cognitive functions via several different mechanisms [1]. Vitamin D receptors have been shown to be widespread in brain tissue [2] and Vitamin D's biologically active form has shown neuroprotective effects including the clearance of amyloid plaques [3]. Although the positive influence of vitamin D on brain function is biologically plausible, few researchers have explore the association between vitamin D status and neuropsychological tests exploring cognitive functioning in elderly people. In the European Male Ageing Study (EMAS), in middle-aged and older men vitamin D levels lower than 35 nmol/L have been found to be associated to slower information processing speed, but not with other cognitive domains such as visuo-constructional ability, memory and recognition [4].

The third National Health and Nutrition Examination Survey (NAHNES III) reported that elderly participants (aged ≥60 years) in the highest 25OHD quintile had the worst cognitive performance after adjustment for possible confounders (such as age, sex, race/ethnicity and activity) [5].

Most of the studies investigating the relationship between vitamin D status and cognitive decline are limited by the cross-sectional design, the small sample sizes or failure to control for multiple confounders [4,6,7,8]. To our knowledge, there are only few prospective cohort studies examining the association between 25-hydroxyvitamin D 25(OH)D levels and cognitive decline, and conflicting results have been reported. In the InCHIANTI population-based study, low baseline levels of vitamin D were associated with subsequent cognitive decline in older aged people studied over a 6-year period [9]. A subgroup of the ongoing ESTHER study in German elders demonstrated that low levels of vitamin D may be associated with reduced cognitive functioning, after 5 years of follow-up [10]. On the contrary, the Osteoporotic Fractures in Men (MrOS) Study on community-dwelling older men, found very little evidence of independent associations between lower 25(OH)D levels and both baseline cognitive impairment and cognitive decline [11].

Longitudinal studies suggesting that vitamin D deficiency may have adverse effects on cognition or behavior are very scarce and there is currently insufficient evidence to draw definitive conclusions on which 25(OH)D serum levels should be considered adequate to prevent or delay cognitive decline in older-aged people.

The aim of the present study was to test the hypothesis that lower 25(OH)D levels are associated with a greater likelihood of cognitive impairment and risk of cognitive decline.

We measured 25(OH)D serum levels and assessed cognitive function using the Mini-Mental State Examination tool in a large sex and age-stratified population of community-dwelling elderly people enrolled in the Pro.VA study [12], and followed them prospectively for an average of 4.4 years for changes in cognitive function.

STUDY POPULATION

The *Progetto Veneto Anziani* (Pro.V.A.) study was designed to identify risk factors for all-cause mortality and disability in older men and women and has been described extensively elsewhere [12]. Briefly, the Pro.VA. study is an observational cohort study on the Italian population aged ≥65 yrs, living in two geographical areas in the North-East of Italy (Camposampiero and Rovigo). The baseline study population included 3099 age- and sex-stratified Caucasian community dwelling participants (1245 men and 1854 women), who were randomly selected between 1995 and 1997, using a multistage stratified method designed to keep the male-to-female ratio at 2:3 and to oversample the oldest possible age-group. Sampling procedures and data collection methods have been fully described elsewhere [12].

A total of 1904 participants completed the follow-up cognitive assessment between 1999 and 2001 and were included in the present analysis (mean [SD] follow-up, 4.4 [1.1] years).

Compared with the sample as a whole, those lost to follow-up (n=1195) were generally older (mean [SD] age, 81.3 [6.8] vs 74.0 [6.7] years), had lower baseline Mini-Mental State Examination [MMSE] score (mean [SD], 20.0 [7.7] vs 24.8 [4.2]) and lower serum 25OHD levels (mean [SD], 58.2 [20.2] vs 75.5 [43.7] nmol/L) (1-way ANOVA test, $p<.0001$ for all comparisons). Moreover those lost to follow-up were more likely than the study participants to have depression symptoms (38% vs 31%), diagnosis of dementia (56.7% vs 31.0%), dependency in ADLs (38.6% vs 13.2%) and cardiovascular diseases (38.4% vs 17.2%) (C*hi*-square test, $p <.0001$ for all comparisons).

The local ethical committees of Padova University and of the Local Health Units USSL) n. 15 and 18 of the Veneto Region approved the study protocol, and participants gave their written informed consent. Subjects unable to give their informed consent were not enrolled.

Clinical and Laboratory Data

Both at baseline and at follow up, participants were examined at the city hospitals by trained physicians and nurses. Information on educational level, physical activity and smoking status was collected during an in-person interview. Educational level (as the total number of years of school attended) was categorized as ≤5 versus >5 years of school. Regular physical activity was defined as > 4 h/week in the previous month of at least moderate physical activity (brisk walking, biking, gardening, dancing, or physical exercising). Smoking status was categorized as “never/former” (for at least 1 year in the past), and “current” smoking.

Disease presence at baseline was determined by board-certified study physicians who examined all of the clinical information collected for each participant in the study, including disease history, self-reported symptoms by standardized questionnaires, medical and hospital records, blood assays, and physical examination. Preexisting major diseases included any of the following: cardiovascular diseases (CVD: congestive heart failure, angina and myocardial infarction, stroke, and peripheral artery disease), diabetes, chronic pulmonary diseases (COPD), cancer, osteoarthritis diseases (including hand/knee/hip osteoarthrosis, hip fracture). Depression was evaluated by using the Geriatric Depression Scale (GDS) [13], and a score of ≥11 was indicative of depressive symptoms. Impaired mobility was defined as a mean gait speed ≤0.4 m/s during 2 timed 4-m walks at normal pace, whereas disability was defined as the inability/need for assistance to perform 1 or more of the activities of daily living (ADL) [14]: bathing, dressing, eating, using the toilet, or transferring.

Venous blood samples were obtained after an overnight fast, centrifuged and stored at -80°C. Routine biochemical tests were performed at the city hospitals, whereas PTH and 25OHD tests were performed at the university laboratory of Padua. Serum 25OHD levels were measured by radioimmunoassay (RIA kit; DiaSorin). The intra-assay and interassay coefficients of variation for 25OHD were 8.1% and 10.2%, respectively. Serum intact PTH levels were measured using a two-site immunoradiometric assay kit (N-tact PTHSP; DiaSorin): the intra-assay and interassay coefficients of variation for PTH were 3.0% and 5.5%, respectively. Serum creatinine was measured using a standard creatinine Jaffe method (Roche Diagnostics, Germany) and glomerular filtration rate (GFR) was calculated with the MDRD formula. Serum albumin was measured using an agarose electrophoretic technique [Hydragel Protein(E) 15/30; Sebia, France].

Cognitive Function Assessment

Cognitive function was evaluated at baseline and at follow-up, by administering the 30-item MMSE [15]. This test is the most widely and validated neuropsychological tool to measure cognitive function in geriatric patients. Moreover it is effective as a screening instrument to separate patients with cognitive impairment from those without it. In addition when used repeatedly the instrument is able to measure changes in cognitive status [16]. Scores for the MMSE range from 0 to 30, with lower scores reflecting worse cognitive function. The crude MMSE score was adjusted for age and formal education using the coefficients proposed for the Italian population [17].

Diagnosis of dementia was performed by geriatricians and psychologists with expertise in cognitive impairment according to criteria set out in the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) [18].

Statistical analysis

Baseline participants' characteristics were summarized using means (± standard deviations) for continuous variables and counts and percentages for categorical variables. Means and proportions were calculated for 25OHD serum levels categorized into clinical groups: 25OHD deficient (<50 nmol/L); 25OHD insufficient (≥50 to <75 nmol/L); and 25OHD sufficient (≥75 nmol/L) [19].

For continuous variables normal distributions were tested using the Shapiro-Wilk test. Differences in categorical variables were examined using the C*hi*-square test, whereas for continuous variables differences between 25OHD levels were checked by using analysis of variance (ANOVA).

Multivariate logistic regression models were used to determine the relationship of serum 25OHD levels to substantial cognitive decline, previously defined as a decline in MMSE score of 3 or more points at follow-up [9].

In unadjusted models, we controlled for baseline MMSE score only. In fully adjusted models, we adjusted for variables that have been identified as potential con founders in studies of cognition or 25OHD levels: age in years, sex, education (>5 years of schooling), smoking habit (never/former vs current smokers), season during which blood samples were obtained (November–February vs March–October), body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters), depressive symptoms, physical activity, GFR, impaired mobility and diagnoses of CVD, diabetes, COPD, osteoarthritis and cancer. Also variables that could act as intermediate factors of altered 25OHD levels- i.e. PTH concentrations - were added to the fully adjusted model.

We used random-effects models to examine the association between serum 25OHD levels and changing in MMSE over the follow-up period. Mean 4y changing in MMSE scores, modeled as a continuous variable, were obtained for each clinical group of 25OHD. Mean MMSE-variations were adjusted for all the baseline covariates included in the fully adjusted model, and their interaction with time (years of follow up) was also considered. Possible 2-way interactions between 25OHD level and changing in cognitive function were tested by including product terms. The association between cognitive performance and vitamin D status was tested considering 25OHD levels both as a continuous variable (per unit of 25OHD) and as a categorical variable. The presence of a nonlinear (quadratic) effect of 25OHD concentrations was examined but did not emerge, so only the linear associations were modeled.

In secondary analysis, we excluded those participants with dementia diagnosed at baseline (n=180).

All analysis were performed using the SAS rel. 9.13 (Cary NC: SAS institute). All statistical tests were two-tailed and statistical significance was assumed for a p-value <.05.

RESULTS

The sample consisted of 1904 community-dwelling elderly subjects (1191 F and 713 M) who completed the follow-up cognitive assessment at 4 y. The mean baseline serum 25OHD level was 65.0 nmol/L (±41.3; range: 2.5-329) in women, and 101.9 nmol/L (±62.4; range: 2.5-441) in men. Vitamin D deficiency was present approximately in the 40% of the women and in the 20% of the men; whereas severe deficiency (25OHD <25 nmol/L; [20]) was identified in 13.5% of women and 5.9% of men.

The prevalence of diagnosed dementia among the Pro.V.A. baseline population (n=3099) was 5.8% (180 subjects). When considering the baseline MMSE scores, those with an impaired cognitive test (MMSE score <24) were 32% (997 subjects). At follow-up, in 2001, dementia affected a total of 244 subjects over a total of 1904 participants (12.8%). The newly diagnosed were 112 subjects. Those with a significant impaired MMSE (<24) were 30.5% (581 subjects).

Baseline characteristics of the study population are listed in Table 1. Unadjusted baseline MMSE scores were significantly lower in those subjects who were 25(OH)D deficient than in those who were 25(OH)D sufficient. Participants who were 25(OH)D deficient were also significantly older than those with higher levels of 25OHD (*p* for trend <.0001), and they were more likely to be female, less active, more depressed and more cognitively impaired than participants in the highest 25OHD level group.

In logistic regression models adjusted only for the baseline MMSE score, participants who were 25(OH)D deficient (<50 nmol/l) or insufficient (>50-<75) were more likely than those who were 25(OH)D repleted to have a 4y substantial cognitive decline on the MMSE scores (Table 2). This association was attenuated but remained significant after controlling for covariates in the deficient 25OHD group only, whereas it was no longer significant in the insufficient 25OHD group. Participants with a 25(OH)D deficiency were approximately 40% more likely than those 25(OH)D sufficient to experience a substantial cognitive decline on the MMSE score at follow-up.

Restricting the sample to participants who were non-demented at baseline (Table 3), the association between 25OHD levels and cognitive decline was even more evident. Participants with both 25OHD deficiency and insufficiency were at higher risk for cognitive decline at 4y.

In random-effects models adjusted for baseline MMSE score only, those who were 25OHD deficient experienced a decline per year of follow-up in cognitive test about twice that observed in participant 25OHD sufficient (Table 4). In fully adjusted models, participants with 25OHD deficiency declined by 0.59 MMSE points per year more than participants who were 25OHD sufficient. The increased rate of decline for those who were 25OHD deficient was statistically significant, as was the linear trend across groups.

The same pattern of associations was observed when we restricted the sample to participants who were non-demented at baseline, even though the amount of the decline and the differences between levels of 25OHD were slightly lower than those observed in all participants.

When the associations between changing in MMSE scores and vitamin D levels were examined using the log-transformed serum 25OHD levels as a continuous variable, a significant linear association emerged in the fully adjusted model: the β coefficient [SE] per unit of log-transformed baseline serum 25OHD was 0.14 [0.01] in all participant, p=.05; and 0.12 [0.01] in non-demented participant, p=.05 (details not shown).

In Figure 1 the adjusted mean MMSE scores at baseline and at follow-up are shown in non-demented participants, according to their 25OHD baseline level. Subjects with 25OHD levels >75nmol/L experienced a decline in MMSE much more slower than those with a 25OHD insufficiency and deficiency. Moreover participant in the highest level, presented mean MMSE score at follow-up higher than 24 (which is considered the clinical cut-off for cognitive impairment).

DISCUSSION

In our population-based study, we found a independent prospective association between low serum levels of 25OHD and the risk of subsequent cognitive decline over 4,4 years. Elderly subjects with vitamin D deficiency, defined as 25OHD levels lower than 50 nmol/L, were at higher risk of experiencing a clinically relevant decline in MMSE scores, independently from their cognitive functioning level. This association remained significant even controlling for a wide range of potential confounders. Moreover, restricting the sample to elderly subjects who were non demented at baseline, even those with vitamin D insufficiency (regarded as 25OHD levels ranging from 50 to 75 nmol/L), were at higher risk for decline in cognitive functions. Though in each vitamin D classes considered in the present study, a decline in MMSE scores over the 4y-follow up does emerge, subjects with vitamin D sufficiency (>75 nmol/l) showed a decline significantly slower than those observed for vitamin D insufficient and deficient subjects; Moreover only participants with the highest vitamin D levels had an adjusted mean follow-up MMSE score greater than 24, which is the clinical cut off commonly used to identify cognitively impaired patients.

Previous studies exploring the relationship between vitamin D and cognitive performance in adults have produced inconsistent findings. Data from NHANES III, a large population-based study on community dwelling subjects, did not show a relationship between serum 25OHD levels and neurocognitive performance in adults [5]. Interestingly, an association between vitamin D status and learning and memory tasks was observed only in the oldest group (60– 90 years).

Also the European Male Ageing Study (EMAS), found that lower level of 25OHD were associated to poorer cognitive performance and the strength of the association was even more significant in those aged 60–69 years and in those aged 70–79 years [4]. These findings allow us to speculate that any ‘potential’ beneficial effect of 25OHD on cognitive function may be evident only in older-aged subjects.

To the best of our knowledge, before our study, only Llewellyn et al. [9] explored the prospective association between vitamin D and the long term risk of cognitive decline in a large sample of elderly subjects. In a total of 858 participants of the InChianti population–based study, levels of serum 25OHD lower than 25 nmol/L, were associated with an increased risk of substantial cognitive decline in MMSE scores over a 6-year period and this association remained after adjusting for potential confounders [9]. In our study a substantial cognitive decline was evident even in subjects with 25OHD levels lower than 50 nmol/l and in non demented people, association between vitamin D status and cognitive decline was apparent even in insufficient vitamin D subjects. The potential confounders considered in our study was similar to those examined in the InChianti study, however the mean values of 25OHD were significantly higher than those observed in the Llewellyn et al study [9]. It is possible that the greater sample size and the higher mean 25OHD serum levels observed in our study have allowed us to test the association with cognitive decline even in the stages of vitamin D deficiency and insufficiency, extending the information obtained in the InChianti study [9].

The present study has limitations. A participation bias probably attenuated the results, since the participants were probably the healthiest and with a higher level of outdoor activity. This might explained why in our population-based sample vitamin D levels were significantly higher than those reported in previous studies on elderly Italian subjects [20-21]. Differences in methods used to measure 25OHD make it difficult to compare optimal levels with those observed in other studies [22], although methodological differences would not affect the linear association seen between 25OHD and cognitive function. Causes of the cognitive changes observed over time were not assessed, and neuro-cognitive functioning was tested by MMSE only: no other neuropsychiatric test were used to explore different cognitive domains. Moreover data on 25OHD levels at follow-up and on vitamin D intake were not available. By the way, in the Pro.V.A. Study [12], less than 1% of the participants were assuming vitamin D integration at the time of enrolment, and less than 2% were vitamin D supplemented during the follow-up period.

The main strengths of our study lie in its population-based design and large sample size, comprising a proportion of men and women representative of the general older population in northern Italy. A further strength relates to the large number of confounders and adjudicated diseases investigated. Moreover the prospective design of the study, the repetition of the analysis excluding those participants who were already demented at baseline, and the absence of a 2-way interactions between 25OHD levels and baseline cognitive function allows us to be more confident that the association observed between vitamin D deficiency and cognitive decline over the follow-up period was not due to reverse causation.

In conclusion we found that elderly subjects with vitamin D deficiency are at higher risk of substantial cognitive decline over a 4.4 -year period and that this association remained after adjusting for potential confounders. Moreover in elderly subjects without cognitive impairment even vitamin D insufficiency might be an independent risk factor of developing cognitive impairment. Whereas level of 25OHD higher than 75 nmol/L seems to be protective from clinically significant cognitive dysfunction. Randomized controlled trials are necessary to confirm whether vitamin D supplementation in elderly subjects with vitamin D deficiency might be beneficial to prevent or delay cognitive decline.

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Table 1: Baseline Characteristics of the Pro.V.A. Participants and MMSE scores at follow-up according to Vitamin D serum levels.

	Serum 25OH vitamin D cut off levels, nmol/L			
Characteristics	<50 n=522	50-75 n= 428	>75 n=954	*p-value*
Age, mean ±SD, (y)	76.4±7.3	74.0±6.6	72.5±5.9	<.0001
Women, n (%)	428(82.0)	311(72.6)	452(47.4)	<.0001
Educational level (< 5y of schooling), n (%)	353(67.7)	84 (19.5)	146(15.3)	<.0001
BMI, mean ±SD, (Kg/m^2)	28.1±5.1	28.1±4.3	27.5±4.2	.006
Current smokers, n (%)	48(9.2)	53(12.4)	138(14.5)	.004
Season tested				.23
From November to February	315(60.3)	221(51.6)	358(37.5)	
From March to October	207(39.7)	207(48.4)	596(62.5)	
Regular physical activity, n (%)	318(63.0)	317(74.8)	809(85.3)	<.0001
Speed, mean ± SD, (m/sec)	0.62±0.20	0.69±0.18	0.76±0.18	<.0001
Dependency in ADL, n (%)	23.2	14.2	7.2	<.0001
GDS score, mean ± SD, (range 0-15)	10.2±6.6	8.7±6.1	7.4±5.7	<.0001
Basal MMSE, mean ± SD,(range 0-30)	23.9±4.9	24.5±4.1	25.4±3.8	<.0001
Follow-up MMSE	21.7±6.6	23.0±5.5	24.2±5.2	<.0001
4y-deltaMMSE	2.15±4.8	1.50±4.10	1.19±4.18	<.0001
Depression, n (%)	208(42.1)	128(31.3)	231(25.1)	<.0001
Dementia (MMSE<24), n (%)	203(39.1)	139(32.5)	239(25.1)	<.0001
Cardiovascular diseases, n (%)	114(22.0)	63(14.7)	146(15.3)	.003
Diabetes, n (%)	45(8.6)	34(7.9)	73(7.6)	.51
COPD, n (%)	41(7.8)	28(6.6)	74(7.8)	.95
Serum 25OHD, mean ± SD, (nmol/L)	32.5±11.6	61.3±7.2	123.4±49.2	<.0001
Calcium, mean ± SD , (mg/dl)	9.5±1.9	9.5±0.4	9.7±5.3	0.35
PTH, mean ± SD, (ng/L)	47.3±29.4	40.7±19.7	34.5±22.6	<.0001
Albumin, mean ± SD, (g/dL)	4.3±0.41	4.4±0.31	4.5±1.7	0.07

Table 2: Logistic Regression Model for the Relative Risk of the 4y-substantial cognitive decline in Pro.V.A. participants, according to 25OHD serum levels. (Substantial cognitive decline: a decline of ≥ 3 points at MMSE).

	Serum 25OH vitamin D cut off levels, nmol/L				
	<50	*p-value*	≥50 and < 75	*p-value*	**≥75**
Model 1	1.96(1.50-2.41)	<.0001	1.46(1.10-1.87)	.01	**1 [reference]**
Model 2	1.37(1.23-1.78)	.05	1.17(0.89-1.55)	.24	**1 [reference]**

Unless otherwhise specified, data are presented as relative risk and 95% confidence interval.

Model 1: adjusted for MMSE score at baseline.

Model 2: adjusted for MMSE score at baseline, plus: age, sex, educational level, BMI, season, smoking, status, regular physical activity, impaired mobility, dependency in ADLs, depression symptoms, GFR, cardiovascular diseases, diabetes, COPD, PTH serum levels.

Table 3: Logistic Regression Model for the Relative Risk of the 4y-substantial cognitive decline **in NON-DEMENTED** Pro.V.A. participants, according to 25OHD serum levels. (Substantial cognitive decline: a decline of ≥ 3 points at MMSE)

	Serum 25OH vitamin D cut off levels, nmol/L				
	<50	*p-value*	≥50 and < 75	*p-value*	**≥75**
Model 1	2.11(1.5-2.7)	<.0001	1.59(1.1-2.0)	.01	**1 [reference]**
Model 2	1.48(1.0-1.9)	.03	1.39(1.0-1.9)	.05	**1 [reference]**

Unless otherwhise specified, data are presented as relative risk and 95% confidence interval.

Model 1: adjusted for MMSE score at baseline.

Model 2: adjusted for MMSE score at baseline, plus: age, sex, educational level, BMI, season, smoking, status, regular physical activity, impaired mobility, dependency in ADLs, depression symptoms, GFR, cardiovascular diseases, diabetes, COPD, PTH serum levels.

Table 4: Random-effects models illustrating the adjusted mean 4y changes in MMSE scores [mean (SE)], by serum 25OHD levels

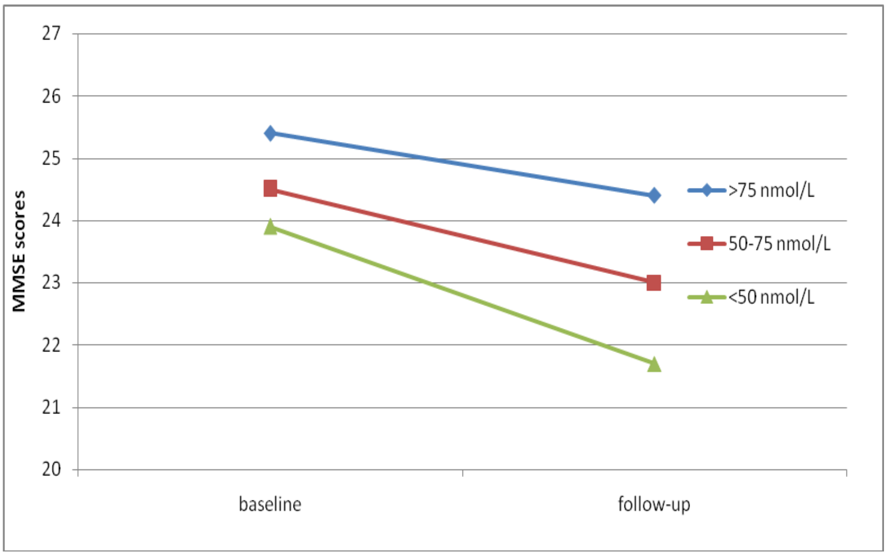
	All participants (n=1904)			**Non-demented participants (n=1724)**	
Serum 25OHD levels, nmol/L	Model1	*Model2*		Model1	*Model2*
≥75	-1.13(0.14)	-1.00(0.14)		-1.24 (0.14)	-1.48(0.14)
≥50 and < 75	-1.51(0.20)	-1.20(0.20)		-1.47(0.22)	-1.52(0.21)
<50	-2.24(0.19)	-1.59(0.20)		-1.97(0.21)	-1.58(0.21)
p-values for linear trend	<.0001	0.04		<.0001	0.05

Unless otherwhise specified, data are presented as adjusted mean differences in MMSE scores and SE

Model 1: adjusted for MMSE score at baseline.

Model 2: adjusted for MMSE score at baseline, plus: age, sex, educational level, BMI, season, smoking, status, regular physical activity, impaired mobility, dependency in ADLs, depression symptoms, GFR, cardiovascular diseases, diabetes, COPD, PTH serum levels.

FIGURE 1. Change in cognitive function IN Pro.V.A.participant accoridng to their 25OHD baseline level. Estimated mean MMSE score are obtained by the use of random-effects model with multivariate adjustment for adjusted for age, sex, educational level, BMI, season, smoking, status, regular physical activity, impaired mobility, dependency in ADLs, depression symptoms, GFR, cardiovascular diseases, diabetes, COPD, PTH serum levels.



TERZA PARTE

Vitamin D and Risk for Depression in Elderly Subjects: The Pro.V.A Study

INTRODUCTION

Chronic low serum 25-hydroxyvitamin D (25OHD) concentrations are common in older-aged people and are associated with numerous non-skeletal diseases. In both humans and animals, vitamin D is a neurosteroid hormone which may regulate neurotransmission, neuroprotection, neuroimmunomodulation and brain processes [1-3]. Nuclear vitamin D receptor (VDR) have been located in the human cortex and hippocampus [4], and it has recently been suggested that vitamin D might act on the hypothalamic core which plays a role in mood regulation [3,5]. It has been hypothesized that hypovitaminosis D may contribute to late life depression [6-8]. Wilkins et al. [9] established that low serum 25OHD oncentrations were closely related to active mood disorders in 80 community-dwelling subjects aged 65 years and older, whereas Milaneschi et al. found that 25OHD levels lower than 50 nmol/l increased the risk of developing depressive mood over a 3- and 6 y of follow up, in a large population based cohort study, particularly in female subjects [10]. In contrast, Oren et al. [11] found no significant differences between serum vitamin D concentrations in 15 depressed subjects compared to 15 healthy controls. Moreover, clinical trials supporting the hypothesis of the efficacy of vitamin D supplementation on mood disorders are very scarce. Dumville et al. [12] did not observe any improvement in SF12 scale scores following 6 months' vitamin D supplementation (800 IU/day) in 1,621 women aged 70 years and older. Therefore, studies exploring the association between vitamin D status and depressive symptoms in older aged people have conflicting findings, the majority of these studies are limited by the cross-sectional design, the limited sample, or the failure to control for possible confounders [8; 13-15].

Vitamin D levels might be interpreted as a biomarker of good health status and good quality of life, therefore the association between hypovitaminosis D and depression may have a common matrix, since both conditions are common in unhealthy older-aged people. We assume that after controlling for the higher number of possible confounders, the association between hypovitaminosis and depression might be no longer significant.

The aim of the present study is to examine the longitudinal relationship between 25OHD levels and depressive symptoms over a 4.4-yr follow-up in a representative group of older-aged men and women. We hypothesized that older-aged subjects with lower 25OHD levels at baseline would not experience a subsequent worsening of depressive symptoms and they would not be at higher risk of developing clinically relevant depressed mood than those with higher 25OHD, after controlling for a large number of comorbidities, functionality and physical performance.

STUDY POPULATION

Data for this analysis are from the *Progetto Veneto Anziani* (Pro.V.A.), an observational cohort study on the Italian population aged ≥65 yrs, living in two geographical areas in the North-East of Italy (Camposampiero and Rovigo), designed to identify risk factors for all-cause mortality and disability in older men and women. Sampling procedure, study design and data collection method have been described extensively elsewhere [16]. Briefly, the baseline Pro.VA study population included 3099 age- and sex-stratified Caucasian community dwelling participants (1245 men and 1854 women), who were randomly selected between 1995 and 1997, using a multistage stratified method designed to keep the male-to-female ratio at 2:3 and to oversample the oldest possible age-group.

Participants who lacked baseline serum 25OHD values (n = 295) and those with missing baseline GDS scores (n=313) – were excluded. Among the remaining 2491 participants, we additionally excluded 582 subjects who were lost to follow-up at 4y, and 234 subjects who do not have available data on depressive symptoms. The final sample consisted of 1675 subjects whose data on vitamin D status and GDS assessment were complete both at baseline and follow-up.

Compared with the sample as a whole, those lost to follow-up were more likely to be women (53.5 vs 46.1%; chi-square test p<.0001) and older (mean [SD] age, 80.6 [6.4] vs 73.1 [6.2] years). They had higher scores at the GDS (mean [SD], 10.2 [6.6] vs 8.2 [6.1]) and lower serum 25OHD levels (mean [SD], 59.2 [20.2] vs 77.5 [43.7] nmol/L) (1-way ANOVA test, *p<.0001* for all comparisons). Moreover those lost to follow-up were more likely than the study participants to have diagnosed depression symptoms (42.5.6% vs 29.5%), diagnosis of dementia (56.3.7% vs 25.0%), dependency in ADLs (32.7% vs 10.2%) and a double prevalence of cardiovascular diseases (36.4.4% vs 15.7%) (C*hi*-square test, *p <.0001* for all comparisons).

The local ethical committees of Padua University and of the Local Health Units USSL) n. 15 and 18 of the Veneto Region approved the study protocol, and participants gave their written informed consent. Subjects unable to give their informed consent were not enrolled.

Clinical and Laboratory Data

Both at baseline and at follow up, participants were examined at the city hospitals by trained physicians and nurses. Information on educational level, physical activity and smoking status was collected during an in-person interview. Educational level (as the total number of years of school attended) was categorized as ≤5 versus >5 years of school. Regular physical activity was defined as ≥ 4 h/week in the previous month of at least moderate physical activity (brisk walking, biking, gardening, dancing, or physical exercising). Smoking status was categorized as “never/former” (for at least 1 year in the past), and “current” smoking. Body weight and height were measured by trained physicians and Body Mass Index (kg/m ^{2}) was calculated. Disease presence at baseline was determined by board-certified study physicians who examined all of the clinical information collected for each participant in the study, including disease history, self-reported symptoms by standardized questionnaires, medical and hospital records, blood assays, and physical examination. Preexisting major diseases included any of the following: cardiovascular diseases (CVD: congestive heart failure, angina and myocardial infarction, stroke, and peripheral artery disease), diabetes, chronic pulmonary diseases (COPD), cancer, osteoarthritis diseases *(including hand/knee/hip osteoarthrosis, hip fracture). Estimation of* glomerular filtration rate (GFR) calculated with the MDRD formula, was also included in the analysis. Cognitive function was evaluated by administering the 30-item Mini Mental State Examination (MMSE).[17] Lower extremity function was evaluated by using the Short Physical Performance Battery according to standard protocol as described elsewhere [18]. Disability was defined as the inability/need for assistance to perform 1 or more of the activities of daily living (ADLs): bathing, dressing, eating, using the toilet, or transferring.

Venous blood samples were obtained after an overnight fast, centrifuged and stored at -80°C. 25OHD and PTH tests were performed at the university laboratory of Padua. Serum 25OHD levels were measured by radioimmunoassay (RIA kit; DiaSorin). The intra-assay and interassay coefficients of variation for 25OHD were 8.1% and 10.2%, respectively. Serum intact PTH levels were measured using a two-site immunoradiometric assay kit (N-tact PTHSP; DiaSorin): the intra-assay and interassay coefficients of variation for PTH were 3.0% and 5.5%, respectively. Serum creatinine was measured using a standard creatinine Jaffe method (Roche Diagnostics, Germany) and GFR was calculated with the MDRD formula. Serum albumin was measured using an agarose electrophoretic technique [Hydragel Protein(E) 15/30; Sebia, France].

Depression and Cognitive Function Assessment

The presence of depressive symptoms was assessed both at baseline and at follow-up by using the Geriatric Depression Scale (GDS) [19]. The Geriatric Depression Scale (GDS) is a 30-item self-report assessment to identify depression and extensively validated in the elderly. Scores for the GDS range from 0 to 30, and a score of ≥11 is indicative of the presence of depressive symptoms. Presence of depression was verified by personnel expert on psychiatric diseases in elderly.

Statistical analyses

Participants’ characteristics were summarized using means (± standard deviations) for continuous variables and counts and percentages for categorical variables. Given the gender-related differences, all data analyses were stratified by sex. Means and proportions were calculated for sex-specific tertiles of the distribution of 25OHD serum levels. For continuous variables normal distributions were tested using the Shapiro Wilk test. Differences in categorical variables were examined using the C*hi*-square test. Age-adjusted *p* values for trends were calculated, checking the differences between means of covariates by tertiles of vitamin D status using analysis of variance (ANOVA). General linear models (GLM) were used to examine the independent association between baseline 25OHD levels and GDS scores at baseline and at follow-up. The presence of a nonlinear (quadratic) effect of 25OHD concentrations was examined but did not emerge, so only the linear associations were modeled. Known factors associated with 25OHD levels and/or with depressive symptoms were examined for inclusion in the analysis. The following confounders and covariates were added in the fully adjusted model: age in years, sex, and educational level, smoking habit, season during which blood samples were obtained (November–February vs March–October), BMI, regular physical activity, SPPB scores and diagnoses of CVD, diabetes, COPD, osteoarthritis and cancer. Also variables that could act as intermediate factors of altered 25OHD levels- i.e. PTH concentrations - were included in the analysis. In all analysis, 25(OH)D status was coded as an indicator variable with the higher level as the reference group.

Cox proportional hazards model was fit to compare risk of developing depressed mood over the follow-up period by Vitamin D status. Participants who do not develop depressed mood were censored at the date of the last follow-up. Hazard ratios (HR) and 95% confidence intervals (CI) were used to compare rates of depressed mood across 25(OH)D tertiles. Multivariable analyses were adjusted for the previously selected covariates that were significantly related to the outcome. All analyses were performed using SAS (version 8.2; SAS Institute, Inc., Cary, NC) with a statistical significance level set at $p < 0.05$.

RESULTS

The sample consisted of 1675 community-dwelling elderly subjects (1039 F and 636 M) who completed the follow-up depression assessment. The baseline prevalence of depressive symptoms among the participants was 40.6% in women and 23.8% in men. No significant variation in the prevalence of depressed mood was found at follow-up, in 2001, with depressive symptoms affecting the 39.3% of women and the 22.5% of men. From baseline to follow-up, newly depressive symptoms were found in 146 women (22.1%), and in 74 men (14.2%). Also Vitamin D deficiency was more frequent in female (405) than in male (20%) participants. Mean serum 25OHD level was 71.7 nmol/L (±42.3) in women, and 101.0 nmol/L (±62.7) in men. Severe deficiency (25OHD <25 nmol/L; [26]) was identified in 13% of women and only 5% of men.

The age-adjusted characteristics of the participants, classified by the tertiles of their 25OHD levels, are shown in Tables 1a and 1b, respectively for women and men. Participants in the lowest 25OHD tertile were significantly older and less active than those with higher levels of 25OHD (*p* for trend <.0001). After adjusting for age, both male and female participants in the lowest tertiles have higher GDS scores, higher rates of disabilities in ADLs, and lower physical performances.

The multivariate adjusted mean GDS scores obtained at baseline and follow-up are shown in Table 2, by sex-specific 25OHD tertiles. A significant linear trend was evident for the GDS scores at baseline in women only (p=.02). Follow-up GDS scores were not associated to baseline Vitamin D status in both genders (p=.19 in women, and p=.63 in men). Participants in tertiles 1 compared with those in the highest tertile, did not show significantly higher GDS scores at follow-up.

Lower baseline serum levels of 25(OH)D were not associated with higher probability of developing depressed mood during the follow-up (Table 3). Compared to women in the highest 25OHD tertile, those in tertiles 1 and 2 did not experience an increased risk for developing depressive symptoms (tertile 1:HR 0.80; 95% CI 0.51-1.27; *P 0.35;* tertile 2 HR 1,00 95%CI 1,53, p0,98). Similarly, men in the lowest tertiles, compared with those in the highest tertile, had no significant hazard (tertile 1 HR 0,95; 95% CI 0,50-1,82; *P* 0,89; tertile 2 HR 1,38; 95% CI 0,74-2,55; *P* 0,30). The most significant 4y-predictors of depressed mood in the Pro.V.A. participants after controlling for possible confounders and covariates were the SPPB scores in both genders and age in men only. The higher the baseline physical performance the lower the risk of developing depressive symptoms at 4 y (HR 0,84, 95%CI 0,98, p=,003 in women; HR 0,80,95%CI 0,68-0,94, p=,009 in men). In male participants, older age was also a significant predictor of depressed mood (HR 1,05, 95%CI 1,01-1,09, p=0,01).

DISCUSSION

Depression is a debilitating chronic illness, and often difficult to treat. It is known to have a seasonal pattern, with summer and winter peaks [20]. Given that vitamin D is widely deficient in Western populations [21-22], and that there is a demonstrated association between mood states and seasonality, several studies have investigated the link between vitamin D and depression.

In our large population-based study, we found no evidence of a prospective independent association between circulating levels of 25(OH)D and depressive symptoms. Participants with low 25(OH)D serum levels did not experience a greater increase in depressive symptoms over a 4yr of follow-up. Low levels of 25OHD were associated to GDS scores only in cross-sectional analysis in female participants. This association was not accounted for by physical health or other potential confounding factors and was not substantially modified by the season of data acquisition and blood collection. The results of our cross-sectional analysis are supported by previous studies on depression in elderly subjects. Wilkins et al. [9] found that 25OHD levels below 20 nmol/l were robustly associated with the presence of mood disorder (odds ratio 11.7, 95% CI 2.0–66.9) in a sample of older aged-subjects. In Hoogendijk et al., 25OHD levels were significantly higher in non-depressed persons when compared to those found in subjects suffering from depressive symptoms [23]. The association between hypovitaminosys D and depression mood was significant even adjusting for potentially confounding factors such as age, sex, BMI, smoking status, and a large number of chronic conditions and was not explained by differences in season of data acquisition, level of physical activity, or use of antidepressants.

Other cross-sectional studies have not observed a relationship between vitamin D and depression. After adjustment for a variety of confounding factors, 25(OH)D levels were not found associated with depressive symptoms in a recent study of 3262 older men and women in China [24]. In a large population-based sample from the NHANES, neither serum vitamin D nor PTH levels were associated with depression after taking the potential confounders into consideration [25]. The inverse associations of vitamin D with moderate-to-severe depression or with major depression observed in the unadjusted model were attenuated to null after further adjusting for other potential confounders, since the coexistence of chronic diseases appeared to be a major confounder [26].

However findings from cross-sectional studies have a limited explanatory power. The evaluation of vitamin D respect to the current mood status, it cannot be ascertained whether observed relationships between low 25(OH)D levels and depression are likely causal. Low 25(OH)D levels might preceded the development of depression or they might be a consequence of dietary and/or behavioral changes resulting from depression. For example, it is plausible that individuals developing depression may reduce their time spent outdoors, participate less in physical activity, change their diet, or increase smoking, all of which may result in lower 25(OH)D levels [27-29].

A small body of research has evaluated whether vitamin D may play a role in the occurrence and the developing of depression mood. Since now only two studies (one of them in children) have evaluated the longitudinal association between vitamin D and depression after a period of follow up [10,30]. A prospective association between vitamin D status and risk for mood disorders was found in Tolppanen et al. study, which considered a population of children. In Milaneschi et al. study, hypovitaminosis D was found to be an indipendent risk factor for the development of depressive symptoms at 6 years, in the large InCHIANTI population based study of elderly people, even after controlling for several confounding factors [10]. In contrast with these findings, we found no evidence of a linear association between baseline 25OHD levels and GDS scores at follow-up in both genders. Low levels of 25OHD were not predictive of subsequent developing of depressive symptoms in our population. To the best of our knowledge, before our study, only the Milaneschi et al. study had deepen the influence of 25OHD on the risk for developing depression after a period of follow up in old people. Even though we had included a set of covariables in our analyses that were almost the same as those used in the study conducted by Milaneschi et al.[10], we do not found any prospective independent association between lower levels of 25OHD and the risk for developing depression mood, both in male then in female older subjects. Differences results might be due to different methodology since depressive symptoms were evaluated by the CES-D questionnaire, and the diagnosis of depression was not confirmed by a clinical psychiatric diagnosis. Another possible explanation might be related to the use of different 25OHD thresholds, since in Milaneschi et al study, tertiles of 25OHD serum levels distribution were computed on the sample as whole, whereas in our study gender-specific 25OHD levels tertiles were obtained due to the difference in mean values between male and female subjects.

Among the other potential confounding factors included in the analysis, even the PTH levels were no associated with depression risk according to other studies [26]. On the contrary, Hoogendijk et al. found that PTH serum levels are associated to depression. However, this association resulted weak and after dividing the population for quartiles of PTH, significant only for subjects in higher quartile [23].

The potential for confounding in studies of vitamin D and depression is great. A variety of factors influence 25(OH)D levels, including age, time spent outdoors, latitude, physical activity, body mass index, smoking, and alcohol use [27-29; 30-32]Many of these factors are also associated with the incidence of depression [33-37] therefore it might be difficult to determine if the observed associations are accurate or may be explained instead by confounding, since controlling for any possible confounding factors might be really hard.

Two considerations sustained our findings. Firstly, the randomized-controlled studies have demonstrated that vitamin D supplements did not substantially improve the depressive symptoms [12;38-40] except for obese subjects [41] and people treated with anti-depressants [42]. Secondly even if nuclear vitamin D receptor (VDR) was found in multiple areas of the human brain involved in pathophysiology of depression [43], studies in animals have demonstrated that mice lacking functioning VDR have increased symptoms referred to anxiety and psychosis but not to depression [44,45]. Also, there is little available evidence concerning how vitamin D relates to the monoamines that were the main neurotransmitters involved in depression [46,47]

Only the level of motor performance, as assessed by the SPPB, had a small predictive effect, confirming that a higher level of performance was associated to a lower risk for depression.

The protective role of a good physical performance status on depression development support the longitudinal studies in old people [48]. At the same time, randomized controlled trials and meta-analysis have demonstrated the antidepressant effect of a good physical performance status and regular physical activity in older adults [49-51].

Our study has both strengths and limitations. The major limitations are linked to the loss of participants at the follow up (32.7%) and that the severity of depression symptoms was evaluated by the use of GDS. Another possible limitation derived from missing values for 25OHD at longitudinal step.. A major strength is the large size of population considered with baseline 25OHD values that is the best indicator of vitamin D status in humans. Moreover, diagnosis of depression was supported by a psychiatrist evaluation. Finally, the analysis were adjusted for a large number of possible confounding factors, including regular physical activity, with specific interest on time spent in outdoor activity (i.e. gardening).

In conclusion, after 4.4 years of follow up, the baseline vitamin D status did not influence the development or the worsening of depressive symptoms in older subjects of the PRO.V.A study. At the same time, the results of our study support the hypothesis that vitamin D status might be interpreted as a biomarker of good physical performance that represents a protective factor on depressive symptoms development.

Table 1a. Participants' baseline characteristics by serum 25-hydroxyvitamin D (25OHD) tertiles in women: the PRO.V.A. Study. Numbers are mean values (and Standard Deviations) or percentage (%), as appropriate.

	25-hydroxyvitamin D (25OHD) tertiles			
	≤49 (n=356)	**>49 *&* ≤81** (n= 335)	**>81** (n= 348)	***Age-adjusted p for trend***
Age (yrs)	74.7(6.4)	76.2(5.8)	71.5(5.2)	<.0001 (unadjusted)
Educational level (<5y of schooling), %	62.5	19.7	15.3	.01
Current smokers, %	6.1	9.2	4.9	.1
Regular activity,%	63.2	78.2	81.3	<.0001
Gardening, %	31.7	46.9	51.4	<.0001
Season of blood collection				.18*
Winter	23.6	25.1	30.5	
Spring	24.1	35.8	33.9	
Summery	30.6	22.1	24.1	
Autumn	21.6	17.0	11.5	
BMI (kg/m^2)	28.7(5.2)	28.4(4.7)	28.0(4.5)	.01
MMSE score	25.0(3.6)	24.9(3.6)	25.5(3.5)	.60
GDS score	10.3(6.8)	8.9(6.3)	8.6(6.1)	.003
ADL$_s$ disabilities	19.9	11.6	4.9	<.0001
Dementia, %	31.8	28.1	22.7	.38
Depressed symptoms, %	41.3	33.7	34.2	.18
Cardiovascular diseases, %	17.5	9.6	10.6	.07
Osteoporosys	56.5	53.7	47.4	.43
Osteoarticular diseases,%	39.5	38.1	32.1	.36
Diabetes ,%	8.4	7.5	8.0	.91
COPD,%	5.6	4.8	2.3	.03
Speed, m/sec	0.62(0.19)	0.68(0.16)	0.72(0.17)	<.0001
SPPB, (0-12 range)	9.5(1.9)	9.9(1.7)	10.1(1.5)	<.0001
Serum levels				
PTH (ng/L)	46.7(29.6)	38.9(18.5)	36.6(30.9)	<.0001
25OHD (nmol/L)	32.9(11.1)	64.9(9.2)	117.8(37.8)	<.0001

* Chi-squared test;

Unless otherwise specified, *p values* are on age-adjusted general linear model or logistic regression as appropriate.

Table 1b. Participants' baseline characteristics by serum 25-hydroxyvitamin D (25OHD) tertiles in men: the PRO.V.A. Study. Numbers are mean values (and Standard Deviations) or percentage (%), as appropriate.

	25-hydroxyvitamin D (25OHD) tertiles			
	≤80 (n=210)	**>80 *&* ≤125** (n= 215)	**>125** (n= 211)	***Age-adjusted p values***
Age (yrs)	74.5(7.2)	72.2(6.3)	72.4(5.8)	<.0001 ***(unadjusted)***
Educational level (< 5y of schooling), %	48.1	15.0	12.1	<.0001
Current smokers, %	24.3	23.7	21.3	.3
Regular activity,%	81.3	84.6	95.6	<.0001
Gardening, %	42.6	49.3	62.9	<.0001
Season of blood collection				.15*
Winter	26.2	28.4	30.8	
Spring	26.2	33.0	26.1	
Summery	25.7	20.	28.4	
Autumn	21.9	18.6	14.7	
MMSE score, (range 0-30)	26.0(3.3)	25.9(3.3)	25.9(3.3)	.40
GDS score, (range 0-30)	6.9(5.0)	6.3(4.8)	5.8(5.0)	.04
ADL_s disabilities,%	9.0	8.4	6.2	.04
Dementia, %	22.9	20.5	20.1	.77
Depressed symptoms, %	20.9	19.1	14.2	.09
Cardiovascular diseases, %	24.8	20.6	17.5	.24
Osteoporosys,%	19.5	19.5	13.3	.16
Osteoarticular diseases,%	27.0	27.7	27.0	.64
Diabetes ,%	9.5	7.9	7.6	.44
COPD,%	12.9	14.4	9.9	.47
Speed, m/sec	0.76(0.19)	0.82(0.15)	0.82(0.16)	.01
SPPB, (0-12 range)	10.6(1.6)	10.9(1.3)	11.1(1.2)	.01
Serum levels				
PTH (ng/L)	39.6(20.3)	35.4(15.9)	28.1(14.0)	<.0001
25OHD (nmol/L)	54.7(18.0)	101.8(12.5)	161.0(57.0)	<.0001

* Chi-squared test;

Unless otherwise specified, *p values* are on age-adjusted general linear model or logistic regression as appropriate.

Table 2: Baseline and follow-up adjusted estimates of GDS scores mean values [mean (SE)] by baseline serum 25-hydroxyvitamin D (25OHD)

	Female		***Male***	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**
Tertile 1	9.5(0.36)	9.8(0.37)	6.2(0.32)	6.64(0.32)
Tertile 2	8.4(0.34)	8.9(0.35)	6.0(0.32)	7.12(0.32)
Tertile 3	8.3(0.33)	9.3(0.34)	5.8(0.32)	6.95(0.32)
p-value for linear trend	*0.02*	0.19	0.65	0.63
p-value tertile 1 vs tertile 3	*0.02*	0.56	0.63	0.82

Adjusted for: age, sex, educational level, season of blood collection, smoking, status, regular physical activity, baseline MMSE score, BMI, dependency in ADLs, cardiovascular diseases, diabetes, COPD, SPPB score, PTH serum levels.

Table 3: Adjusted Hazard Ratio (95%CI) for depressed mood at 4 year in Pro.V.A. participants, according to baseline 25OHD serum levels.

	Female		***Male***	
	HR (95%CI)	***p –value***	***HR (95%CI)***	***p –value***
Tertile 1 vs 3	0.80(0.51-1.27)	0.35	0.95(0.50-1.82)	0.89
Tertile 2 vs 3	1.00(0.65-1.53)	0.98	1.38(0.74-2.55)	0.30

Unless otherwhise specified, data are presented as relative risk and 95% confidence interval.
Adjusted for: age, sex, educational level, season of blood collection, smoking, status, regular physical activity, baseline MMSE score, dementia, BMI, dependency in ADLs, cardiovascular diseases, diabetes, COPD, SPPB score, PTH serum levels

Table 4: Significant predictors of depressed mood at 4 year in the Pro.V.A. participants (Hazard Ratio (95%CI)

	Female		***Male***	
	HR (95%CI)	***p –value***	***HR (95%CI)***	***p –value***
SPPB	0.84(0.75-0.98)	0.003	0.80(0.68-0.94)	0.009
Age, y	-	-	1.05(1.01-1.09)	0.01

adjusted for: age, sex, educational level, season of blood collection, smoking, status, regular physical activity, baseline MMSE score, dementia, BMI, dependency in ADLs, cardiovascular diseases, diabetes, COPD, SPPB score, PTH serum levels

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