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published in
Clinical Endocrinology
2005

DOI (link to publisher)
10.1111/j.1365-2265.2005.02355.x

document version
Publisher's PDF, also known as Version of record

Clinical Endocrinology (2005) **63**, 403–411 doi: 10.1111/j.1365-2265.2005.02355.x

ORIGINAL ARTICLE

Endocrine and inflammatory markers as predictors of frailty

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Summary

Objective To examine the association of serum concentrations of 25-hydroxyvitamin D [25(OH)D], interleukin-6 (IL-6), C-reactive protein (CRP) and IGF-1 with prevalent and incident frailty.

Design The Longitudinal Aging Study Amsterdam (LASA), a prospective cohort study with 3-yearly measurement cycles.

Setting General population-based sample.

Participants The respondents were men and women aged 65 and over, who participated at T_1 (1995/1996, $N = 1720$) and T_2 (1998/1999, $N = 1509$). Blood samples were obtained at T_1 ($N = 1271$).

Measurements The presence of frailty at T_1 and 3-year incidence of frailty. Frailty is defined as the presence of three out of nine frailty indicators.

Results At T_1, 242 (19·0%) of all respondents were frail. Those who were frail at T_1 had higher CRP and lower 25(OH)D levels. Serum 25(OH)D remained associated with frailty after adjustment for potential confounders with an odd ratios (OR) of 2·60 [95% confidence interval (95% CI) 1·60–4·21] for 25(OH)D < 25 nmol/l and 1·72 (95% CI 1·19–2·47) for 25(OH)D 25–50 nmol/l *vs.* high levels of 25(OH)D. Of the nonfrail at T_1, 125 respondents (14·1%) became frail at T_2. After adjustment, moderately elevated CRP levels (3–10 μg/ml) (OR 1·69, 95% CI 1·09–2·63) and low 25(OH)D (OR 2·04, 95% CI 1·01–4·13) were associated with incident frailty. No consistent associations were observed for IL-6 and IGF-1.

Conclusion Low 25(OH)D levels were strongly associated with prevalent and incident frailty; moderately elevated levels of CRP were associated with incident frailty.

(Received 9 March 2005; returned for revision 30 March 2005; finally revised 21 April 2005; accepted 13 July 2005)

Introduction

Frail is a term used to describe an older person at risk for adverse outcomes such as physical decline,[1] disability,[1,2] nursing home admission[2] and mortality.[1,3,4] Frailty consists of multisystem decline,[1,5] and is considered to be a consequence of changes in neuromuscular, endocrine and immune changes that occur as people age.[5,6] Fried *et al*. hypothesized a negative spiral in which inflammation, neuroendocrine deregulation and sarcopaenia results in frailty.[7] However, there is little empirical evidence for the role of endocrine and inflammatory markers of frailty.

There are several reasons to expect that inflammatory and endocrine markers are associated with frailty. First, studies have shown that the levels of inflammatory markers, such as interleukin-6 (IL-6) and C-reactive protein (CRP), increase with ageing, and that elevated levels are associated with disability and mortality.[8–11] High levels of cytokines may induce skeletal muscle loss and aggravate neuroendocrine deregulation.[7,12] Furthermore, vitamin D deficiency is common in older people, with a gradual decline in levels from healthy to dependent and institutionalized individuals.[13] Low serum 25-hydroxyvitamin D [25(OH)D] is associated with muscle weakness,[14] sarcopaenia,[15] falls[16] and disability.[13] GH and IGF-1 decrease with age[17] and may play an role in the maintenance of muscle mass and functioning with ageing.[18] Interaction between IGF-1 and IL-6 in relation to disability has also been reported.[8]

Only a few investigators have studied the direct relationship between biological markers and frailty.[19–21] Most studies investigating endocrine and inflammatory markers so far have focused on outcomes such as disability, mobility and mortality.[8–11,22] Furthermore, the relationship between frailty, endocrine markers and inflammation has been investigated in cross-sectional studies only, which makes it difficult to draw conclusions on the predictive value of the endocrine and inflammatory markers for frailty. The aim of this study was to examine the associations between endocrine and inflammatory markers and frailty, cross-sectionally and prospectively in the subsequent 3 years in a population-based study of men and women aged 65 and over.

Methods

Study population

The data were collected in the context of the Longitudinal Aging Study Amsterdam (LASA). LASA is an ongoing multidisciplinary study on predictors and consequences of changes in physical, cognitive, emotional and social functioning in older people in the Netherlands. A random sample stratified by age and gender according

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to expected mortality after 5 years was drawn from population registers of 11 municipalities in three geographical areas in the Netherlands. At each cycle, data were collected in a face-to-face main interview, carried out in the subjects' home or institutional residence, by specially trained interviewers, followed by a medical interview 2–6 weeks later. The details of the LASA study have been described elsewhere[23] (see also http://ssg.scw.vu.nl/lasa/). The Medical Ethics Committee of the VU University Medical Centre approved the study and informed consent was obtained from all respondents.

The sample for this study consisted of respondents who participated in the main interview at the first follow-up measurement T_1 (1995/1996) and were asked to participate in a medical interview (inclusion criterion for a medical interview 1995/1996 age 65 years and older, $N = 1720$). Of the 1720 respondents who were eligible for the medical interview, 1509 participated (87·7%). Blood samples were obtained from 1321 respondents. In 1285 of these respondents all four serum markers were determined (74·7%). For the cross-sectional analyses, 14 respondents were excluded because of missing covariates, leaving a sample of 1271 respondents. The nonresponders at baseline (1995/1996) were older, had more cognitive problems and chronic disease, and a lower education level. There were no sex differences in nonresponse.

For the prospective analyses with 3 years of follow-up, 231 of the 1271 respondents were lost to follow-up; 159 respondents died, 12 respondents refused, 11 were not able to participate due to physical or cognitive problems, six respondents could not be contacted and 43 respondents were excluded because no information on the frailty indicators was available. Of the remaining 1040 respondents for the prospective analyses, the respondents who were frail at baseline were excluded from the study on the effect of serum endocrine and inflammatory markers on incident frailty, leaving a sample of 885 respondents. Those lost to follow-up more often had higher levels of IL-6 and CRP, and lower levels of IGF-1 and 25(OH)D. Those lost to follow-up were older, had more cognitive problems, more depressive symptoms, more chronic diseases and more frailty markers present at baseline.

Serum endocrine and inflammatory markers

Morning blood samples were obtained in 1995/1996. The participants were allowed only tea and toast. The samples were centrifuged and serum was stored at −70 °C until measurement.

Serum 25(OH)D was measured according to a competitive binding protein assay (Nichols Diagnostics, San Capistrano, CA, USA). IGF-1 was determined by immunoradiometric assay after extraction (DSL, Webster, TX, USA). These analyses were carried out at the Endocrine Laboratory of the VU University Medical Centre, Amsterdam.

The serum concentrations of CRP and IL-6 were determined using a sensitive enzyme-linked immunosorbent assay (ELISA) at Sanquin Research, Amsterdam. The IL-6 ELISA was obtained from the Business Unit Immune Reagents of Sanquin, and performed according to the manufacturer's instructions. CRP levels were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and a biotinylated monoclonal antibody (mAb) against CRP (CLB anti-CRP-2) as the detecting antibody. CRP and IL-6 were measured in duplicate, and averages were reported.

The detection limit was 10 nmol/l for 25(OH)D, 1 nmol/l for IGF-1, 0·8 ng/ml for CRP, and 5·0 pg/ml for IL-6. Recombinant IL-6, purified CRP and pooled human plasma were used as standards in the respective assays. The interassay coefficient of variation (CV) was < 4·2% for CRP, < 5% for IL-6, < 14% for IGF-1 and < 15% for 25(OH)D.

Frailty

Nine frailty indicators were used to determine frailty. Both physical and psychological frailty indicators were included (see[4] for an extensive description). The nine frailty indicators included *low body mass index* (BMI < 23 kg/m^2), *low peak expiratory flow* (lowest quintile ≤ 270 l/min[24]), *cognitive functioning* [Mini-mental State Examination (MMSE) score < 24[25]], *poor distant vision and hearing problems* (able to see or hear with much difficulty or not able[26]), *incontinence*,[27] *low sense of mastery* (short version of the Pearlin and Schooler mastery scale, lowest quintile ≤ 14[28]), d*epressive symptoms* (CES-D score ≥ 16[29]) and *physical activity* (LASA Physical Activity Questionnaire, lowest quintile < 66 min/day[30]).

The definition of frailty in this study was the presence of three or more out of nine frailty indicators. Also the number of frailty indicators was used as an outcome.

Covariates

The analyses were adjusted for age, sex, education, season of blood sampling, use of prescribed anti-inflammatory drugs [nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin and corticosteroids], use of oestrogens, smoking status, alcohol consumption, obesity, high-intensity physical activity, levels of PTH and chronic disease.

The presence of chronic disease, use of oestrogens, smoking, obesity and alcohol consumption increase the levels of inflammatory markers and are associated with frailty.[31–34] High-intensity physical activity decreases the production of inflammatory markers and is inversely associated with frailty.[31,34] The season of blood sampling was included (spring/summer *vs.* autumn/winter) because the serum concentrations of 25(OH)D are influenced by sunlight exposure.[13] High serum concentrations of PTH are associated with low 25(OH)D.[15] Lower education levels are associated with more chronic disease, a less healthy lifestyle and frailty,[32] and were therefore included in all analyses.

The respondents were asked about their highest level of education attained, which was categorized into three categories (low, middle and high). Serum concentration of PTH was measured by immunoradiometric assay (Incstar Corp., Stillwater, MN, USA) and was used as a continuous variable in the analyses. The interviewers inspected medication bottles, and the medication was recorded if it was prescribed by a general practitioner and used in the 2 weeks before the interview. Smoking status was divided into never smoker *vs.* other, and alcohol consumption was divided into never drinker *vs.* other. Alcohol use was also examined with more categories (moderate and excessive drinking), but preliminary analyses showed that these groups did not differ in their associations with frailty and they were therefore grouped together. Obesity was defined as a BMI ≥ 30 kg/m^2. High-intensity physical activity (yes/no) was based on

the following activities with a metabolic equivalent score (MET) ≥ 5·0: distance walking, cycling, swimming, dancing, jogging, rowing, playing tennis, soccer, basketball, volleyball and winter sports. Seven self-reported chronic diseases were examined: chronic obstructive pulmonary diseases, cardiac disease (myocardial infarction, arrhythmias, congestive heart failure, angina pectoris and narrowing of the coronary arteries), peripheral arterial disease, diabetes mellitus, cerebrovascular accidents, rheumatoid arthritis or osteoarthritis (both conditions were grouped together because respondents found it difficult to differentiate between them) and cancer. These chronic diseases are the most frequent in the Dutch older population with a prevalence of at least 5%. Agreement between respondents' self-reported data and data from the general practitioner has been shown to be satisfactory or good for most diseases studied.[35] Respondents could answer yes or no.

Statistical analyses

Serum 25(OH)D was categorized into three groups: < 25, 25–50 and > 50 nmol/l.[13] The highest group was the reference group. IGF-1 was dichotomized at the lowest 10% (below 7·7 nmol/l) as these levels were shown to be associated with low walking speed.[36] IL-6 was dichotomized at the detection limit (5 pg/ml), with low as the reference group. Because of the large numbers of respondents below the detection limit it was not possible to divide IL-6 into more categories. CRP was categorized into: < 3, 3–10 and > 10 μg/ml. Values > 3 μg/ml are frequently used to indicate an increased risk of adverse outcomes[37] while values > 10 μg/ml indicate clinically relevant inflammation.[38] The low group (< 3 μg/ml) was used as the reference group.

Both *t*-tests and χ^2-tests were performed to assess differences between those who were frail and those who were not frail at baseline. For the examination of the cross-sectional association, logistic regression analyses were performed for each of the endocrine and inflammatory markers with the presence of frailty as the outcome measure. The first model included only the single serum markers, sex and age. In the second model, season of blood sampling (only for 25(OH)D), use of anti-inflammatory drugs (only for CRP and IL-6), smoking status, alcohol use, oestrogen use, obesity (only for CRP and IL-6) and physical activity (only for CRP and IL-6) were included. For all serum markers, the interaction with sex and the interactions between the serum markers were studied ($P < 0{\cdot}10$). In the final model, chronic disease and PTH (PTH only for 25(OH)D) were added, to study whether PTH and chronic diseases mediated the relationship between endocrine and inflammatory markers and frailty. All serum markers were finally included in a single model to study their associations with frailty adjusted for each other.

For the examination of the prospective association, logistic regression analyses were performed to study whether serum markers predicted incident frailty. The consecutive logistic regression models were similar to those of the cross-sectional analyses.

As an additional outcome variable, the total number of frailty indicators was used and associations were tested with multinomial logistic regression analysis. The group without any frailty indicators was the reference group. For the cross-sectional analysis, respondents with four or more frailty indicators were grouped together because of small numbers. In the prospective analysis, respondents with three or more frailty indicators were grouped together because of small numbers. Analyses were adjusted for baseline number of frailty indicators and for the confounders listed above.

Results

Characteristics of the sample

There were 242 (19·0%) frail respondents at baseline (1995/1996). Frail respondents were more often women, older (79·2 *vs.* 74·5 years), had more chronic disease, and more often had low serum concentration of 25(OH)D and higher serum concentration of CRP (Table 1, left segment). Frail respondents also more often had a lower level of education, higher BMI, higher serum PTH, and smoked and used alcohol less often.

One hundred and twenty-five respondents (14·1%) who were not frail at baseline became frail after 3 years (T_2, 1998/1999). They were older, and more often had lower serum concentrations of 25(OH)D and IGF-1 and higher serum concentrations of CRP at baseline (Table 1, right segment).

Cross-sectional analyses of frailty

Low serum 25(OH)D concentrations were significantly associated with frailty when adjusting for sex and age (model 1, Table 2). Compared to high serum 25(OH)D, the odds ratio (OR) for low serum 25(OH)D was 2·95 [95% confidence interval (95% CI) 1·87–4·65], and 1·85 (95% CI 1·31–2·60) for moderately low serum 25(OH)D. The ORs decreased to 2·60 (95% CI 1·60–4·21) and 1·72 (95% CI 1·19–2·47) (model 3) when adjusting for all confounders. There was no significant cross-sectional association of CRP, IGF-1 and IL-6 with frailty when adjusting for all confounders. When the serum markers were adjusted for each other, the results were similar. There was no interaction between the serum markers or between the serum markers and sex cross-sectionally.

Prospective analyses of frailty

Moderately elevated serum concentrations of CRP (3·0–10·0 μg/ml) predicted frailty, with an OR of 1·77 (95% CI 1·15–2·68) *vs.* low serum concentrations of CRP when adjusting for sex and age (Table 3). The OR decreased to 1·69 (95% CI 1·09–2·63) when adjusting for all confounders. Low serum 25(OH)D was also significantly associated with incident frailty with an OR of 2·04 (95% CI 1·01–4·13) *vs.* high serum 25(OH)D when adjusting for all confounders. When all biological markers were included in a model, the OR of serum CRP did not change but the OR for low serum 25(OH)D changed to 1·90 (95% CI 0·92–3·95). There was no significant prospective association of serum IGF-1 and serum IL-6 with incident frailty when adjusting for all confounders. Again, there was no interaction between the serum markers or between the serum markers and sex.

Additional analyses of number of frailty indicators

From cross-sectional analyses (Table 4, left segment), it can be seen that low serum concentrations of 25(OH)D were associated with

Table 1. Characteristics of the study sample

Baseline characteristics	Cross-sectional analyses			Prospective analyses		
	Not frail at T_1 $N = 1029$	Frail at T_1 $N = 242$	Overall *P*-value	Not frail at T_2 $N = 760$	Frail at T_2 $N = 125$	Overall *P*-value
Endocrine and inflammatory markers						
25(OH)D < 25 nmol/l	85 (8·3%)	56 (23·2%)	0·000	46 (6·1%)	20 (16·0%)	0·000
25(OH)D 25–50 nmol/l	355 (34·5%)	116 (47·9%)		254 (33·4%)	51 (40·8%)	
25(OH)D > 50 nmol/l	589 (57·2%)	70 (28·9%)		460 (60·5%)	54 (43·2%)	
IGF-1 ≤ 7·7 nmol/l	97 (9·4%)	33 (13·6%)	0·052	60 (7·9%)	19 (15·2%)	0·008
IL-6 ≥ 5·0 pg/ml	111 (10·8%)	28 (11·6%)	0·725	69 (9·1%)	15 (12·1%)	0·302
CRP < 3·0 μg/ml	525 (51·0%)	95 (39·3%)	0·004	424 (55·8%)	53 (42·4%)	0·006
CRP 3·0–10·0 μg/ml	359 (34·9%)	102 (42·1%)		242 (31·8%)	58 (46·4%)	
CRP > 10·0 μg/ml	145 (14·1%)	45 (18·6%)		94 (12·3%)	14 (11·3%)	
Number of frailty indicators present at baseline						
0	362 (35·2%)	0	0·000	309 (40·7%)	13 (10·4%)	0·000
1	399 (38·8%)	0		303 (39·9%)	43 (34·4%)	
2	268 (26·0%)	0		148 (19·5%)	69 (55·2%)	
3	0	136 (56·2%)		0	0	
4	0	106 (43·8%)				
Covariates						
Women (%)	498 (48·4%)	151 (62·4%)	0·000	378 (49·7%)	69 (55·2%)	0·258
Mean age (SD)	74·5 (6·3)	79·2 (6·2)	0·000	73·4 (5·9)	78·2 (6·2)	0·000
Low level of education	399 (38·8%)	132 (54·5%)	0·000	277 (36·4%)	57 (45·6%)	0·082
Middle level of education	481 (46·7%)	76 (31·4%)		378 (49·7%)	49 (39·2%)	
High level of education	149 (14·5%)	34 (14·0%)		105 (13·8%)	19 (15·2%)	
Mean no. chronic diseases (SD)	1·1 (1·0)	1·6 (1·1)	0·000	1·0 (0·9)	1·3 (1·1)	0·002
BMI > 30 kg/m^2	196 (19·0%)	62 (25·6%)	0·022	147 (19·3%)	29 (23·2%)	0·317
Mean PTH (pmol/l) (SD)	3·5 (1·9)	4·2 (2·5)	0·000	3·4 (1·8)	3·6 (1·4)	0·185
High-intensity physical activity	211 (20·5%)	18 (7·4%)	0·000	181 (23·8%)	18 (14·4%)	0·019
Never smoked	339 (33·0%)	111 (45·9%)	0·000	255 (33·6%)	47 (37·6%)	0·376
Ever smoked	690 (67·1%)	131 (54·1%)		505 (66·4%)	78 (62·4%)	
No alcohol use	224 (21·8%)	89 (36·8%)	0·000	159 (20·9%)	24 (19·2%)	0·660
Alcohol use	805 (78·2%)	153 (63·2%)		601 (79·1%)	101 (80·8%)	
Use of anti-inflammatory drugs	277 (26·9%)	88 (36·4%)	0·003	183 (24·1%)	43 (34·4%)	0·014
Use of oestrogens	9 (0·9%)	4 (1·7%)	0·279	6 (0·8%)	2 (1·6%)	0·375

three and four or more frailty indicators (Fig. 1). Moderate levels of serum 25(OH)D were associated with three frailty indicators only. Low serum IGF-1 was associated with four or more frailty markers. From the prospective multinomial logistic regression analyses (Table 4, right segment), it can be seen that low serum 25(OH)D was associated with one and three or more frailty indicators (Fig. 1). Furthermore, moderately elevated serum CRP was associated with three or more frailty indicators. The results did not change when all serum markers were adjusted for each other (results not shown).

Discussion

To our knowledge, this study is the first to examine the association of four endocrine and inflammatory markers with prevalent and incident frailty in a large population-based sample. Low serum concentrations of 25(OH)D were associated with prevalent and incident frailty with a clear dose–response relationship. In the prospective analyses moderately elevated levels of CRP (3·0–10·0 μg/ml) predicted the incidence of frailty. These associations were independent of each other and independent of the effects of smoking, drinking, high BMI, intense physical activity, use of anti-inflammatory drugs, chronic disease and education.

The mechanism explaining the relationship between low levels of 25(OH)D and frailty is not yet clear. Low 25(OH)D levels have been shown to be associated with low muscle strength, falls and disability.[13,16] In a previous report based on LASA, low 25(OH)D levels were found to be associated with sarcopaenia,[15] showing that both vitamin D deficiency and insufficiency may cause loss of muscle mass and strength. As a result of loss of muscle mass and muscle weakness, older persons may become less active, accelerating the frailty process. A reverse pathway is also possible: older people often may not go outside and may be physically inactive as a consequence of their frail health, resulting in very low sunlight exposure, which subsequently causes vitamin D deficiency. However, this pathway is not supported by our longitudinal analyses. Thus the first pathway seems the most likely. Although the LASA is a prospective cohort study, as in most prospective cohort studies it remains difficult to investigate causal relationships. Randomized clinical trials are

Table 2. Odds ratios (with 95%CI) from cross-sectional logistic regression analyses of the association of four serum markers and prevalent frailty ($n = 1271$)

N	Serum marker	Model 1	Model 2	Model 3	Model 4
1132	IL-6 < 5 pg/ml	1	1	1	1
139	IL-6 ≥ 5 pg/ml	0·98 (0·62–1·57)	0·96 (0·60–1·53)	0·94 (0·58–1·53)	0·75 (0·44–1·27)
620	CRP < 3·0 μg/ml	1	1	1	1
461	CRP 3·0–10·0 μg/ml	1·35 (0·99–1·89)	1·27 (0·91–1·78)	1·20 (0·85–1·69)	1·14 (0·80–1·61)
190	CRP > 10·0 μg/ml	**1·64 (1·07–2·50)**	1·46 (0·95–2·25)	1·37 (0·88–2·13)	1·37 (0·85–2·19)
141	25(OH)D < 25 nmol/l	**2·95 (1·87–4·65)**	**3·04 (1·92–4·82)**	**2·60 (1·60–4·21)**	**2·55 (1·56–4·17)**
471	25(OH)D 25–50 nmol/l	**1·85 (1·31–2·62)**	**1·88 (1·32–2·67)**	**1·72 (1·19–2·47)**	**1·66 (1·15–2·40)**
659	25(OH)D > 50 nmol/l	1	1	1	1
130	IGF-1 ≤ 7·7 nmol/l	1·02 (0·65–1·60)	0·98 (0·62–1·54)	1·01 (0·64–1·61)	0·88 (0·54–1·41)
1141	IGF-1 > 7·7 nmol/l	1	1	1	1

Bold text = $P < 0·05$

Model 1: single endocrine and inflammatory markers, adjustment for age and sex.

Model 2: single endocrine and inflammatory markers, additional adjustment for IL-6 and CRP for education, use of anti-inflammatory drugs, use of oestrogen, obesity, physical activity, smoking status, and alcohol consumption. For 25(OH)D, additional adjustment for education, season of blood sampling, smoking status and alcohol consumption. For IGF-1, additional adjustment for education, smoking status and alcohol consumption.

Model 3: single endocrine and inflammatory markers, additional adjustment for the self-reported chronic diseases: cardiac disease, peripheral arterial disease, diabetes mellitus, arthritic disease, chronic obstructive pulmonary disease, stroke and cancer. For 25(OH)D, additional adjustment for PTH.

Model 4: All endocrine and inflammatory markers in model, additional adjustment for the self-reported chronic diseases: cardiac disease, peripheral arterial disease, diabetes mellitus, arthritic disease, chronic obstructive pulmonary disease, stroke and cancer.

Table 3. Odds ratios (with 95% CI) from prospective logistic regression analyses of the association of four serum markers and incident frailty ($n = 885$)

N	Serum marker	Model 1	Model 2	Model 3	Model 4
801	IL-6 < 5 pg/ml	1	1	1	1
84	IL-6 ≥ 5 pg/ml	1·08 (0·58–2·02)	1·00 (0·53–1·89)	1·03 (0·54–1·97)	0·93 (0·47–1·84)
477	CRP < 3·0 μg/ml	1	1	1	1
300	CRP 3·0–10·0 μg/ml	**1·77 (1·15–2·68)**	**1·72 (1·11–2·65)**	**1·69 (1·09–2·63)**	**1·70 (1·09–67)**
108	CRP > 10·0 μg/ml	1·27 (0·66–2·45)	1·23 (0·63–2·39)	1·17 (0·69–2·31)	1·13 (0·56–2·27)
66	25(OH)D < 25 nmol/l	1·89 (0·98–3·63)	**2·02 (1·03–3·94)**	**2·04 (1·01–4·13)**	1·90 (0·92–3·95)
305	25(OH)D 25–50 nmol/l	1·14 (0·73–1·77)	1·21 (0·77–1·89)	1·30 (0·82–2·07)	1·24 (0·77–2·00)
514	25(OH)D > 50 nmol/l	1	1	1	1
79	IGF-1 ≤ 7·7 nmol/l	1·42 (0·79–2·57)	1·53 (0·84–2·80)	1·47 (0·79–2·72)	1·40 (0·74–2·62)
806	IGF-1 > 7·7 nmol/l	1	1	1	1

Bold text = $P < 0·05$

Model 1: single endocrine and inflammatory markers, adjustment for age and sex.

Model 2: single endocrine and inflammatory markers additional adjustment for IL-6 and CRP for education, use of anti-inflammatory drugs, use of oestrogen, obesity, physical activity, smoking status, alcohol use. For 25(OH)D, additional adjustment for education, season of blood sampling, smoking status and alcohol use. For IGF-1, additional adjustment for education, smoking status and alcohol use.

Model 3: single endocrine and inflammatory markers additional adjustment for the self-reported chronic diseases: cardiac disease, peripheral arterial disease, diabetes mellitus, arthritic disease, chronic obstructive pulmonary disease, stroke and cancer. For 25(OH)D, additional adjustment for PTH.

Model 4: All endocrine and inflammatory markers in model, additional adjustment for the self-reported chronic diseases: cardiac disease, peripheral arterial disease, diabetes mellitus, arthritic disease, chronic obstructive pulmonary disease, stroke and cancer.

necessary to investigate whether vitamin D supplementation can prevent frailty. It is known that a low serum 25(OH)D concentration can be easily corrected by sunlight exposure or vitamin D supplementation of 400–800 IU/day. Supplementation has been shown to effectively improve vitamin D status, bone mineral density and muscle strength in older people.[13,16] However, no trials have been performed focusing on new frailty.

In the only other large cross-sectional study investigating the relationship between frailty and biological serum markers, Walston *et al.* found that persons who had CRP levels > 5·77 mg/l had an OR of 3·5 for prevalent frailty, in contrast to our study, which showed no association between CRP and prevalent frailty.[19] However, in our study, moderately elevated levels of CRP (3–10 μg/ml) were associated with incident frailty in both men and women after 3 years. With ageing, the levels of circulating cytokines increase, equivalent to a low-grade systemic inflammation, but not necessarily to the levels of acute infections.[17] CRP levels above 10 μg/ml are generally associated with acute disease. This is supported by our data: the respondents with the highest CRP levels had more chronic disease, used more anti-inflammatory drugs, had more frailty indicators present at

Table 4. Odds ratios (with 95% CI) from multinomial logistic regression analysis of the association of single serum markers and the number of frailty indicators cross-sectionally ($n = 1271$) and prospectively ($n = 885$)

	Cross-sectionally				Prospectively		
Serum marker	1 *vs.* 0*	2 *vs.* 0*	3 *vs.* 0*	≥ 4 *vs.* 0*	1 *vs.* 0†	2 *vs.* 0†	≥ 3 *vs.* 0†
IL-6 < 5 pg/ml	1	1	1	1	1	1	1
IL-6 ≥ 5 pg/ml	0·97	0·99	1·10	0·73	1·28	0·90	1·11
	(0·60–1·56)	(0·58–1·70)	(0·58–2·14)	(0·33–1·61)	(0·70–2·34)	(0·42–1·91)	(0·48–2·53)
CRP < 3 μg/ml	1	1	1	1	1	1	1
CRP 3·0–10·0 μg/ml	0·84	0·98	1·30	0·83	1·17	0·98	**1·88**
	(0·58–1·13)	(0·67–1·44)	(0·81–2·09)	(0·48–1·43)	(0·79–1·73)	(0·60–1·59)	**(1·08–3·26)**
CRP > 10 μg/ml	0·64	0·84	0·88	1·28	0·94	1·08	1·10
	(0·40–1·01)	(0·51–1·38)	(0·46–1·69)	(0·67–2·44)	(0·54–1·64)	(0·56–2·08)	(0·48–2·52)
25(OH)D < 25 nmol/l	1·22	1·16	**2·51**	**3·37**	**2·83**	1·92	**5·05**
	(0·66–2·24)	(0·59–2·26)	**(1·19–5·30)**	**(1·56–7·29)**	**(1·17–6·84)**	(0·70–5·23)	**(1·80–14·14)**
25(OH)D 25–50 nmol/l	1·00	1·26	**2·06**	1·56	1·04	0·94	1·27
	(0·71–1·40)	(0·86–1·84)	**(1·25–3·41)**	(0·88–2·79)	(0·70–1·54)	(0·58–1·50)	(0·73–2·24)
25(OH)D > 50 nmol/l	1	1	1	1	1	1	1
IGF-1 ≤ 7·7 nmol/l	1·58	1·52	0·96	**2·15**	1·29	1·07	1·72
	(0·91–2·76)	(0·83–2·80)	(0·44–2·07)	**(1·05–4·42)**	(0·65–2·56)	(0·49–2·35)	(0·76–3·92)
IGF-1 > 7·7 nmol/l	1	1	1	1	1	1	1

Bold text = $P < 0·05$

*Adjustment for age and sex education, smoking status, alcohol consumption, use of anti-inflammatory drugs (IL-6 and CRP), use of oestrogen (IL-6 and CRP), obesity (IL-6 and CRP), physical activity (IL-6 and CRP), season of blood sampling (25(OH)D), PTH (25(OH)D), and the self-reported chronic diseases: cardiac disease, peripheral arterial disease, diabetes mellitus, arthritic disease, chronic obstructive pulmonary disease, stroke and cancer.

†Adjustment for all variables mentioned above and number of frailty indicators present at baseline.

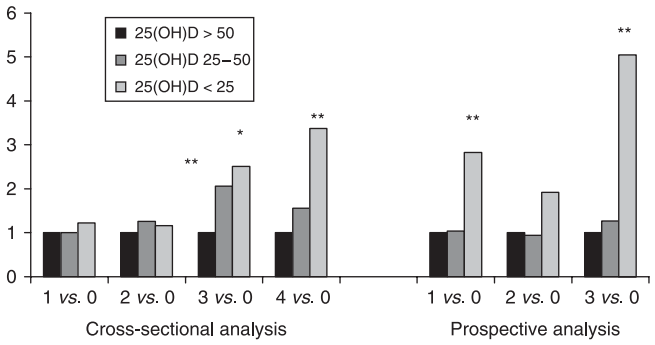

Fig. 1 Odds ratios from multinomial logistic analyses of the association between 25(OH)D and frailty. The group with 25(OH)D > 50 nmol/l is the reference group. *$P < 0·05$, **$P < 0·01$. Adjustment for age, sex, education, number of frailty indicators present at baseline, smoking status, alcohol consumption, use of anti-inflammatory drugs (IL-6 and CRP), use of oestrogen (IL-6 and CRP), obesity (IL-6 and CRP), physical activity (IL-6 and CRP), season of blood sampling (25(OH)D), PTH (25(OH)D), and the following self-reported chronic diseases: cardiac disease, peripheral arterial disease, diabetes mellitus, arthritic disease.

baseline and were more often lost to follow-up (data not shown). High CRP levels have been shown to be associated with a high risk of cardiovascular events and mortality.[37] A possible explanation of why we did not find an association with frailty in subjects with high levels of CRP, but only in subjects with moderately elevated levels of CRP, is selective dropout. Nevertheless, the finding that moderately elevated levels of CRP are associated with incident frailty is in agreement with the hypothesis that frailty is a result of chronic low-grade inflammation.

In this study, low serum IGF-1 was significantly associated with the presence of four or more frailty indicators in cross-sectional multinomial regression analysis. In logistic regression analysis, a tendency for an association between low IGF-1 and frailty was seen. This finding is in line with reports that IGF-1 is associated with disability and mobility decline.[8,36]

In contrast to other studies in which the association between IL-6 and frailty was examined,[20,21] we found no association between IL-6 and frailty. A possible explanation for this lack of association is the high detection limit of our assay. Associations between IL-6 and health outcomes have been observed at levels far below the detection limit of our study.[22,34]

Leng *et al.* found an inverse relationship between IL-6 and IGF-1, suggesting an interaction between endocrine and immune functioning.[21] IL-6 plays an important role in the inflammatory response by inducing the synthesis of acute-phase proteins, such as CRP, and inhibiting the synthesis of IGF-1.[39] In this study we found no interaction between IL-6 and IGF-1. However, this could be the result of the high detection limit for IL-6, limiting the number of respondents in which the interaction could be examined. Results from other studies have shown that vitamin D has important effects on the function of the immune system.[40] Vitamin D deficiency has been shown to occur in patients with inflammatory bowel disease,[41] and vitamin D status was associated with cancer and autoimmune diseases.[42] Studies in mice have shown that supplementation with vitamin D can protect

mice against developing insulin-dependent diabetes mellitus.[43,44] More research is needed to study the functional relationship between the endocrine and immune system in humans to gain more insight into the development of frailty.

In this study and in other studies, a low BMI and a low physical activity score were used as frailty indicators. The analyses were adjusted for high-intensity physical activity and a high BMI. This procedure might have led to over-adjustment, because physical activity has been shown to be associated with lower inflammation levels[31,34,45] as opposed to obesity, which is associated with greater inflammation.[33] However, the associations did not change when not adjusting for these factors. Of interest is our finding that those who were frail at baseline were more often obese than the nonfrail. Moreover, those who became frail were more often obese than those who did not become frail. These results suggest that the concept of frailty might need to be adjusted. Possibly, not only low body weight but also obesity should be included as a frailty indicator. Obesity increases the risk of arteriosclerosis and cardiovascular disease, both of which have been suggested as possible pathways leading to frailty.[46] The role of the potentially U-shaped relationship between BMI and frailty should be examined in future studies.

Recent studies showed potential benefit of physical activity with regard to the levels of inflammatory markers, as physical activity was associated with lower levels of inflammation.[12,34,45] Physical inactivity is also an important contributor to the development of frailty and loss of muscle mass. In addition to observational studies, trials are necessary to investigate the effect of physical activity on inflammation. So far, to our knowledge, no trials have been performed on the direct relationship between physical activity and serum inflammatory markers.

The presence of seven self-reported chronic diseases was studied as a potential mediator of the relationship between the serum endocrine and inflammatory markers and frailty. The chronic diseases included in this study were chronic obstructive pulmonary disease, diabetes mellitus, arthritic diseases, peripheral arterial disease, cardiac diseases, cancer and stroke. These chronic diseases are characterized by increased inflammation. Chronic diseases are also related to frailty.[1] However, no mediating effect of these diseases on the association between the serum markers and frailty could be demonstrated. This suggests that the serum markers examined had an independent effect on prevalent and incident frailty. However, it remains possible that other chronic diseases, not included in this study, are mediators in the relationship, leading to an overestimation of the effect of the serum markers.

A further limitation of this study is the relatively long follow-up interval of 3 years and the determination of the biological serum markers at baseline only. Frailty is thought to be a dynamic process and therefore multiple assessments of frailty and the biological serum markers using short time intervals might have shown more precisely the effect of biological serum markers on the development of frailty.[7]

In conclusion, this study shows that low serum 25(OH)D concentrations are associated with prevalent and incident frailty. The respondents with moderately elevated serum CRP were at risk of becoming frail after 3 years. No consistent associations were observed for IL-6 and IGF-1.

Acknowledgements

This study was based on data collected in the context of the Longitudinal Aging Study Amsterdam (LASA), which is largely funded by the Ministry of Health, Welfare and Sports of the Netherlands. This research of M.T.E.P. has been made possible by the Vrije Universiteit 'Frailty and Vitality in Ageing' programme. The research of M.V. has been made possible by a fellowship from the Royal Netherlands Academy of Arts and Sciences. We thank the Endocrinological Laboratory of the VU University Medical Centre Amsterdam for the assessment of 25(OH)D and IGF-1, and Sanquin Research Laboratory (head: Prof C. E. Hack) for the assessment of CRP and IL-6. There is no conflict of interest.

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