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► **To cite this version:**

Terezia Pincikova, Kristina Nilsson, Inger Elisabeth Moen, Ferenc Karpati, Gjermund Fluge, et al..
Inverse relation between vitamin D and serum total IgG in the Scandinavian Cystic Fibrosis Nutritional
Study. *European Journal of Clinical Nutrition*, 2010, 10.1038/ejcn.2010.194 . hal-00578708

HAL Id: hal-00578708

<https://hal.science/hal-00578708>

Submitted on 22 Mar 2011

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Title Page

Inverse relation between vitamin D and serum total IgG in the Scandinavian Cystic Fibrosis Nutritional Study^{1,2}

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¹ Supported by the Swedish Heart Lung Foundation, *Stiftelsen Frimurare-Barnhuset i Stockholm*, Karolinska Institutet, Norwegian and Swedish Cystic Fibrosis Associations, and by an unrestricted grant from Solvay Pharma, which has made the meetings of the SCFSC possible. Financial support was further provided through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and the Karolinska Institutet.

² Author disclosures: T. Pincikova, K. Nilsson, I. E. Moen, F. Karpati, G. Fluge, A. Hollsing, P. K. Knudsen, A. Lindblad, L. Mared, T. Pressler, and L. Hjelte, no conflicts of interest.

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Running Title:

Inverse relation between vitamin D and serum IgG

¹⁰ Abbreviations used: BW, bodyweight; CF, cystic fibrosis; CFRD, CF-related diabetes; FEV1, forced expiratory volume in one second; [MLR, multiple linear regression](#); 25OHD, 25-hydroxyvitamin D.

Page count for the manuscript file: 32

Total word count: 3117 words

Number of tables: 3

Number of figures: 3

Number of references: 42

Supplementary online material submitted: 4 tables and 3 figures

Abstract Page

ABSTRACT

Background/Objectives: The hallmark of cystic fibrosis (CF) is chronic lung inflammation. The severity of lung disease is closely correlated with IgG levels. Beyond its contribution to the bone health, the importance of vitamin D has not been fully recognized due to lack of human studies providing evidence of its benefit. In context of the recently described immunomodulatory functions of vitamin D, we aimed to assess the relationship between vitamin D and IgG levels.

Subjects/Methods: 896 CF patients were included (0.53 – 65.9 years) from 7 centers in Denmark, Norway and Sweden. Serum 25-hydroxyvitamin D (25OHD) and total IgG were measured, spirometry was carried out and vitamin D intake data were gathered using a seven-day dietary food record. Multiple linear regression analyses were performed for IgG and FEV1 as dependent variables, and serum 25OHD, daily food and supplemented vitamin D sources of intake as independent variables. The model was controlled for age, gender, genotype, CF-related diabetes, *season*, infection/colonization status, long-term oral corticosteroid treatment, long-term treatment with macrolide antibiotics, pancreatic insufficient phenotype and Body Mass Index z-score.

Results: Serum total IgG levels were negatively associated with serum 25OHD (*adjusted* $R^2=0.376$; $\beta=-0.02$; $p<0.001$), supplemented vitamin D intake per kg bodyweight (*adj.* $R^2=0.375$; $\beta=-0.82$; $p<0.001$), and total vitamin D intake per kg bodyweight (*adj.* $R^2=0.398$; $\beta=-0.60$; $p=0.002$). Serum 25OHD was positively associated with FEV1 (*adj.* $R^2=0.308$; $\beta=0.0007$; $p=0.025$).

Conclusions: Increasing vitamin D intake may positively modulate inflammation in CF. The study supports the proposed role of vitamin D in the immune system during infection and substantiates prospective studies.

Word count for the abstract: 250 words

KEYWORDS:

cystic fibrosis; food; forced expiratory flow rates; immunity; immunoglobulin G; vitamin D

Introduction

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive condition in Caucasians. Progressive pulmonary disease is the major cause of morbidity and mortality in CF patients. Both the severity of lung disease and long-term prognosis are closely correlated with total IgG levels in children and adults with CF (Levy *et al.*, 2007; Wheeler *et al.*, 1984). Bacterial infection and chronic colonization of the lungs are the primary stimuli for inflammation in the CF airway. The inflammatory process is associated with low levels of the potent anti-inflammatory cytokine IL-10 in the CF lung (Bonfield *et al.*, 1995). The infection/colonization persists and, without treatment, irreversibly damages the airways (De Rose, 2002). Breaking the vicious circle of infection and inflammation with anti-inflammatory agents delays respiratory failure (Konstan *et al.*, 1995).

Classical function of the active form 1,25-dihydroxyvitamin D is maintaining calcium homeostasis. However, several recent observations have initiated a re-evaluation of the physiologic actions of vitamin D (Mora *et al.*, 2008; Hansdottir *et al.*, 2008). This was followed by subsequent description of “non-classical functions of vitamin D”, which include immunomodulatory properties of vitamin D, firmly supported by numerous *in vitro* and animal experiments (Bouillon *et al.*, 2008). The *in vitro* findings consistently suggest that vitamin D enhances antimicrobial activity at early stages of infection (Liu *et al.*, 2006; Urry Z *et al.*, 2009) and provides negative feedback at later stages (Sadeghi *et al.*, 2006). The inhibitory effects of vitamin D on adaptive immune response include induction of the potent anti-inflammatory cytokine IL-10. This complex immunomodulatory role of vitamin D has been reviewed in detail elsewhere (Mora *et al.*, 2008). So far there are no large human studies on the proposed role of vitamin D in the immune system during infection.

Most CF patients have suboptimal serum levels of 25OHD (below 75 nmol/L), despite following the currently recommended vitamin D supplementation regimens (Stephenson *et al.*, 2007; Rovner *et al.*, 2007). Literature review indicates that vitamin D status of CF populations may have been improving over the last 15 years, but mean 25OHD serum concentration continues to be suboptimal (Hall *et al.*, 2010). Vitamin D supplementation in CF is given to avoid osteoporosis. Because of improved treatment of bone disease with bisphosphonates, the level of vitamin D in CF patients worldwide is seldom monitored. In Scandinavia, no specific recommendations for vitamin D supplementation for CF population are followed. There is no evidence for benefit of vitamin D supplementation in cystic fibrosis patients yet (Ferguson & Chang, 2009).

We hypothesize that chronic vitamin D insufficiency may contribute to the ongoing CF lung inflammation, which is associated with high IgG levels, low IL-10 levels, and poor lung function. The aim of this analysis was to study the relation between vitamin D and serum IgG, and secondarily, between vitamin D and lung function. This was studied in a large, well-characterized Scandinavian CF population.

Materials and Methods

Study population

The Scandinavian CF Nutritional Study is a cross-sectional multi-centre study that was initiated to investigate the nutritional status of the Scandinavian CF patients as well as their energy and macronutrient intake data. Seven of the eight CF-centers in Scandinavia participated with 898 patients included in the study (Sweden: Stockholm 164, Gothenburg 131, Lund 115, Uppsala 73; Denmark: Copenhagen 236; Norway: Oslo 144, Bergen 35). The Regional Ethics Committees in each country approved the study and informed written consent was obtained from all the adult patients and from parents of all the children.

Patients were included consecutively from 05-09-2003 to 25-05-2006 when in clinically stable condition. Inclusion criteria were confirmed CF diagnosis based on clinical features, repeatedly (\geq two) positive sweat tests ($Cl > 60$ mmol/L children, > 80 mmol/L adults), and/or the presence of a known disease-causing mutation on each CFTR gene. Exclusion criterion was pregnancy.

Methods

The internationally accepted measure of vitamin D status, serum 25OHD, was measured in blood taken at inclusion. The analysis of all the samples was done by high-performance liquid chromatography with UV detection method at The Research Laboratory, Department of Pediatrics, Haukeland University Hospital, Bergen, Norway. A modified version of the method described by Aksnes was used (Aksnes, 1994). [This methodology gives results comparable with both liquid chromatography-tandem mass spectrometry as well as with](#)

radioimmunoassay technology (Aksnes, personal communication; Lensmeyer *et al.*, 2006; Roth *et al.*, 2008).

Food vitamin D daily intake was obtained by a seven-day food record. Nationally designed and validated pre-coded forms were used, in which household measures and photographs described food portion sizes. All three food records were designed to assess the entire diet over the previous 7-day period, and they have been described in several studies (Lillegaard & Andersen, 2005; Becker & Johansson, 1987; Becker *et al.*, 1998).

In our study, the patients and/or parents were instructed by a CF dietitian how to complete the food records. Food recording was done in patients \geq four years of age followed at all the centers in the study except the CF centre in Gothenburg due to lack of resources. 406 patients completed the food record. Response rates were 86% (115/134), 84% (76/91), 79% (27/34) and 76% (93/122) for Stockholm, Lund, Bergen and Oslo, respectively. The records were coded and analyzed for energy and nutrients. In Sweden this was done at the National Food Administration using the software MATs (Rudans Lättdata) with the database PC-Kost, in Denmark at Danish National Food Institute using the GIES software, and in Norway at the Department of Nutrition, University of Oslo, using the KBS software.

Supplemented vitamin D daily intake was calculated from the data on the mean daily intake of the different vitamin and mineral supplements by dietitians, who were taking into account forgotten or skipped doses (728 patients). The patients were asked how often they forgot or deliberately did not take their supplements and mean daily dose was counted: (daily dose taken x number of days a week when the dose was taken) divided by 7 days. Total vitamin D daily intake was calculated as sum of daily food vitamin D and daily supplemented vitamin D

(389 patients). Due to the wide bodyweight (BW) range (7.0 – 131.0 kg) we further adjusted vitamin D intake as vitamin D intake per kg BW.

IgG was measured at respective hospital laboratories using routine in-house methods of protein electrophoresis.

Lung function test was performed at inclusion (\geq seven years) by dynamic spirometry at each center. From measured forced expiratory volume in one second (FEV1) in liters, percentage of predicted values were calculated using Solymar and Quanjer reference equations for patients < 19 and ≥ 19 years, respectively (Solymar *et al.*, 1980; Quanjer & Tammeling, 1983).

For cytokine analysis, enzyme immunoassay was performed in sera from 124 randomly chosen patients from Stockholm, according to the manufacturer's instructions: IL-10, High Sensitivity ELISA (Quantikine® HS) kit, from R&D Systems, Abingdon, U.K.

All variables included in the multiple linear regression (MLR) analysis as independent variables were assessed at the inclusion into the study. Patients using pancreatic enzymes were classified as pancreatic insufficient. Z-BMI was used as a measure of nutritional status. Infection/colonization status was considered positive if the patient had positive bacterial/fungal isolate at time of inclusion. Those were patients with either chronic lung infection or current colonization/infection of the airways. Sputum and nasopharyngeal swabs were cultured at each center. In accordance with previous studies at northern latitudes, the summer season was defined as May-October and the winter season as November-April (Snellman *et al.*, 2009).

Statistical analysis

Univariate linear regression analysis was used to examine the relation between vitamin D, and serum IgG levels or FEV1, using the following independent variables: serum 25OHD, total vitamin D intake, total vitamin D intake per kg BW, food vitamin D intake, food vitamin D intake per kg BW, supplemented vitamin D, and supplemented vitamin D per kg BW.

MLR analyses were performed for IgG and FEV1 as dependent variables, and serum 25OHD, food and supplemented vitamin D sources of intake as independent variables. To consider possible confounding factors, the model constructed was controlled for age, gender, genotype, CF-related diabetes (CFRD), [season](#), infection/colonization status, long-term treatment with oral corticosteroid, oral long-term treatment with macrolide, pancreatic insufficient versus sufficient phenotype, and z-BMI. Categorical variables were coded (gender male 1/female 2; F508del homozygous genotype 0/other genotype 1; [winter 0/summer season 1](#); all other variables: no 0/yes 1 (**Table 1**)). [All patients enrolled in the Scandinavian CF Nutritional Study were included in the analyses except patients younger than 6 months, because their IgG levels are predominantly maternally derived.](#)

IL-10 values were log-transformed ($\ln(\text{IL-10})$) to have approximately normal distribution of data. Pearson correlation analysis was used to determine the relation between serum 25OHD and $\ln(\text{IL-10})$.

Statistical analyses used STATISTICA (version 7) and SPSS (version 17). All tests were two-sided and $p < 0.05$ was considered statistically significant.

Results

Among the 896 Scandinavian CF patients aged 0.53 – 65.9 years, the mean serum 25OHD was 52.0 nmol/L (range: 1.5–206.9 nmol/L). Data on serum 25OHD were available for 787 patients; 84% of those had suboptimal 25OHD level (<75 nmol/L). The estimated median total daily vitamin D intake was 17 µg (680 IU). Majority of the patients were using pancreatic enzymes, were not born with meconium ileus, did not have CFRD diagnosis, and were not on long-term oral corticosteroid or macrolide treatment (**Table 1**). In this patient dataset, serum 25OHD levels were not determined by latitude zone (zone 1: 54° – 59°; zone 2: 60° – 65°; zone 3: 66° – 71° North) or by belonging to a specific CF centre (ns).

Univariate linear regression analyses showed significant negative correlations between IgG and 25OHD, food vitamin D intake per kg BW, supplemented vitamin D intake, supplemented vitamin D intake per kg BW, total vitamin D intake, and total vitamin D intake per kg BW (**SI: Table 1; SI: Figure 1**). Significant positive correlations were found between FEV1 and 25OHD, supplemented vitamin D intake per kg BW, and total vitamin D intake per kg BW (**SI: Table 2; SI: Figure 2**).

In the MLR model of IgG controlled for age, gender, genotype, CFRD, season, infection/colonization status, corticosteroid treatment, macrolide treatment, pancreatic function, and z-BMI, associations remained statistically significant with serum 25OHD (**Table 2; Figure 1**), supplemented vitamin D intake per kg BW (**Table 3; SI: Figure 3**), and total vitamin D intake per kg BW (**Table 3; Figure 2**) as independent variables. Food vitamin D intake, food vitamin D intake per kg BW, supplemented vitamin D intake, and total vitamin D intake did not show significant relation to IgG in this model (**Table 3**).

In the [MLR](#) model controlled for age, gender, genotype, CFRD, [season](#), infection/colonization status, corticosteroid treatment, macrolide treatment, pancreatic function, and z-BMI, serum 25OHD remained as a significant determinant of FEV1 values (**SI: Table 3; Figure 3**). None of the various vitamin D sources of intake showed significant relation to FEV1 in this model (**SI: Table 4**).

Pearson correlation analysis revealed no statistically significant correlation between serum 25OHD and log-transformed IL-10 serum level in 124 randomly chosen CF patients from Stockholm ($r=0.053$; $p=0.561$).

Discussion

Our study was performed in a [large](#) cohort of CF patients, treated in specialized Scandinavian CF centers and living at northern latitudes (54°–71° North). Most of them had [suboptimal](#) serum 25OHD concentration. [This finding is](#) in line with studies of other CF patient populations (Stephenson *et al.*, 2007; Rovner *et al.*, 2007; Neville & Ranganathan, 2009). With supplementation or UV-B exposure, 25OHD could, however, be safely increased over time (Stephenson *et al.*, 2007; Neville & Ranganathan, 2009; Khazai *et al.*, 2009).

The wide range of serum 25OHD levels in our sample population (1.5–206.9 nmol/L), the relative homogeneity of the patient sample with regard to treatment strategies and lifestyle, and the chronic inflammation as common characteristics of all the patients, render our study population sensitive for studying the proposed immunomodulatory effect of vitamin D. Our study raises the question whether vitamin D insufficiency may contribute to the progress of the airway infection/inflammation in CF. In support of this hypothesis, our data have shown 25OHD to be inversely related to serum total IgG and positively related to FEV1.

Furthermore we found that supplemented vitamin D per kg BW and total vitamin D intake per kg BW were also inversely related to serum total IgG. This supports the notion that low 25OHD levels and high IgG levels are not merely markers of disease severity, but rather causally related, since if anything pancreatic insufficient patients, who are more severely ill than pancreatic sufficient patients, are recommended more intensively to take vitamin supplementation. This interpretation is well compatible with our finding that supplemented vitamin D per kg BW and total vitamin D intake per kg BW were strongly related to serum IgG in our model, but [they](#) failed to show any connection with FEV1, which reflects the severity of the lung disease in CF. [On the other hand, serum 25OHD was associated with](#)

FEV1. Theoretically, more severely ill patients might spend more time outdoors and thus have higher serum 25OHD. However, the associations of vitamin D intake variables can not be subjected to the same criticism. Importantly, the associations found were clinically relevant as regards the IgG concentration *per se*. Therefore we can state that the consistent association between IgG and several vitamin D variables is a strong indication of the importance of vitamin D for immune regulation.

The vitamin D intake in form of food only did not prove to be a significant determinant of serum IgG concentration. However, food vitamin D constituted **only 27%** of the total vitamin D intake in our patient group, in spite of the fact that margarines, butter and low-fat milk are fortified with vitamin D both in Sweden and Norway. Additionally, none of the food records used to assess the vitamin D intake in food had been validated for vitamin D intake, implying that the precision of the assessment might be limited.

FEV1 is the best surrogate outcome measure in CF, with little within-subject variability and high relative precision (Kerem *et al.*, 1992; Cooper *et al.*, 1990). Serum total IgG levels represent a marker of the chronic inflammatory process in CF and correlate inversely with the patients' lung function and long-term prognosis (Levy *et al.*, 2007; Wheeler *et al.*, 1984). Therefore data were analyzed using the same **MLR** model for both IgG and FEV1 as dependent variables. FEV1 decreases and IgG increases with factors contributing to lung morbidity and overall mortality in CF. These were consistently shown to be age, female gender, pancreatic insufficient phenotype, infection/colonization, worse nutritional status (Konstan *et al.*, 2007), F508del homozygous genotype (Kerem *et al.*, 1990), and additional diagnosis of CFRD (Schaedel *et al.*, 2002). On the contrary, IgG and FEV1 were reported to decrease and increase, respectively, upon treatment with anti-inflammatory agents, such as

oral steroids (Auerbach *et al.*, 1985), and are most likely being positively influenced by the recently documented immunomodulatory effect of macrolides (Shinkai *et al.*, 2008). At northern latitudes, vitamin D cannot be synthesized in the skin between late autumn through April (Webb *et al.*, 1988). Therefore, a season variable was included in the MLR model.

In our MLR model with 25OHD as independent variable we have confirmed age, serum 25OHD, infection/colonization status, and nutritional status assessed by z-BMI as significant determinants of both serum total IgG and lung function measured by FEV1. Additionally, gender, steroid treatment, and macrolide treatment were identified as risk-indicator variables for serum total IgG levels. Long-term treatment with macrolide antibiotics was previously shown to positively affect lung function (Hansen *et al.*, 2005; Anwar *et al.*, 2008) and exert complex immunomodulatory effect (Shinkai *et al.*, 2008; Steinkamp *et al.*, 2008), which justified inclusion of this variable into our model. Surprisingly, macrolide treatment showed a positive and a negative relation to serum total IgG and FEV1, respectively, probably as a result of selection bias and little immunomodulatory effect. Importantly, season did not show to be a determinant of serum total IgG or FEV1, which indicates that it is rather 25OHD level *per se* that is associated with the studied outcome variables, and not season as such. These results suggest that season might be associated with serum total IgG or FEV1, but only if it influences serum 25OHD levels.

Several previous studies have addressed the relation between vitamin D status and lung function. These have found a positive association between serum 25OHD and FEV1. However, analyses were either not controlled for any confounders or controlled for only a few relevant factors despite the substantial heterogeneity within the subject population (Wolfenden *et al.*, 2008; Green *et al.*, 2008; Black & Scragg, 2005). The positive association

between 25OHD and FEV1 in our MLR model confirmed these previous observations in our large population of well-defined patients. The absolute amplitude of the association in this model is small. This is not surprising in the context of the well-described other, stronger lung function determinants in this patient group, all of which are included in our model together with vitamin D. Therefore, despite the considerable support for the concept of vitamin D as an immunomodulator provided by this study, the clinical relevance of this finding remains unclear.

In search for the underlying mechanism behind the inverse relation between 25OHD and serum IgG, we subsequently analyzed 124 randomly chosen sera from the Stockholm CF patients and determined IL-10 level. Vitamin D has been shown to induce the production of IL-10 *in vitro* (Mora *et al.*, 2008; Xystrakis *et al.*, 2006). We did not observe any correlation between serum 25OHD and IL-10. However, vast majority of the randomly chosen sera samples had suboptimal 25OHD concentrations and serum IL-10 might not necessarily reflect IL-10 level in epithelial lining fluid in the lung.

If the observed probable immunomodulatory effect can not be explained by increase in IL-10 levels, there are several other mechanisms of vitamin D immunomodulation which may be related to it. Expression of CYP27B1, a 25(OH)D-activating enzyme, has been reported in normal human respiratory epithelial cells (Hansdottir *et al.*, 2008), and active vitamin D has been found to induce antimicrobial peptide expression in CF bronchial epithelial cells (Yim *et al.*, 2007). Moreover, vitamin D was demonstrated to primarily inhibit production of the proinflammatory cytokines by adaptive immune cells and to decrease B cell proliferation, plasma-cell differentiation, and IgG secretion (Mora *et al.*, 2008). We speculate that this direct effect on antibody production might explain our findings to some extent.

In conclusion, our study suggests that increasing vitamin D intake is likely to positively modulate inflammation in CF. Future research should focus on intervention studies to test whether the associations represent causal relationships. Our findings clearly indicate that elaborate prospective studies with hard end-points are fully justified, in order to assess the amplitude of the absolute clinical benefit of vitamin D supplementation.

Acknowledgement Section

Acknowledgements

We thank all the participating dietitians for their work with the food records, all the nurses for their practical input into the nutritional study, the CF-patients in each center who have patiently participated in this study, Jan Kowalski for reviewing the statistical methods and Professor Åke Nilsson for valuable remarks on the final version of the manuscript.

Funding

This work was supported by Swedish Heart Lung Foundation, *Stiftelsen Frimurare-Barnhuset i Stockholm*, Karolinska Institutet, Norwegian and Swedish Cystic Fibrosis Associations, and by an unrestricted grant from Solvay Pharma, which has made the meetings of the SCFSC possible. Financial support was further provided through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and the Karolinska Institutet.

Role of the sponsors

The funding sources did not participate in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Statement of Authors' Contributions to Manuscript

T.Pi. had full access to all of the data in the study and takes responsibility for the integrity of the data, the accuracy of the data analysis and final content of the paper. T.Pi. and L.H. had the main responsibility for the vitamin D study. The study was designed by T.Pi. and L.H. T.Pi. developed the statistical model for data analysis, analyzed data, interpreted results and wrote the manuscript with the contribution of K.N. and L.H. Patients were enrolled by all co-authors. F.K. was in charge of the IL-10 analyses. Food records were analyzed by K.N. and I.E.M. All authors critically read and commented on the manuscript. All authors read and approved the final manuscript. The Scandinavian CF Nutritional Study was designed by the Scandinavian CF Consortium, L.H. being main responsible.

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Supplementary information is available at the journal's website.

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Tables

Table 1.

Descriptive statistics for demographic, biochemical, and vitamin D intake data for the CF patients included in the study, n= 896

Continuous variables with normal distribution	N	Mean (SD)	
BMI z-score	891	-0.22	(1.15)
Serum total IgG (g/L)	868	11.2	(4.4)
FEV1 (% of predicted)	802	0.79	(0.24)
Serum 25OHD (nmol/L)	787	52	(26.7)
Continuous variables with skewed distribution	N	Median (quartiles)	
Age (yrs)	896	18.4	(10.4; 29.5)
Total vitamin D intake (μ g)	389	17	(10.54; 26.55)
Total vitamin D intake (IU)	389	680	(422; 1062)
Total vitamin D intake per kg BW (μ g/kg)	388	0.37	(0.2; 0.72)
Total vitamin D intake per kg BW (IU/kg)	388	15	(8; 29)
Food vitamin D intake (μ g)	406	4.59	(2.95; 6.8)
Food vitamin D intake (IU)	406	184	(118; 272)
Food vitamin D intake per kg BW (μ g/kg)	405	0.1	(0.06; 0.17)
Food vitamin D intake per kg BW (IU/kg)	405	4	(2.4; 6.8)
Supplemented vitamin D intake (μ g)	728	10	(5.18; 20)
Supplemented vitamin D intake (IU)	728	400	(207; 800)
Supplemented vitamin D intake per kg BW (μ g/kg)	727	0.24	(0.11; 0.53)
Supplemneted vitamin D intake per kg BW (IU/kg)	727	9.6	(4.4; 21.2)
Categorical variables	N	No	Yes
Adult (\geq 18 yrs)	896	440	456
Female gender	896	467	429
Use of pancreatic enzymes	896	127	769
Meconium ileus	896	784	112
CFRD	896	808	88
Current bacterial/fungal infection/colonization	896	476	420
Chronic bacterial/fungal infection/colonization	896	402	494
F508del homozygote genotype	896	442	454
Long-term oral corticosteroid treatment ¹	896	839	57
Long-term oral macrolide treatment ¹	896	647	249
Suboptimal serum 25OHD level (<75 nmol/L)	787	126	661
Sampling done during summer season (May-Oct)	896	533	363

Conversions: To convert serum total IgG to mg/dL, multiply by 100; serum 25OHD to ng/mL, divide by 2.5.

¹ Long-term oral treatment is classified as “Yes” if it is being given at the time of sampling in long-term manner

Table 2.

Multiple linear regression model of serum 25OHD and IgG (full model), [adjusted R² = 0.376](#)

N=777	Estimate of beta	95% CI	p-level
Intercept	8	6.61 to 9.39	<0.001
Gender	0.59	0.1 to 1.09	0.019
Age	0.13	0.11 to 0.15	<0.001
Genotype	-0.45	-0.97 to 0.08	0.096
CFRD	0.69	-0.16 to 1.53	0.112
Infection/colonisation status	1.48	0.86 to 2.1	<0.001
Steroid treatment	-2.09	-3.16 to -1.01	<0.001
Macrolide treatment	1.29	0.7 to 1.88	<0.001
Serum 25OHD	-0.02	-0.03 to -0.01	<0.001
Pancreatic suff./insuff.	-0.69	-1.49 to 0.11	0.093
Z-BMI	-0.63	-0.86 to -0.4	<0.001
Summer season	0.19	-0.32 to 0.71	0.463

Table 3.

Results of multiple linear regression analyses¹ of serum 25OHD or vitamin D sources of intake and IgG.

Independent variables	N	Estimate of beta	95% CI	p-level	adj. R ²
25OHD	777	-0.02	-0.030 to -0.01	<0.001	0.376
Food vitamin D	398	0.008	-0.057 to 0.073	0.806	0.387
Food vitamin D/kg BW	398	-1.92	-4.251 to 0.417	0.108	0.391
Supplemented vitamin D	719	-0.006	-0.016 to 0.004	0.262	0.358
Supplemented vitamin D/kg BW	719	-0.82	-1.170 to -0.462	<0.001	0.375
Total vitamin D	382	-0.009	-0.020 to 0.002	0.122	0.386
Total vitamin D/kg BW	382	-0.6	-0.967 to -0.225	0.002	0.398

¹The models used the same variables, with the exception of the vitamin D terms. The common independent variables include: age, gender, genotype, CFRD, [summer season](#), infection/colonization status, steroid treatment, macrolide treatment, pancreatic sufficiency/insufficiency and z-BMI.

Legends for Figures

Figure 1.

Partial plot for relation between serum 25OHD and IgG from **MLR** model with age, gender, genotype, CFRD, **summer season**, infection/colonization status, long-term treatment with oral corticosteroid, oral long-term treatment with macrolide, pancreatic insufficient versus sufficient phenotype, serum 25OHD and z-BMI entered as independent variables, and IgG entered as dependent variable (**adjusted R²=0.376**; **p<0.001**).

Figure 2.

Partial plot for relation between total vitamin D intake per kg BW and IgG from **MLR** model with age, gender, genotype, CFRD, **summer season**, infection/colonization status, long-term treatment with oral corticosteroid, oral long-term treatment with macrolide, pancreatic insufficient versus sufficient phenotype, total vitamin D intake per kg BW and z-BMI entered as independent variables, and IgG entered as dependent variable (**adjusted R²=0.398**; **p=0.002**).

Figure 3.

Partial plot for relation between serum 25OHD and FEV1 from **MLR** model with age, gender, genotype, CFRD, **summer season**, infection/colonization status, long-term treatment with oral corticosteroid, oral long-term treatment with macrolide, pancreatic insufficient versus sufficient phenotype, serum 25OHD and z-BMI entered as independent variables, and FEV1 entered as dependent variable (**adjusted R²=0.308**; **p=0.025**).

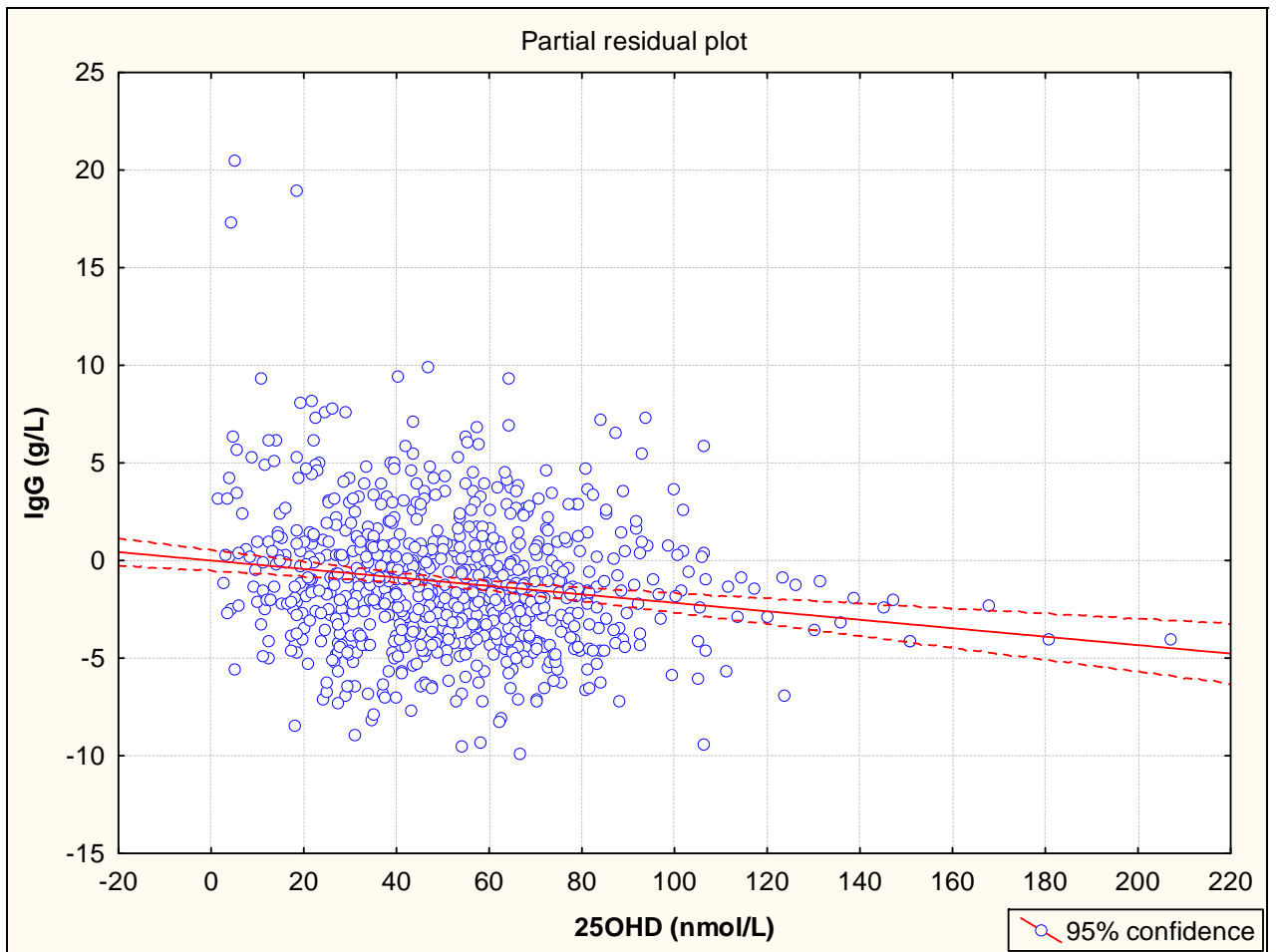


Figure 1.

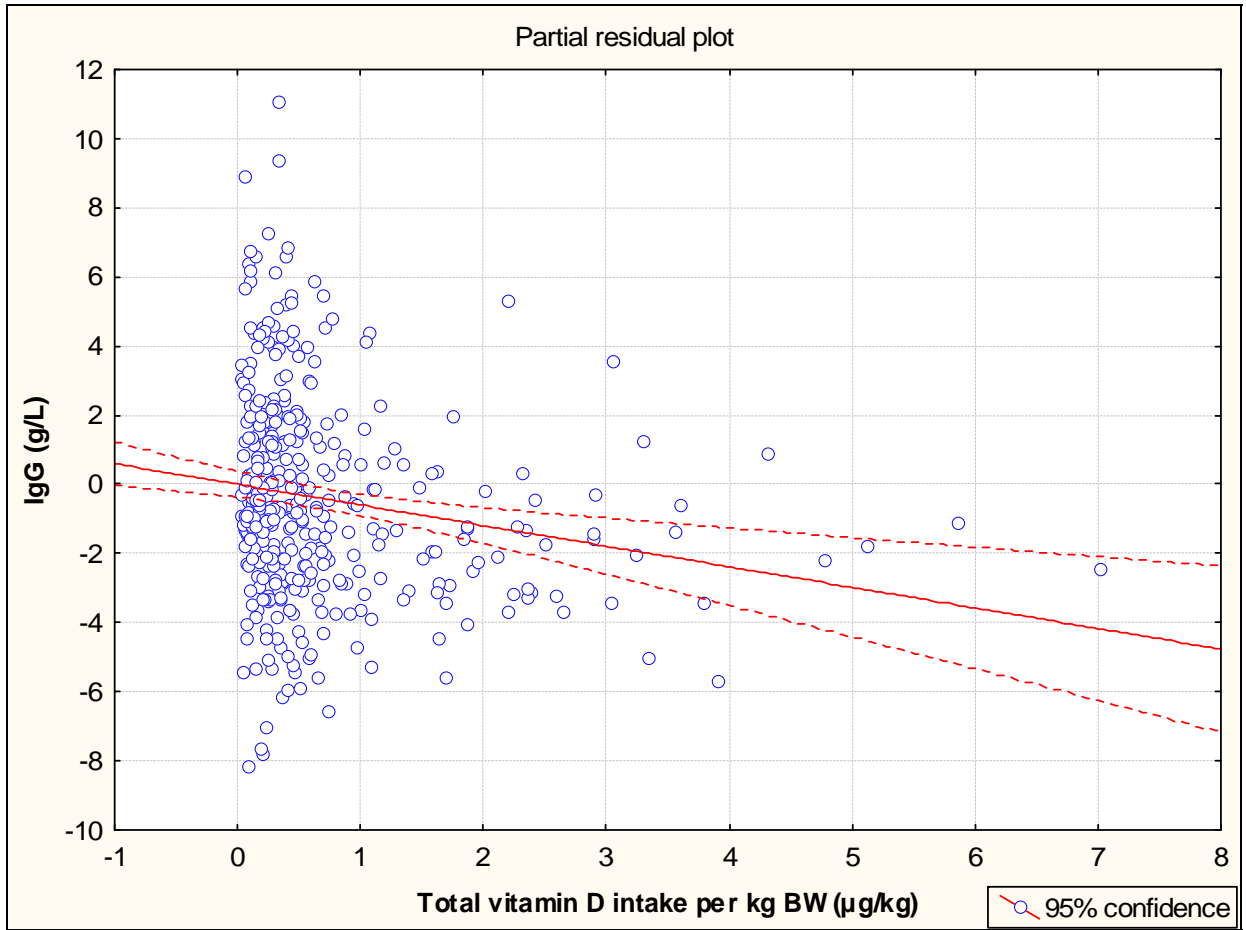


Figure 2.



Figure 3.