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Original article

Serum 25-hydroxyvitamin D and fatty acids in relation to the risk of microbial infections in children: The TRIGR Divia study

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SUMMARY

Background & aims: Nutrient status may affect the risk of microbial infections and play a role in modulating the immune response against such infections. The aim of this study was to determine whether serum 25-hydroxyvitamin D [25(OH)D] and serum fatty acids in infancy are associated with microbial infections by the age of 18 months.

Methods: Altogether 576 newborn infants from Trial to Reduce IDDM in the Genetically at Risk (TRIGR) born between 2002 and 2007 were included. The concentration of 25(OH)D vitamin and proportions of 26 fatty acids (presented as % of total fatty acids) were analyzed in cord blood serum and in sera taken at 6, 12, and 18 months of age. The cord blood samples and mean of 6–18-month values were used as exposures. Infections were detected by screening IgG antibodies against 10 microbes using enzyme immunoassay and antibodies against 6 coxsackievirus B serotypes by plaque neutralization assay in serum samples taken at 18 months of age.

Results: A higher proportion of *n*-3 polyunsaturated fatty acids (PUFAs) and especially long-chain *n*-3 PUFAs at birth and at the age of 6–18 months was associated with decreased risk of coxsackievirus B2 infection unadjusted and adjusted for region, case-control status, and maternal type 1 diabetes. Higher proportion of docosapentaenoic acid (DPA, 22:5 *n*-3) at birth was associated with a decreased risk of respiratory syncytial virus infection. 25(OH)D vitamin concentration was not consistently associated with the risk of infections. When only infected children were included docosahexaenoic acid (DHA, 22:6 *n*-3) and arachidonic acid (20:4 *n*-6) proportions were positively associated with IgG antibody levels against influenza A virus. 25(OH)D vitamin concentration showed an inverse association with rotavirus IgG levels among children with rotavirus seropositivity.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BSA, bovine serum albumin; CVB, Coxsackievirus B; DGLA, dihomogammalinolenic acid; DPA, Docosapentaenoic acid; EIA, Enzyme immunoassay; EIU, Enzyme immunoassay units; GMK cells, Green Monkey Kidney cells; RSV, respiratory syncytial virus; TRIGR, Trial to Reduce IDDM in the Genetically at Risk.

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Conclusions: In young children with increased susceptibility to type 1 diabetes, long-chain *n*-3 PUFAs may influence the risk of viral infections and immune response against the infections. However, this association may depend on the type of virus suggesting virus-specific effects.

Vitamin D and long chain *n*-3 fatty acids have been identified as important players in the immune system regulation [1,2] and the use of supplementation with these nutrients has been suggested to decrease infection incidence in children [3–5].

A recent meta-analysis based on randomized controlled trials comparing vitamin D supplementation with placebo found a modest protective effect of vitamin D supplementation on acute respiratory infections in all participants, as well as in a subgroup including only children [3]. The definition and assessment of infections varied between the studies included and was often based on severe infections with physician or laboratory confirmation (e.g., influenza, pneumonia), or parent-reported infections. The evidence on the effect of vitamin D supplementation on other types of infections, such as gastrointestinal infections in children is limited [6].

Fatty acids have been studied less, however, some intervention studies [4,5], and some prospective observational studies [7,8] suggest that milk products supplemented with specific fatty acids (DHA, DHA + arachidonic acid, or DHA + EPA) may decrease number of episodes of respiratory illness or diarrhea in children. In addition, maternal supplementation with DHA + EPA during pregnancy decreased risk of the lower respiratory tract infections in the offspring [9]. The role of other fatty acids in childhood infections is unclear.

We have recently reported the associations of serum 25-hydroxyvitamin D [25(OH)D] concentration [10] and fatty acid proportions [11] in early childhood with the risk of type 1 diabetes related islet autoimmunity in the Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study population.

Now, our aim was to study whether serum 25(OH)D vitamin and serum fatty acids in early childhood are associated with common microbial infections, including infections which have been linked to type 1 diabetes in previous studies (Coxsackievirus B [CVB], rotavirus) [12–14].

1. Methods

1.1. Study design and population

This study is based on samples collected in the TRIGR study (ClinicalTrials.gov registration no. NCT00179777). TRIGR is a double-blind randomized clinical trial of 2159 infants recruited between 2002 and 2007 in 15 countries (Australia, Canada, Czech Republic, Estonia, Finland, Germany, Hungary, Italy, Luxembourg, Netherlands, Poland, Spain, Sweden, Switzerland, USA) [15]. The inclusion criteria were having at least one first-degree relative with type 1 diabetes, a risk HLA genotype for type 1 diabetes, and parental consent [15]. Exclusion criteria have been described before [15]. All participants were observed until the youngest child turned 10-year-old in 2017. Written informed consent was collected from all families, signed by the legal guardian of the child. The study was approved by the ethics committees of all participating centers. There was no difference in the incidence of islet autoimmunity [16] or type 1 diabetes [15] between participants randomized to receive extensively hydrolyzed casein infant formula and those randomized to receive regular cow's milk-based one. The study formula powder contained 250 IU of vitamin D and 25 g of fat/100 g, and respective quantities in control formula were 300 IU and 27 g. Neither of the formulas contained long-chain *n*-3 PUFAs.

Within the TRIGR cohort, an ancillary case-control selection including 244 case children with islet autoimmunity and two age- and country-matched control children (n = 488) was done in 2016 to study dietary and viral factors associated with islet autoimmunity [11]. In this study, we use the data from this nested case-control study as a cohort, adjusting for case-control status. Among the 732 children in TRIGR ancillary study, altogether 576 children had at least one successfully analyzed dietary biomarker (25(OH)D vitamin, fatty acids) and virus antibody data and were included in the present study (Supplementary Fig. 1).

At 18 months of age, when microbe outcomes of this study were assessed by the presence of microbial antibodies in serum, 529 (91.8%) children had not seroconverted to any islet autoantibodies (of GAD, IAA, IA2A and ZnT8A), while 40 children (6.9%) had seroconverted to one and seven children (1.2%) to two or more islet autoantibodies.

1.2. Assessments

The cord blood was collected according to hospital practices and was a mixture of arterial and venal cord blood. The follow-up blood samples were collected at 3–12 months' intervals at study visits [15]. The serum samples were stored frozen at −70 °C until analyzed.

Serum fatty acids at ages 0, 6, 12, and 18 months were determined by gas-chromatography using an Agilent 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) with a split injector and hydrogen as the carrier gas at the Finnish Institute for Health and Welfare, Finland [11]. We employed a capillary column Omegawax 320 (length: 30 m, I.D.: 0.32 mm, phase layer: 0.25 μm; Supelco, Bellefonte, PA, USA).

Altogether 26 individual fatty acids were detected and reported as proportions (%) of total fatty acids. Furthermore, we calculated the sum of *n*-3 long chain PUFAs [EPA 20:5*n*-3, docosapentaenoic acid (DPA) 22:5*n*-3, and DHA 22:6*n*-3], *n*-3 total (*n*-3 long chain and alphalinolenic acid 18:3*n*-3), and *n*-6 total (linoleic acid 18:2*n*-6, dihomogammalinolenic acid 20:3*n*-6 (DGLA), arachidonic acid 20:4*n*-6, and docosatetraenic acid 22:4*n*-6).

Vitamin D was measured as 25-hydroxyvitamin D [25(OH)D] concentrations determined by a chemiluminescent microparticle immunoassay by Architect *i* system (Abbott Laboratories, Abbott Park, IL, USA) [10]. The laboratory regularly participates in the Vitamin D External Quality Assessment Scheme (DEQAS, Charing Cross Hospital, UK).

Microbial infections by the age of 18 months were assessed with two methods in serum samples collected at the age of 18 months.

The serum samples were analyzed for the presence of IgG antibodies against adenovirus, enterovirus, cytomegalovirus, influenza A virus, mycoplasma, norovirus, parainfluenza virus, respiratory syncytial virus (RSV), rhinovirus and rotavirus using EIA, as previously described [17,18]. All the antigens and their concentrations are described in Supplementary Table1. After coating with the given antigen, the wells were blocked with 0,1% bovine serum albumin

(BSA). Serum samples were analyzed in 1/200 (mycoplasma), 1/500 (RSV) or 1/1000 dilution (all other antigens) in PBS + 1% BSA + 0,05% Tween 20, except for rhinovirus where the serum samples were diluted in pH 9,4 carbonate buffer. Binding of antibodies was documented using peroxidase-conjugated antihuman IgG (Rabbit Anti-Human IgG/HRP (Dako P0214; Dako, Copenhagen, Denmark). Finally, orto-phenylenediamine-dihydrochloride substrate was added and color reaction measured at 490 nm. EIU (enzyme immunoassay unit, the relative antibody reactivity of the sample compared with positive and negative reference sera included in each assay) values were calculated for each sample. Infection by the age of 18 months was defined as seropositivity with cut-off ≥15 EIU in the 18-month sample.

Neutralizing antibodies against each of the six CVB serotypes were analyzed using plaque neutralization assay in mycoplasma-free African Green Monkey Kidney (GMK) cell line as previously described [19] in the serum samples collected at the age of 18 months. CVB infection by the age of 18 months for each CVB serotype was defined as the presence of neutralizing antibodies specific for that CVB type. Briefly, in this assay serum samples were first incubated for 1h at +37 °C with a standardized amount of CVB generating ¼ serum dilution, and this mixture was then transferred on GMK cells on a 12-well plate. The number of plaques were calculated after 2 days incubation at +37 °C, and serum samples showing at least 80% reduction in the number of virus plaques as compared to the control wells that were infected with untreated virus were considered positive for neutralizing antibodies.

The clinical and background variables were derived from hospital records, questionnaires, and assessments during follow-up. The following variables were considered as potential confounders: region (Northern Europe, Central and Southern Europe, North America, Australia), maternal type 1 diabetes (yes, no), case-control status (islet autoimmunity case, control), child's HLA-conferred risk for type 1 diabetes (high, moderate, mild), sex (female, male), duration of any breastfeeding (<6 months; ≥6 months), season of birth (summer, fall, winter, spring), having an older sibling (yes, no), and cord blood seropositivity to the microbe studied (yes, no).

1.3. Statistical analyses

The 25(OH)D vitamin concentration and fatty acid proportions in the cord blood samples and the mean values of 6, 12 and 18-month samples were utilized as exposure variables. For statistical comparisons, the fatty acid measures were center log-ratio transformed. 25(OH)D was considered as an exposure variable both log-transformed continuous and categorized serum (<50 nmol/l vs ≥ 50 nmol/l).

To study the association between nutrients and risk of infections, logistic regression analyses (specific infections), or ordinal logistic regression (number of infections) were utilized. Linear regression analyses were used to study the associations between nutrients and microbe-specific log-transformed IgG levels (EIU) among seropositive children. All analyses were done unadjusted and adjusted for region, case-control status, and maternal type 1 diabetes. Region and maternal type 1 diabetes were associated with several exposure and outcome variables (data not shown) and chosen as covariates. Case-control status was chosen as covariate due to the specific study design. Other covariates (listed above) that based on literature could have been confounders were excluded from the final model, either because they were not associated with the exposure and outcome variables, or because inclusion of them in the model did not change the interpretation of the results.

Based on a family-wide error rate of 0.05 and the use of the Sidak correction to account for multiple testing, p-values < 0.00002 were considered statistically significant. As no association met that threshold, comparisons at a significance level of $p < 0.01$ and $p < 0.001$ are presented.

The statistical analyses were performed with SAS version 9.4 and the visualization with RStudio Version 1.4.1103.

2. Results

Study participants are described in Table 1. Mean 25(OH)D concentrations and fatty acid proportions by age are presented in Supplementary Tables 2 and 3, respectively. Number (%) of children with 25(OH)D concentration <50 nmol/l and ≥50 nmol/l was 429 (74.5%) and 147 (25.5%) at 0 months and 123 (21.4%) and 453 (78.6%) at 6–18 months of age, respectively.

The numbers of participants with different microbial infections by the age of 18 months are presented in Table 2. Altogether 99.1% of the children were positive for at least one microbial IgG antibody, and the median (IQR) number of microbial infections was 4.0 (2.0–5.0) per child. Altogether 38.9% of the children had antibodies for at least one of the six CVB serotypes while the median number of different CVB infections was 0 (0.0–1.0). Altogether 91 children (15.8%) had been vaccinated against influenza A virus and 12 children (2.3%) against rotavirus.

3. Association between nutrients with risk of infections

Figure 1 summarizes the unadjusted associations between nutrient levels at birth and at 6–18 months of age with the risk of microbial infections by the age of 18 months. The proportion of *n*-3 PUFAs was associated with several infections. Most consistent association was seen between higher *n*-3 PUFAs and decreased risk of CVB2 infection, any CVB infection, and number of CVB infections. In addition, higher proportion of some *n*-3 PUFAs was associated with decreased risk of adenovirus infection and increased risk of cytomegalovirus infection. Further, there were some associations between 25(OH)D vitamin, SFA, MUFA, *n*-6 PUFA, or other fatty acids

Table 1
Characteristics of the 576 participants, the TRIGR Divia study.

	Number of children (%)
Sex	
Female	274 (47.6)
Male	302 (52.4)
Region	
Northern Europe	164 (28.5)
Central and Southern Europe	150 (26.0)
North America	237 (41.2)
Australia	25 (4.3)
Type 1 diabetes-related HLA risk†	
High risk	157 (27.3)
Moderate risk	244 (42.4)
Mild risk	175 (30.4)
Mother has type 1 diabetes	
Yes	269 (46.7)
No‡	307 (53.3)
Treatment group in TRIGR study	
Casein Hydrolysate	288 (50.0)
Control Formula	288 (50.0)
Duration of breastfeeding, months	
<3	146 (25.4)
3-5.9	69 (12.0)
≥6	361 (62.7)

†High risk: HLA-DQB1*0302/DQB1*02; Moderate risk: HLA-DQB1*0302/x (x not DQB1*02, DQB1*0301, or DQB1*0602); Mild risk: HLA-DQA1*05-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603) and HLA-DQA1*03-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603).
‡ Children without a mother with type 1 diabetes but having another 1st degree relative with type 1 diabetes.

Table 2
Number and proportion of the children with infections by the age of 18 months, the TRIGR Divia study.

	Number (%) of children with an infection	Number of children with an analyzed sample
Microbe		
Adenovirus	211 (39.4)	535
Enterovirus	402 (75.6)	532
Cytomegalovirus	108 (20.3)	533
Influenza A	422 (79.3)	532
Mycoplasma	83 (15.6)	531
Norovirus	373 (70.0)	533
Parainfluenza	156 (29.2)	534
Respiratory syncytial virus	219 (41.4)	529
Rhinovirus	302 (56.7)	533
Rotavirus	188 (35.4)	531
Any IgG seropositivity	530 (99.1)	535
Coxsackievirus B (CVB)		
CVB1	108 (18.8)	574
CVB2	107 (18.6)	574
CVB3	29 (5.0)	576
CVB4	24 (4.2)	576
CVB5	23 (4.0)	574
CVB6	19 (3.3)	572
Any CVB seropositivity	224 (38.9)	576

and specific infections but no clear pattern was seen for any other nutrient than *n*-3 PUFAs (Fig. 1).

After adjustment for region, case-control status, and maternal type 1 diabetes, higher proportion of several *n*-3 PUFAs was associated with decreased risk of CVB2 infection (Fig. 2). However, the associations of *n*-3 PUFAs with any CVB, number of CVBs, adenovirus, and cytomegalovirus infections were no longer associated at the $p < 0.01$ level (Fig. 2). Other associations that were associated at the $p < 0.01$ level with and without adjustments were those between cis vaccenic acid and rotavirus (direct association), oleic acid and CVB2 (direct), linoleic acid and number of CVB infections (inverse), DGLA acid and rotavirus (inverse), as well as the association between DPA and RSV (inverse).

When serum 25(OH)D vitamin concentration was categorized, we found that higher (≥50 nmol/l) compared to lower (<50 nmol/l) serum 25(OH)D concentration at 0 months was associated with higher number of CVB infections (OR 1.65: 95% CI 1.13–2.39, $p = 0.009$). The association remained similar after adjustment for region, case-control status, and maternal diabetes (1.58: 1.06–2.35, $p = 0.03$). Higher (≥50 nmol/l) compared to lower (<50 nmol/l) serum 25(OH)D concentration at 6–18 months was associated with increased risk of adenovirus infection (1.86: 1.19–2.91, $p = 0.006$) unadjusted, but not when adjusted (1.50: 0.94, 2,42, $p = 0.09$). No other associations ($p < 0.01$) were observed between categorized serum 25OHD (≥50 vs. <50 nmol/l) and microbial infections.

3.1. Association of nutrients with viral IgG antibody levels

Next, we studied the associations between the nutrients and IgG antibody levels (EIU) among children with microbial infections (Fig. 3). We observed that higher cord blood and 6-18-month proportions of DHA (a long-chain *n*-3 PUFA), long-chain *n*-3 PUFA, and 6-18-month total *n*-3 PUFA levels were associated with higher levels of IgG antibodies for influenza A. After adjustment for region, case-control status, and maternal type 1 diabetes the associations between *n*-3 PUFA and influenza A IgG levels slightly weakened (Fig. 4). Other associations that were associated at the $p < 0.01$ level in unadjusted and adjusted analyses were those between 25(OH)D vitamin and rotavirus IgG (inverse association), DGLA acid and parainfluenza IgG (inverse), and arachidonic acid and influenza A IgG (direct) (Figs. 3 and 4).

When serum 25(OH)D vitamin concentration was categorized, we found that higher compared to lower (≥50 nmol/l vs. <50 nmol/l) concentration at 6–18 months of age was associated with lower rotavirus IgG levels (β −0.282, $p = 0.001$) among seropositive children unadjusted and with adjustment for region, case-control status, and maternal type 1 diabetes (β −0.272, $p = 0.003$).

4. Discussion

We observed that higher serum proportion of several *n*-3 PUFAs in newborn infants and young children was associated with a decreased risk of CVB infections, especially CVB2 infection by the age of 18 months. In addition, higher DPA at birth was associated with decreased risk of RSV infection. Serum 25(OH)D vitamin was not consistently associated with microbial infections.

The strengths of this study include a unique prospectively collected dataset that comprises a wide panel of nutrients and viral antibodies assessed in a large study population of young children from different countries. Most importantly, we could study the associations between nutrients and microbial infections and include relevant adjustments into the models.

A limitation is that the study was not originally designed to study associations between nutrients and microbial infections. However, we tried to take this into account by adjusting for the case-control status of the participants. In addition, there might be residual confounding that could explain the observed or null associations. However, we carefully considered several potential confounders before choosing the final adjustments. Further, the study included multiple tests and results presented at the level $p < 0.01$ may include some false discoveries. Finally, this study was exploratory and not powered sufficiently for the number of outcomes considered. As a result, there is a possibility for type 2 error, that a negative result was accepted even though it is incorrect.

Since microbial infections were defined as the presence of microbial antibodies at a single time point (18 months), we don't know the exact time of the infections. The analyses between cord blood nutrients and infections by the age of 18 months are truly prospective. For the analyses including the later age points of nutrient assessment, there is a possibility of reverse causality. For example, infection could lead to lower serum *n*-3 PUFA proportion and not vice versa. However, we combined the nutrient assessments of 6–18 months to describe the nutrient status over a longer period of time to average possible short-term changes in fatty acid proportions.

An advantage of using the 18-month time point for serological screening of infections is that maternal antibodies have already disappeared by that age and could not have biased this comparison. However, we might have missed some early infections if antibodies related to them had also disappeared by the age of 18 months. The timing of the infection could have also influenced the antibody levels as recently infected children are likely to have higher IgG levels than those who were infected at an earlier age.

This study included children with a high genetic and familial risk of type 1 diabetes, and it is not known whether the current findings would apply to the general population. However, only a minority of the children developed islet autoantibodies during the 18-month follow-up. This study population consist of well-nourished children and differences in nutrient levels and their association with infections could be different to those in study populations with malnourished children.

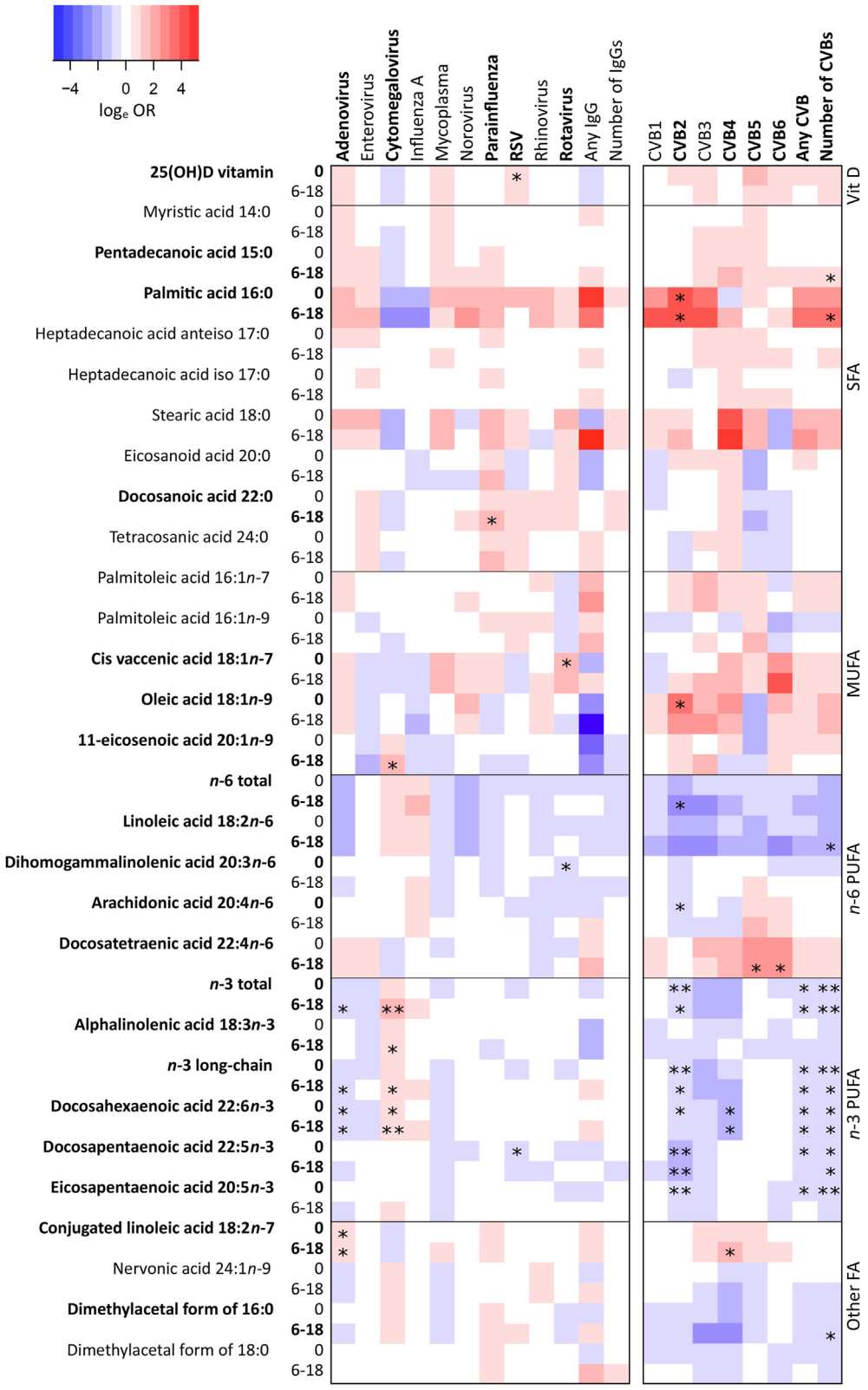

Fig. 1. Associations of serum 25(OH)D vitamin and fatty acid levels at birth (0 months) and at 6–18 months with the risk of microbial infections by the age of 18 in 576 children. Results indicate unadjusted odds ratios based on logistic regression analyses, or ordinal logistic regression analyses. Red color indicates increased risk and blue decreased risk of infection. *p < 0.01,**p < 0.001. CVB, coxsackievirus B; RSV, respiratory syncytial virus; FA, fatty acid.

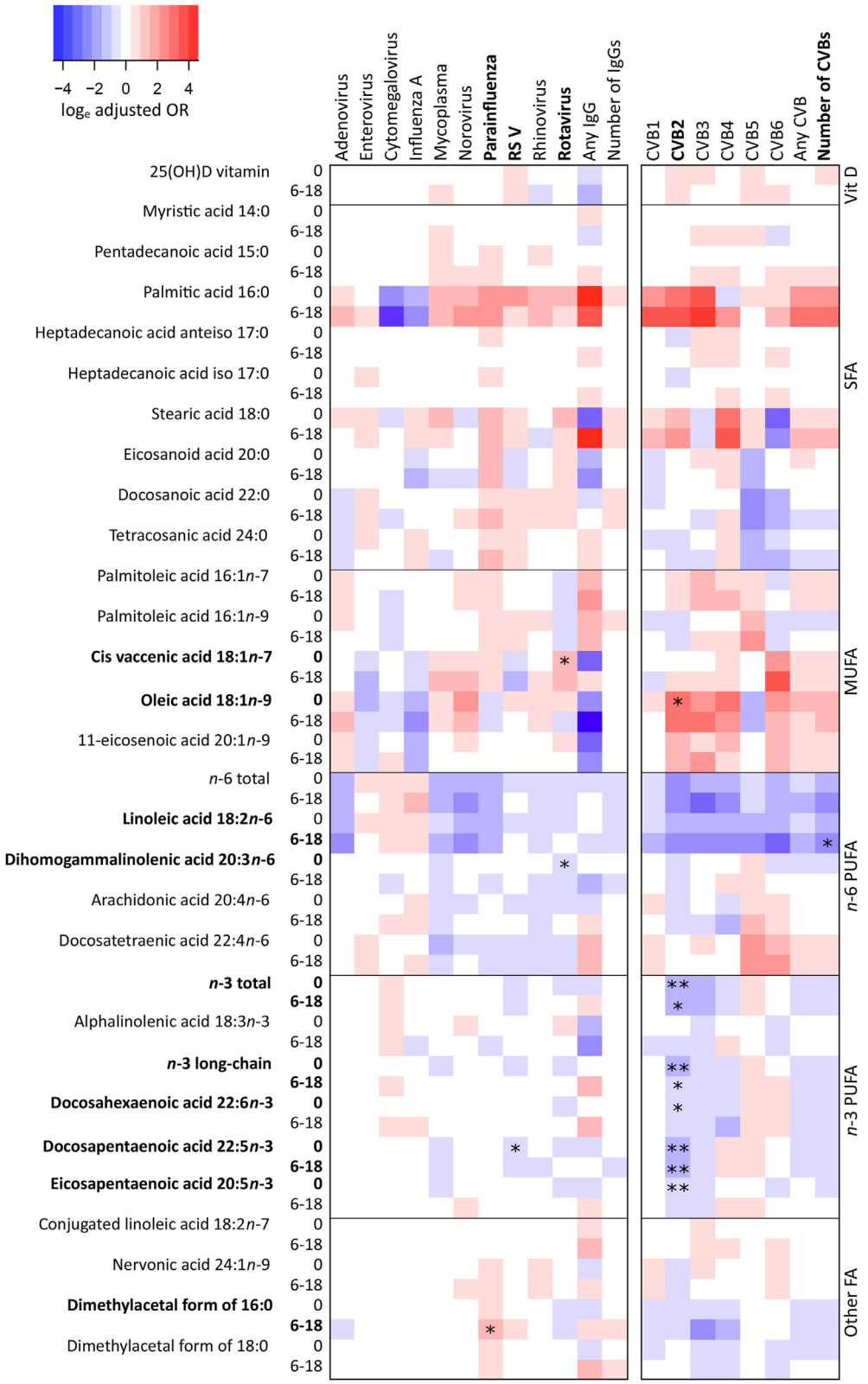

Fig. 2. Associations of serum 25(OH)D vitamin and fatty acid levels at birth (0 months) and at 6–18 months with the risk of microbial infections by the age of 18 in 576 children. Results indicate adjusted odds ratios based on logistic regression analyses, or ordinal logistic regression analyses. Adjusted for region, case-control status, and maternal type 1 diabetes. Red color indicates increased risk and blue decreased risk of infection. *p < 0.01,**p < 0.001. CVB, coxsackievirus B; RSV, respiratory syncytial virus; FA, fatty acid.

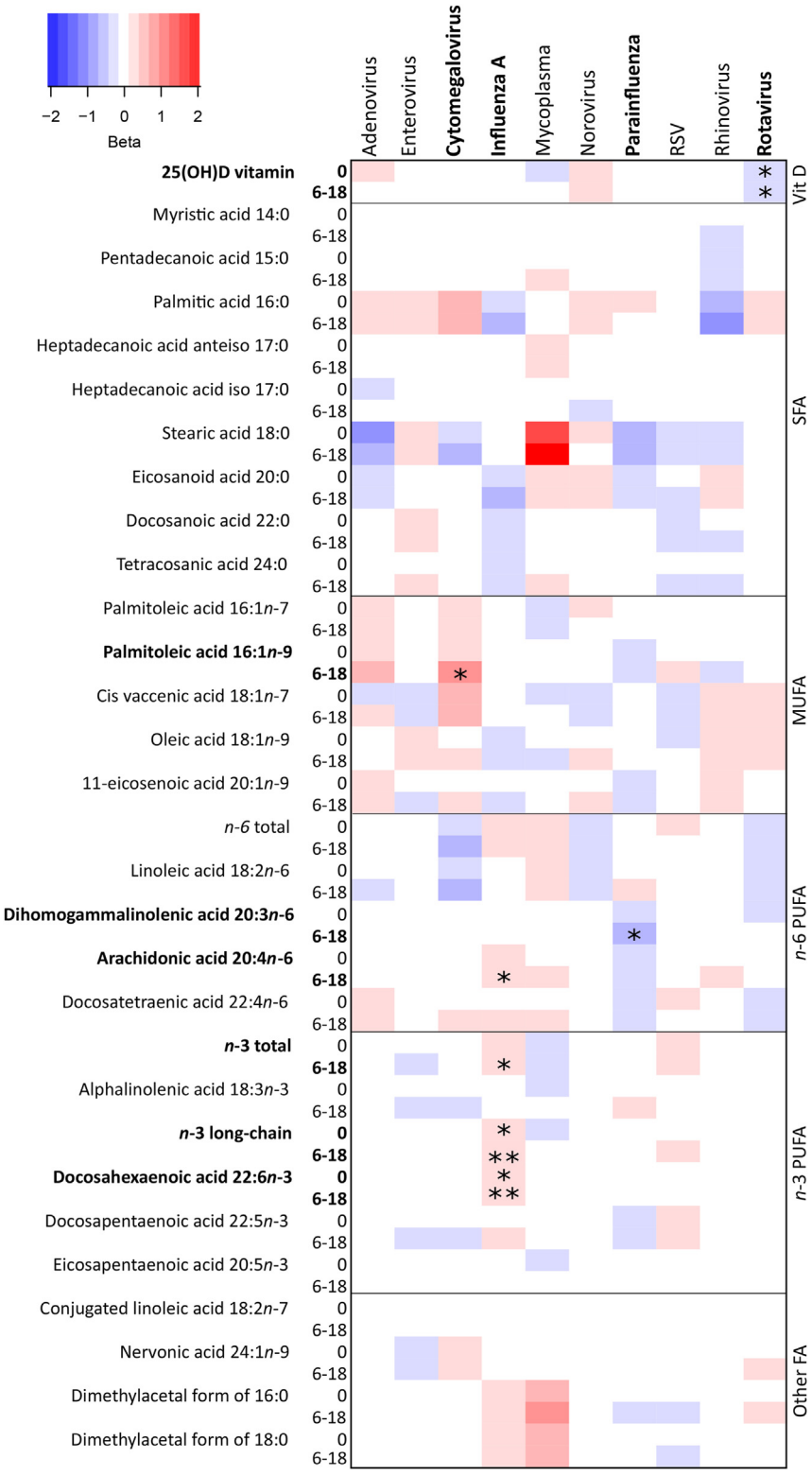

Fig. 3. Associations of serum 25(OH)D vitamin and fatty acid levels at birth (0 months) and at 6–18 months with microbe-specific IgG levels (EIU) assessed at 18 months among seropositive children (EIU levels ≥15 of the specific IgG, n in Table 2). Results indicate unadjusted beta coefficients based on linear regression analyses. Red color indicates direct and blue color indirect association. *p < 0.01, **<0.001 RSV, respiratory syncytial virus; FA, fatty acid.

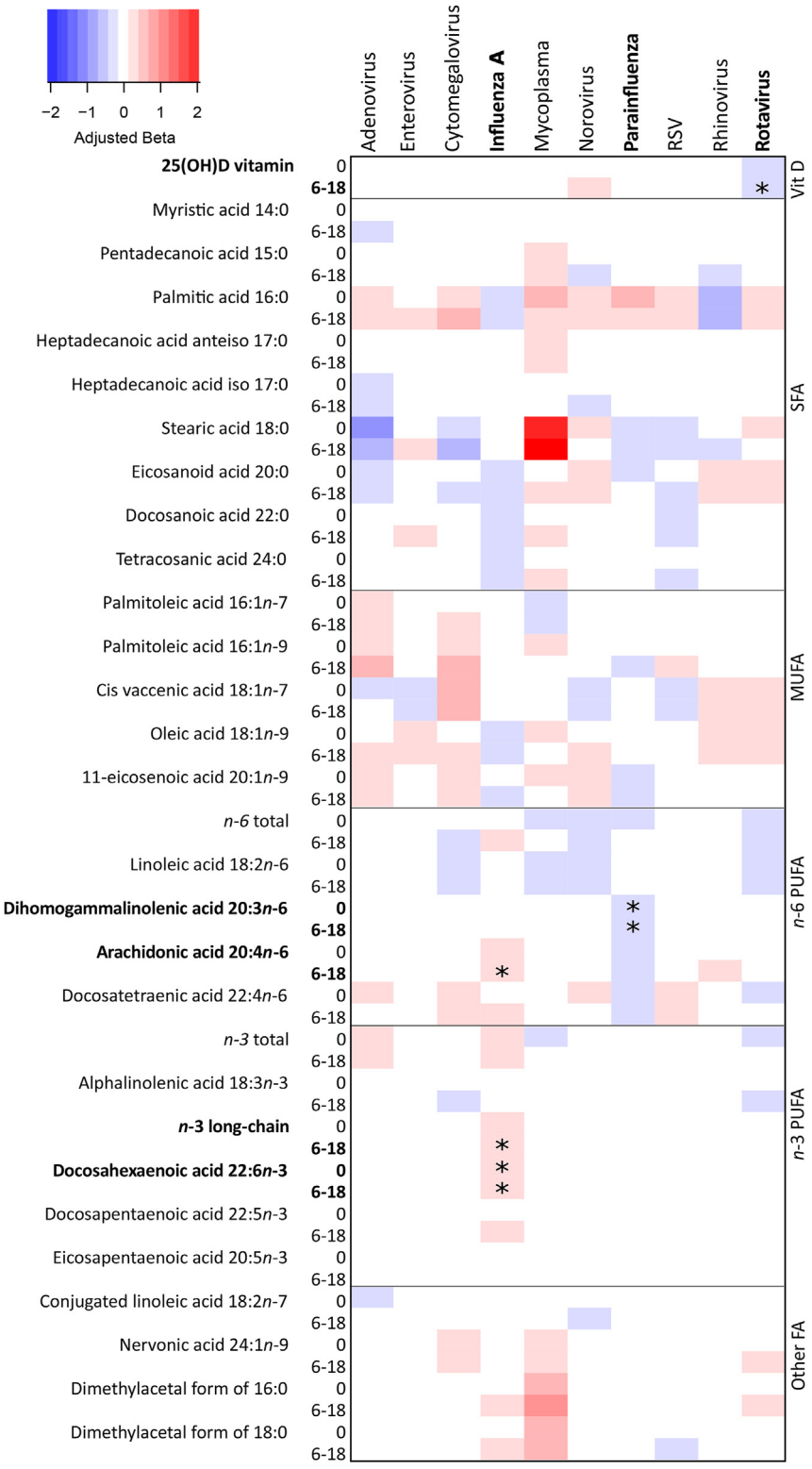

Fig. 4. Associations of serum 25(OH)D vitamin and fatty acid levels at birth (0 months) and at 6–18 months with microbe-specific IgG levels (EIU) assessed at 18 months among seropositive children (EIU levels ≥15 of the specific IgG, n in Table 2). Results indicate beta coefficients based on linear regression analyses adjusted for region, case-control status, and maternal type 1 diabetes. Red color indicates direct and blue color indirect association. *p < 0.01, **<0.001 RSV, respiratory syncytial virus; FA, fatty acid.

4.1. n-3 PUFA and other fatty acids

Our finding that higher *n*-3 PUFAs, and especially long-chain *n*-3 PUFAs were associated with decreased risk of some microbial infections is in line with previous studies that have included long-chain *n*-3 PUFA-supplementation or supplemented drinks and symptomatic or severe respiratory infections [4,5,7,8]. Our findings are also in agreement with a recent biobank study that suggested that higher plasma *n*-3 PUFAs and DHA were associated with decreased risk of SARS-CoV-2 infection [20]. In the present study, most consistent protective associations regarding *n*-3 PUFAs were seen for several *n*-3 PUFAs and risk of CVB2, and DPA at birth and risk of RSV.

The mechanisms of long-chain *n*-3 PUFAs in preventing or alleviating microbial infections have been suggested to include changes in immune cell signaling and function, inhibition of excessive inflammation, and inhibiting virus replication [21]. Long-chain *n*-3 PUFAs inhibited coxsackievirus replication in an *in vitro* study, and the inhibition may be related to intracellular membrane remodeling that takes place during enteroviral replication [22]. Thus, inhibition of virus replication could be one mechanism behind our observation related to *n*-3 PUFAs and CVB2 infections. A review based on animal studies suggested that the protective effect and the mechanisms of *n*-3PUFAs can be pathogen-specific [21] which could explain that we observed protective associations only for some viruses.

In unadjusted analyses, higher *n*-3 PUFA proportion was associated with increased risk of cytomegalovirus infection. However, that association seemed to be confounded by one or more factors included in the adjustment. Breastfeeding could explain this association too, as breastfeeding leads to higher serum *n*-3 PUFA proportions [11] and cytomegalovirus is known to transmit form mothers to infants by breastfeeding [23].

Our finding that *n*-3 PUFAs, and especially long-chain *n*-3 PUFAs were associated with higher levels of influenza A IgG among seropositive children is in agreement with the view that *n*-3 PUFAs can improve the immune response [21]. Similarly, we found that arachidonic acid was positively associated with influenza A IgG levels among influenza A seropositive children. There are not many studies to reflect this finding to, however, an intervention study in adult men showed that high vs. low arachidonic acid diet induced higher postimmunization peripheral blood mononuclear cell proliferation in response to influenza vaccine [24]. However, no effect on influenza antibody titers was observed in that context [25]. Again, fatty acid-contributed effect on immune response may be pathogen-specific [21].

We observed that higher DGLA proportion at birth was associated with a decreased risk of rotavirus infection by 18 months of age, and that DGLA proportion was inversely associated with parainfluenza IgG among seropositive children at 18 months of age. These are novel findings, and their meaning is unclear. Very little is known about DGLA and its derivates in microbial infections, however, this fatty acid may play a role in prostaglandin production and therefore affect the course of infection and immune response [26].

4.2. Vitamin D

Our finding that serum 25(OH)D concentration was not associated with risk of microbial infections in adjusted analyses, and that higher vs. lower 25(OH)D category was associated with higher number of CVB infections samples do not support the acknowledged protective effect of vitamin D on acute respiratory infections [3]. However, several factors could explain the negative finding in the present study in comparison to previous reports. First, we assessed seropositivity to microbes, which reflects both symptomatic and asymptomatic infections. Most previous studies have included symptomatic respiratory infections or severe infections [3]. In addition, studies that has shown the strongest protective effect of vitamin D supplementation on infection incidence have often included children with low vitamin D levels at baseline [27] and/or increased susceptibility to severe infections [27,28]. However, one observational study in children showed a protective association of 25(OH)D vitamin at level $\geq$75 nmol/l vs. <75 nmol/l with symptomatic laboratory confirmed upper respiratory tract infections [29] suggesting that vitamin D effect may not just be about avoiding deficiency. Thus, it is possible that the inclusion of asymptomatic infections and non-respiratory infections could partly explain that no protective association between 25(OH)D vitamin concentration and microbial infections was observed in the present study.

Our observation that higher 25(OH)D vitamin was associated with lower rotavirus IgG levels among rotavirus seropositive children is in line with findings from a Bangladeshi study that found an inverse association between serum vitamin D and rotavirus IgA [30]. In addition, in a study in pigs, vitamin D supplementation alleviated the immune response to rotavirus challenge [31]. The knowledge on the role of vitamin D in antibody response to infection or vaccination in children is limited, but the current evidence, based mainly on studies with influenza vaccinations, does not imply an increased antibody response by increased vitamin D [32–35]. However, the effects of vitamin D on course of infection might be pathogen specific and depend on vitamin D related genetics as well [36].

Conclusions

To conclude, this large prospective international study in children with increased susceptibility to type 1 diabetes suggests that early *n*-3 PUFA status could play a role in infection risk and immune response to infections. The role of vitamin D status was less clear in this study, but the overall findings suggest that nutrient effects on infections and antibody response to infections may be pathogen-specific. Research on this field is scarce and additional studies are needed to understand the role of nutrients, their mechanisms of action in the course of different infections, and to understand the role of nutrient–microbe interactions on the risk of chronic diseases such as type 1 diabetes.

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Author contributions

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Data sharing

Data described in the manuscript can be made available upon request pending application and approval by TRIGR central coordinating committee.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.10.017.

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