Antibody response to SARS-CoV-2 mRNA vaccination in Danish adults exposed to perfluoroalkyl substances (PFASs)

The ENFORCE study

Timmermann, Amalie; Johansen, Isik S; Tolstrup, Martin; Heilmann, Carsten; Budtz-Jørgensen, Esben; Tolstrup, Janne S; Nielsen, Flemming; Grandjean, Philippe

Published in:
Environmental Research

DOI:
10.1016/j.envres.2024.120039

Publication date:
2024

Document version:
Final published version

Environmental Research 263 (2024) 120039

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Antibody response to SARS-CoV-2 mRNA vaccination in Danish adults exposed to perfluoroalkyl substances (PFASs): The ENFORCE study

Amalie Timmermann [a,*], Isik S. Johansen [b,c], Martin Tolstrup [d], Carsten Heilmann [e], Esben Budtz-Jørgensen [f], Janne S. Tolstrup [a], Flemming Nielsen [g], Philippe Grandjean [g,h]

[a] National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark
[b] Department of Infectious Diseases, Odense University Hospital, Odense, Denmark
[c] Research Unit of Infectious Diseases, Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[d] Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[e] Department of Pediatrics and Adolescent Medicine, Pediatric Hematopoietic Stem Cell Transplantation and Immunodeficiency, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark
[f] Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark
[g] Research Unit of Clinical Pharmacology, Pharmacy and Environmental Medicine, University of Southern Denmark, Odense, Denmark
[h] Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, United States

ARTICLE INFO

Keywords:
PFAS
Immune response
Corona virus
SARS-CoV-2
COVID-19

ABSTRACT

Introduction: Per- and polyfluoroalkyl substances (PFASs) have immunotoxic effects in children while studies in adults, including recent studies on the SARS-CoV-2 vaccine response have been less consistent. In a cohort of 50–69-year-olds repeatedly vaccinated against COVID-19 in Denmark from early 2021, we aimed to assess the association between serum-PFAS concentrations and SARS-CoV-2 antibody responses.

Methods: We assessed serum-PFAS concentrations among 371 middle-aged adults from the National Cohort Study of Effectiveness and Safety of SARS-CoV-2 vaccines (ENFORCE) who had received their first vaccination against COVID-19. Following the second dose and the booster (third) Pfizer-BioNTech mRNA vaccination, we measured the specific spike IgG antibody response. Associations between serum-PFAS concentrations at inclusion and spike IgG antibody concentrations after vaccination were assessed using median regression, and analyses were adjusted for age, sex, presence of diabetes, number of vaccines received, and time since vaccination. We further examined the associations between serum-PFAS concentrations at inclusion and changes in spike IgG antibody concentration between the second dose and booster (third) vaccination.

Results: Serum-PFAS concentrations were not associated with spike IgG antibody concentrations after the SARS-CoV-2 vaccinations, but the increase in response after the booster (third) vaccination compared to after the second vaccination was consistently lower at higher serum-PFAS concentrations. Each doubling in the concentration of seven serum-PFASs was associated with a 802 BAU/mL lower median increase in spike IgG antibody response after the booster (third) vaccination (95% CI: −1812; 208) adjusted for confounders.

Discussion: As many adults were probably not immunological naïve prior to vaccination, our results were likely affected by individual variability in immune response to the vaccination. Despite this uncertainty, the diminished increase in SARS-CoV-2 spike antibody response after the booster (third) vaccination at higher PFAS exposure may potentially reflect an immunotoxic impact of the PFASs.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs), a group of persistent chemicals, have been continuously used in a variety of products since the 1950's (National Academies of Sciences, 2022). The chemicals are disseminated in the environment and due to their slow breakdown,

* Corresponding author. National Institute of Public Health, University of Southern Denmark, Studiestræde 6, 1455, København K. Denmark.
E-mail addresses: atimmermann@health.sdu.dk (A. Timmermann), Isik.Somuncu.Johansen@rsyd.dk (I.S. Johansen), marttols@rm.dk (M. Tolstrup), Carsten.Heilmann@regionh.dk (C. Heilmann), ebj@sund.ku.dk (E. Budtz-Jørgensen), jest@sdu.dk (J.S. Tolstrup), FNielsen@health.sdu.dk (F. Nielsen), PGrandjean@health.sdu.dk (P. Grandjean).

https://doi.org/10.1016/j.envres.2024.120039
Received 27 June 2024; Received in revised form 8 September 2024; Accepted 18 September 2024
Available online 24 September 2024

PFASs are now found in oceans, surface waters, soil, and rainwater (Cousins et al., 2022) as well as in fish, wildlife (The Environmental Working Group (EWG), 2023), and in humans across the planet (ATSDR, 2021; Sunderland et al., 2019; Uhl et al., 2023). PFASs are still used in multiple applications, including oil- and water-repellent textiles, food packaging, microelectronics, and firefighting foam (ATSDR, 2021).

Within the last decade, PFASs have been associated with numerous adverse health effects including immunotoxic effects, as reflected by increased risk of childhood infection and reduced antibody response after routine childhood vaccinations (ATSDR, 2021; Crawford et al., 2023; Zhang et al., 2022). Antibody response after vaccination is a sensitive marker of immune function, and using this outcome, we have observed immunotoxic effects of PFASs among children with elevated exposures (Grandjean et al., 2012, 2017; Timmermann et al., 2022) as well as within background levels (Timmermann et al., 2020). In adults, the associations have been less consistent, but higher serum-PFOA concentrations were associated with reduced response after vaccination against influenza (Looker et al., 2014), hepatitis (Shih et al., 2021), and tetanus-diphtheria (Kielsen et al., 2016). Overall, decreased immune responses have been shown both for toxoid vaccines and live attenuated vaccines.

Following the COVID-19 pandemic, SARS-CoV-2 mRNA vaccination programs were rolled out across the world, and robust antibody responses to these vaccines were key in reducing disease severity, preventing hospitalization, and curbing community transmission. While factors such as age, sex, and underlying health conditions have been studied extensively, research on the potential impact of environmental factors, such as PFAS exposure, on the SARS-CoV-2 mRNA vaccine response remain limited. To our knowledge, four studies have examined the association between PFAS exposure and SARS-CoV-2 mRNA vaccine response, but findings were not consistent across studies (Andersson et al., 2023; Bailey et al., 2023; Hollister et al., 2023; Porter et al., 2022). The aim of the present study was to examine if serum-PFAS concentrations were associated with reduced responses to repeated SARS-CoV-2 mRNA vaccines among adults in Denmark.

2. Material and methods

The National Cohort Study of Effectiveness and Safety of SARS-CoV-2 vaccines (ENFORCE) is a clinical study designed to evaluate the effect and safety of vaccines against coronavirus among Danish adults (Staerke et al., 2022). Adults with a scheduled appointment for vaccination, were invited through a letter sent via the vaccination centres. One research interest for the original ENFORCE study was to investigate the vaccine effect in persons at increased risk of SARS-CoV-2 exposure and those at increased risk of a serious course of infection. Hence, invites were sent to healthcare workers and selected risk groups including cancer patients, patients with immunodeficiencies, and patients with other underlying disease (Staerke et al., 2022).

Between February 3rd and August 5th, 2021, 6,943 adults from all five regions of Denmark were included from 14 days to 30 min before they received their first dose of a SARS-CoV-2 vaccine. Data on age, sex, and recent medical history (in the past year) were obtained at inclusion, and uses of medication (in the past 24 h) were obtained at each visit (Staerke et al., 2022). Blood samples were obtained at inclusion and at follow-up visits scheduled after each vaccination (Figure S1).

For the present study, we selected 477 participants aged 50–69 years who had been given the Pfizer-BioNTech mRNA vaccine BNT162n2 (Pfizer, New York, USA; BioNTech SE, Mainz, Germany), had a blood sample obtained at the first follow-up (before the second vaccination), and had antibodies assessed after vaccination.

A large proportion of the ENFORCE participants were recruited among patients. In addition, BNT162b2 was the first vaccine to be used when the Danish national vaccine campaign was initiated. The most vulnerable groups including older people and people with chronic illnesses were the first to be vaccinated, and some enrolled participants may therefore have a relevant underlying disease or immune dysfunction.

For the present study, we excluded 80 participants who had HIV, other immunosuppressive disorders or active or treated malignancy at baseline. Antibody responses in these individuals are likely highly affected by their disease with substantial interindividual variability that would be difficult to account for in statistical models. Including these individuals would thus limit our ability to detect possible associations with contaminants. We likewise excluded participants who received potentially immunosuppressive treatment 24 h before the baseline visit or before one of the two first follow-up visits planned 0–5 days prior to receiving the second vaccine dose and 3 months after receiving the first vaccine dose (Figure S2).

2.1. PFAS assessment

PFAS concentrations in serum obtained immediately prior to participants receiving the second vaccine dose were analyzed at the Environmental Medicine laboratory, University of Southern Denmark using on-line solid phase extraction followed by liquid chromatography and triple quadropole mass spectrometry as previously described (Haug et al., 2009; Nielsen et al., 2024). Excess serum sample material from the European Human Biomonitoring Initiative (HBM4EU), organized by the German Environment Agency; NIST 1957 as well as in-house made quality control samples were included in the sample series for quality control. The between-batch imprecision for all compounds in the series of samples was <7.5%, and the bias ranged from – 8.4 to 12.2%. The limit of detection (LOD) was 0.03 ng/mL for all components, and values below the LOD were replaced by LOD/2. The accuracy of the utilized PFAS analysis is continuously secured by regular participation in the German Quality Assessment program (G-EQUAS) organized by the German Society of Occupational Medicine. The samples were screened for perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPeS), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonic acid (PFHxS), perfluoroheptanoic acid (PFHpA), perfluoroheptane sulfonic acid (PFHpS), perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorononane sulfonate (PFNS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), N-methylperfluorooctanesulfonamidoacetic acid (N-MeFOSAA) and N-ethyl perfluorooctane sulfonamido acetic acid (N-EtFOSAA). Only PFASs with concentrations above the limit of detection in at least 50% of the individuals were included for statistical analysis.

2.2. Humoral immune response following SARS-CoV-2 mRNA vaccination

Spike receptor binding domain, full spike and nucleocapsid directed IgG and ACE-2 competition were quantified in plasma using a Multi-antigen Serology Assay (Meso Scale Diagnostics, Maryland, USA) at the Research Laboratory at the Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark (Staerke et al., 2022). In the present study, we relied on full spike IgG antibodies obtained after second vaccination and after booster (third) vaccination.

Individuals that have been SARS-CoV-2 infected have a different response to the vaccine than those that have not previously met the virus, and their response will depend on timing and severity of the infection. Including previously infected individuals would thus add substantial imprecision to the analyses. In Denmark, SARS-CoV-2 tests were free of charge and publicly available from April 2020. All test results were registered in national databases and linked to the study participants using the Danish personal identification number (CPR). We excluded participants who had tested positive for SARS-CoV-2 using a polymerase chain reaction (PCR)-test or antigen test at least once prior to blood sampling. If participants had been tested with both a PCR-test and an antigen test on the same day and the test results did not match,

we relied on the result from the PCR-test. Few (<3) subjects tested positive before the blood sampling performed after the second vaccine dose and were thus excluded from all analyses. Further 56 subjects tested positive before the blood sampling performed after the booster (third) vaccination and were thus excluded from analyses of antibody concentrations after the booster vaccination (Figure S2).

2.3. Statistics

A joint PFAS measure (∑PFAS) was calculated as the sum of PFASs detected in at least 50% of the samples (PFHxS, PFHpS, PFOS, PFOA, PFNA, PFDA, and PFUnDA). Individual serum-PFAS, ∑PFAS, and antibody concentration distributions (medians, 5th, and 95th percentiles) were calculated by age at enrolment, sex, and diabetes in the past year. Antibody concentration distributions were additionally calculated by time between vaccination and blood sampling, and we calculated median, minimum, and maximum number of days between vaccination and blood sampling.

Associations between serum-PFAS concentrations (individual and ∑PFAS) at inclusion and spike IgG antibodies after vaccination were assessed using median regression with clusters for the same persons being included in the analyses after both the second and the booster (third) vaccination (Parente and Silva, 2016). Serum-PFAS concentrations were log-2-transformed to avoid high serum-PFAS concentrations being overly influential, and regression estimates thus expressed differences in median IgG for each doubling in serum-PFAS concentrations. The models assumed that covariates had the same effect on IgG concentrations after the second vaccination and the booster (third) vaccination. We tested this assumption by including interaction terms between serum-PFAS concentrations and number of vaccinations, and we performed sensitivity analyses with separate median regression analyses for antibody responses after the second vaccination and the booster (third) vaccination.

The IgG concentration was strongly correlated to the number of vaccinations and time since last vaccination, and all regression models were thus adjusted for number of vaccines received and number of days since last vaccination. We further performed sensitivity analyses excluding nine individuals vaccinated more than 100 days prior to blood sampling.

Age and sex can affect serum-PFAS concentrations and also the antibody response to vaccination, thus acting as confounders. Diabetes might also affect the antibody response, and a diabetic diet could affect PFAS exposure or PFAS exposure could affect the risk of diabetes (Khoury et al., 2024; Roth and Petriello, 2022; Valvi et al., 2021). Diabetes might thus either act as a confounder or mediate the association by mechanisms not relevant for the present study. In addition to performing basic analyses adjusted for number of vaccines received and number of days since last vaccination, we therefore additionally included age, sex, and the presence of a diabetes diagnosis in adjusted models.

Although we excluded individuals who had tested positive for SARS-CoV-2 prior to blood sampling, some individuals could have been exposed to the virus but not tested or diagnosed, and underlying disease might also affect the antibody response. To account for individual variability in antibody response, we applied a difference-in-difference approach and conducted median regression analyses for the associations between serum-PFAS concentrations at inclusion and changes in spike IgG antibody concentration between the second and booster (third) vaccination. All analyses were conducted using Stata version BE18.

3. Results

In this population consisting of middle-aged adults in Denmark, who had received their first vaccination against COVID-19, PFHxS, PFOS, PFOA, PFNA, and PFDA were detected in all 371 serum samples, while PFHpS and PFUnDA were below the LOD in 10 and 15 samples, respectively (Table 1). Median PFAS concentrations ranged from 0.11 ng/mL (PFUnDA) to 5.32 ng/mL (PFOS), and four individuals had serum-PFOS concentrations above 20 ng/mL.

Older participants had higher median serum concentrations of most PFASs (Table 1). Compared to women, men had higher median serum concentrations of most PFASs, although median serum-PFDA concentrations were similar across sex, and PFUnDA concentrations were slightly higher among women. No association was observed between serum-PFAS concentrations and having a diabetes diagnosis (Table 1).

The median time between vaccination and blood sampling for antibody assessment was 64 days (range 36–200 days) after the second vaccination and 29 days (range 16–166 days) after the booster (third) vaccination. Spike IgG antibody concentrations were higher after the booster (third) vaccination than after the second vaccination, and men, older participants, and those with diabetes tended to have lower spike IgG antibody concentrations after vaccination (Table 2).

In basic analyses with no confounder adjustment, higher serum concentrations of PFHxS, PFHpS, PFOS, PFOA, PFNA, PFDA and ΣPFAS were associated with slightly lower median spike IgG antibody response after vaccination, but confidence intervals were wide, and the associations were diminished or even reversed after adjustment for potential confounding factors (Table 3). Excluding individuals vaccinated more than 100 days prior to blood sampling did not materially change the results (Table S1). No significant interactions were found between serum-PFAS concentrations and the number of vaccines received. However, when examining the effects of PFASs on spike IgG antibody concentrations after the second vaccination and booster (third) vaccination separately, higher serum-PFAS concentrations were found to be weakly associated with higher median spike IgG antibody response after receiving the second vaccine dose. Each doubling in serum-∑PFAS concentration was thus associated with a 436 BAU/mL higher median spike IgG antibody response after the second vaccination (95% CI: −669; 1541, p = 0.44) after adjustment for potential confounders. In contrast, higher serum-PFAS concentrations were weakly associated with a lower median spike IgG antibody responses after the booster (third) vaccination. Each doubling in serum-∑PFAS concentration was thus associated with a 77 BAU/mL lower median spike IgG antibody response after the booster vaccination (95% CI: −486; 332, p = 0.71) after adjustment for potential confounders. However, the associations were not statistically significant for any of the PFASs (Table S2).

When examining the change in spike IgG antibody concentration from the second vaccination to the booster (third) vaccination, higher serum-concentrations of all PFASs were associated with a decreased increase in spike IgG antibody response following the booster (third) vaccination compared to the response following the second vaccination, but the associations did not reach statistical significance (Table 3). Each doubling in serum-∑PFAS concentration was thus associated with a 802 BAU/mL lower median increase in spike IgG antibody response after the booster (third) vaccination compared to the response following the second vaccination (95% CI: −1812; 208, p = 0.12) after adjustment for potential confounders (Table 3).

4. Discussion

In this study of 371 middle-aged adults in Denmark who had received their first vaccination against COVID-19 and had not previously been affected with SARS-CoV-2 virus, we did not observe consistent associations between serum-PFAS concentrations and total SARS-CoV-2 spike antibody responses after the second and booster (third) vaccination. Nevertheless, the increase in SARS-CoV-2 spike IgG antibody response following the booster (third) vaccination compared to the response following the second vaccination was consistently lower at higher serum-PFAS concentrations. Although the observed trend was not statistically significant, this tendency may reflect a PFAS-linked weakness of the immune response.

In accordance with our initial findings, three other recent studies did

Table 1
Serum-PFAS concentrations by study sample characteristics.

		PFAS (ng/mL), median (5th-95th percentile)							
	n (%)	PFHxS	PFHpS	PFOS	PFOA	PFNA	PFDA	PFUnDA	∑PFAS
Total	371 (100%)	0.71 (0.26–1.69)	0.15 (0.05–0.39)	5.32 (1.85–12.32)	1.08 (0.43–2.56)	0.54 (0.22–1.18)	0.18 (0.07–0.40)	0.11 (0.03–0.31)	8.25 (3.26–17.71)
n < LOD (%)		0 (0%)	10 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (4%)	0 (0%)
Sex									
Men	180 (49%)	0.98 (0.37–1.89)	0.23 (0.09–0.48)	6.98 (2.49–14.00)	1.32 (0.48–2.99)	0.56 (0.26–1.20)	0.18 (0.07–0.39)	0.10 (0.02–0.29)	10.86 (4.64–19.15)
Women	191 (51%)	0.53 (0.20–1.12)	0.11 (0.03–0.28)	4.75 (1.74–10.57)	0.93 (0.43–2.01)	0.52 (0.22–1.15)	0.18 (0.07–0.41)	0.13 (0.03–0.33)	7.09 (3.03–14.43)
Age (years)									
50–54	129 (35%)	0.65 (0.20–1.39)	0.12 (0.01–0.32)	5.03 (1.78–10.87)	0.95 (0.43–1.91)	0.52 (0.21–0.94)	0.18 (0.06–0.33)	0.11 (0.01–0.28)	7.73 (2.86–15.34)
55–59	106 (29%)	0.67 (0.29–1.72)	0.14 (0.06–0.39)	5.23 (2.03–13.51)	1.00 (0.46–2.76)	0.52 (0.25–1.13)	0.17 (0.08–0.40)	0.11 (0.04–0.31)	7.94 (4.11–17.96)
60–64	56 (15%)	0.79 (0.31–1.96)	0.18 (0.06–0.57)	5.82 (1.74–15.77)	1.21 (0.43–2.56)	0.54 (0.29–1.30)	0.20 (0.07–0.41)	0.12 (0.01–0.32)	8.59 (3.26–22.51)
65–69	80 (22%)	0.83 (0.26–1.80)	0.19 (0.06–0.42)	6.57 (2.42–14.82)	1.32 (0.56–2.98)	0.64 (0.26–1.32)	0.20 (0.08–0.43)	0.11 (0.03–0.33)	9.66 (3.95–21.26)
Diabetes diagnosis									
No	338 (91%)	0.71 (0.26–1.65)	0.15 (0.05–0.39)	5.34 (1.79–12.38)	1.07 (0.43–2.75)	0.54 (0.22–1.18)	0.18 (0.07–0.41)	0.11 (0.03–0.31)	8.26 (3.26–17.80)
Yes	33 (9%)	0.89 (0.31–1.99)	0.20 (0.07–0.50)	5.26 (1.86–11.22)	1.21 (0.44–2.35)	0.46 (0.18–1.23)	0.16 (0.06–0.27)	0.09 (0.01–0.19)	7.65 (2.86–14.83)

Table 2
SARS-CoV-2spike IgG antibody concentration after vaccination by study sample characteristics.

	After 2nd vaccination		After 3rd vaccination	
	n(%)	Median spike IgG antibody concentration, BAU/mL (5th-95th percentile)	n(%)	Median spike IgG antibody concentration, BAU/mL (5th-95th percentile)
Total	371 (100%)	5,527 (1,022–16,123)	284 (100%)	16,075 (4,494–18,072)
Sex				
Men	180 (49%)	4,564 (792-15,023)	139 (49%)	15,961 (3,393–17,954)
Women	191 (51%)	5,933 (1,319–16,701)	145 (51%)	16,188 (6,581–18,149)
Age (years)				
50–54	129 (35%)	7,169 (1,755–16,583)	96 (34%)	16,181 (2,630–18,077)
55–59	106 (29%)	6,017 (995-15,402)	82 (29%)	16,119 (4,749–18,147)
60–64	56 (15%)	4,355 (585-14,953)	37 (13%)	16,132 (8,524–17,954)
65–69	80 (22%)	4,388 (925-17,040)	69 (24%)	15,922 (4,336–18,070)
Diabetes diagnosis				
No	338 (91%)	5,662 (1,041–16,263)	257 (90%)	16,143 (4,336–18,107)
Yes	33 (9%)	4,413 (878-15,062)	27 (10%)	15,510 (8,524–17,866)
Days since vaccination				
16–30	0 (0%)	–	146 (51%)	16,236 (6,717–18,147)
30–59	121 (33%)	9,447 (1,960–16,673)	100 (35%)	16,067 (5,782–17,847)
60–74	199 (54%)	4,429 (1,007–15,543)	0 (0%)	–
75–200	51 (14%)	3,793 (542-14,200)	38 (13%)	14,941 (2,165–18,260)

Table 3
Difference in median SARS-CoV-2spike IgG antibody concentration (BAU/mL) and SARS-CoV-2spike IgG antibody concentration change between the second and third vaccination with each doubling in serum-PFAS concentrations.

	Difference in median spike IgG antibody concentration, BAU/mL (95% CI) N = 655, clusters = 371[a]	Difference in spike IgG antibody concentration change between 2nd and 3rd vaccination, BAU/mL (95% CI) N = 284[b]
PFHxS		
Basic	−236 (−555; 82)	−379 (−1315; 557)
Adjusted[c]	−123 (−492; 246)	−774 (−1783; 235)
PFHpS		
Basic	−153 (−432; 126)	−486 (−1287; 314)
Adjusted[c]	69 (−308; 445)	−726 (−1595; 143)
PFOS		
Basic	−130 (−445; 185)	−564 (−1426; 297)
Adjusted[c]	47 (−303; 397)	−777 (−1677; 123)
PFOA		
Basic	−67 (−420; 287)	−524 (−1513; 466)
Adjusted[c]	122 (−279; 524)	−713 (−1765; 340)
PFNA		
Basic	−87 (−503; 329)	−692 (−1773; 389)
Adjusted[c]	93 (−307; 493)	−790 (−1872; 293)
PFDA		
Basic	−38 (−443; 368)	−671 (−1733; 390)
Adjusted[c]	77 (−289; 444)	−759 (−1764; 247)
PFUnDA		
Basic	93 (−209; 395)	−517 (−1291; 256)
Adjusted[c]	63 (−216; 342)	−656 (−1412; 99)
∑PFAS		
Basic	−161 (−522; 200)	−601 (−1573; 372)
Adjusted[c]	47 (−354; 448)	−802 (−1812; 208)

[a] Adjusted for number of vaccines received and days since last vaccination.
[b] Adjusted for time from booster vaccination 1 to blood sampling, time from booster vaccination 2 to blood sampling, and time between the two blood samplings.
[c] Additionally adjusted for age at enrolment, sex, and diabetes diagnosis.

not find clear associations between PFAS exposure and immune response following COVID-19 vaccination among essential workers in United States (Hollister et al., 2023), and populations in America (Bailey et al., 2023) and Sweden (Andersson et al., 2023) highly exposed to PFAS through drinking water. Of note, Sweden instituted few barriers to virus transmission and may therefore have achieved some degree of herd immunity (Vogel, 2020) that may have affected the subsequent vaccine response. Still, it is possible that mRNA-based vaccines such as the COVID-19 vaccine depend on immune mechanisms that are less affected

by PFAS exposure.

However, our findings of slightly diminished increase in CoV-2 spike IgG antibody response after the booster (third) vaccination could indicate that increased PFAS exposure may dampen or inhibit the immune response. This finding is consistent with the associations observed between higher serum-PFAS concentrations and lower IgG antibody concentrations after one or two coronavirus vaccinations among current and former employees at the 3M PFAS production plant in the United States (Porter et al., 2022). However, the associations observed in the study of 3M employees were more pronounced than those observed in our study of lower exposure levels and smaller ranges of PFAS concentrations. Such potential immunotoxicity may be related to PFASs reducing activation of T-cells (Maddalon et al., 2023), which plays a pivotal role in the immune response.

One limitation of our study is the lack of information about the participants' socioeconomic status (SES), which could potentially affect the results. PFAS exposure might vary with SES (Buekers et al., 2018), and SES could also indirectly affect response to COVID-19 vaccine. The strengths of our study include a prospective design, with repeated blood sampling for SARS-CoV-2 serology. Further, we had detailed information about time from vaccination to blood sampling. All participants in our study received the same dose and same type of vaccine, thus providing some standardization in triggering the antibody response. By using the Danish national data on SARS-CoV-2 testing, we were able to account for changes in antibody concentrations due to individuals having been recently infected with SARS-CoV-2 virus. However, some individuals might have been infected but not tested or diagnosed. Further, the possible presence of cross-reactive T cells might contribute to a more rapid and effective immune response upon exposure to SARS-CoV-2 (Karlsson et al., 2020), potentially also resulting in improved antibody responses that were not accounted for in our analyses.

A major limitation in this study compared to studies on vaccine responses in children is the greater variability in initial immune response among adults, as they are likely not immunological naïve and may have differed in their ability to respond to the vaccine. The potentially higher individual variability in adults might explain why studies in adults generally do not show the same clear pattern of PFAS immunotoxicity as studies in children.

There is no plausible biological explanation for PFAS not affecting the vaccine response but affecting only the change in vaccine response between the second and booster (third) vaccination in our study. However, when analyzing associations between serum-PFAS concentrations and changes in spike IgG antibody concentration between the second and booster (third) vaccination, we took some of the individual variability into account by applying a difference-in-difference approach. The difference-in-difference approach minimizes bias from individual variability, that could have masked the associations in the other analyses. The ability to account for some of the individual variability may thus explain why these analyses demonstrated more pronounced associations with immunotoxicant exposures. Still, the individual variability in immune response introduces substantial imprecision, and as our study sample size is relatively small, our results are subject to wide confidence intervals.

Based on the results from our studies and other similar studies, it is not clear if PFAS affects the adult immune system. However given the large individual variability in immune responses among adults, we suggest that more studies use difference-in-difference approaches when assessing antibody responses in adults.

5. Conclusions

Among middle-aged adults in Denmark with no previous SARS-CoV-2 virus infection, we found no clear association between serum-PFAS concentrations and SARS-CoV-2 mRNA vaccine response. Nevertheless, increased serum-PFAS concentrations were weakly associated with a diminished subsequent response after the booster (third) vaccination. This finding suggests that PFAS exposure may potentially influence the immune system response to the COVID-19 vaccine.

Funding

Funding for the ENFORCE study was granted by the Danish Ministry of Health (legal deeds 150 28/1 2021 and 263 3/6 2021). PG is supported by the National Institute of Environmental Health Sciences (P42ES027706).

Ethics

The ENFORCE study was approved by the Danish Medicines Agency (no. 2020-006003-42) and the National Committee on Health Research Ethics (no. 1-10-72-337-20). All participants gave written informed consent to participate in the study. Re-use of blood samples for PFAS analyses in the present study were approved by the scientific ethical committee of the Region of Southern Denmark (no. S-20220029).

CRediT authorship contribution statement

Amalie Timmermann: Writing – original draft, Methodology, Formal analysis. **Isik S. Johansen:** Writing – review & editing, Resources, Methodology. **Martin Tolstrup:** Writing – review & editing, Methodology, Investigation. **Carsten Heilmann:** Writing – review & editing, Methodology. **Esben Budtz-Jørgensen:** Writing – review & editing, Methodology. **Janne S. Tolstrup:** Writing – review & editing, Methodology. **Flemming Nielsen:** Writing – review & editing, Methodology, Investigation. **Philippe Grandjean:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Philippe Grandjean reports financial support was provided by the Danish Ministry of Health and by the National Institute of Environmental Health Sciences. Philippe Grandjean has served as a health expert in lawsuits on environmental PFAS exposures that includes: paid expert testimony. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgements

The authors thank all members of the ENFORCE study group for their contribution to the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.120039.

References

Andersson, A.G., et al., 2023. High exposure to perfluoroalkyl substances and antibody responses to SARS-CoV-2 mRNA vaccine-an observational study in adults from ronneby, Sweden. Environ. Health Perspect. 131, 87007.

ATSDR, 2021. Toxicological Profile for Perfluoroalkyls. Agency for Toxic Substances and Disease Registry (ATSDR).

Bailey, J.M., et al., 2023. Immune response to COVID-19 vaccination in a population with a history of elevated exposure to per- and polyfluoroalkyl substances (PFAS) through drinking water. J. Expo. Sci. Environ. Epidemiol. 33, 725–736.
Buekers, J., et al., 2018. Socio-economic status and health: evaluation of human biomonitored chemical exposure to per- and polyfluorinated substances across status. Int J Environ Res Public Health 15.
Cousins, I.T., et al., 2022. Outside the safe operating space of a New planetary boundary for per- and polyfluoroalkyl substances (PFAS). Environ. Sci. Technol. 56, 11172–11179.
Crawford, L., et al., 2023. Systematic review and meta-analysis of epidemiologic data on vaccine response in relation to exposure to five principal perfluoroalkyl substances. Environ. Int. 172, 107734.
Grandjean, P., et al., 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 307, 391–397.
Grandjean, P., et al., 2017. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J Immunotoxicol 14, 188–195.
Haug, L.S., et al., 2009. A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring. J. Chromatogr. A 1216, 385–393.
Hollister, J., et al., 2023. Serum per- and polyfluoroalkyl substance concentrations and longitudinal change in post-infection and post-vaccination SARS-CoV-2 antibodies. Environ. Res. 239, 117297.
Karlsson, A.C., et al., 2020. The known unknowns of T cell immunity to COVID-19. Sci Immunol. 5.
Khoury, N., et al., 2024. Dietary intake of Perfluorooctanesulfonic acid (PFOS) and glucose homeostasis parameters in a non-diabetic senior population. Environ. Int. 186, 108565.
Kielsen, K., et al., 2016. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. J Immunotoxicol 13, 270–273.
Looker, C., et al., 2014. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol. Sci. 138, 76–88.
Maddalon, A., et al., 2023. Mixtures of per- and poly-fluoroalkyl substances (PFAS) reduce the in vitro activation of human T cells and basophils. Chemosphere 336, 139204.
National Academies of Sciences, Engineering, and Medicine, 2022. Guidance on PFAS Exposure, Testing, and Clinical Follow-Up. National Academies Press, Wahington, DC.
Nielsen, F., et al., 2024. Occurrence of major perfluorinated alkylate substances in human blood and target organs. Environ. Sci. Technol. 58, 143–149.
Parente, P.M.D.C., Silva, J.M.C.S., 2016. Quantile regression with clustered data. J. Econom. Methods 5, 1–15.
Porter, A.K., et al., 2022. Antibody response to COVID-19 vaccines among workers with a wide range of exposure to per- and polyfluoroalkyl substances. Environ. Int. 169, 107537.
Roth, K., Petriello, M.C., 2022. Exposure to per- and polyfluoroalkyl substances (PFAS) and type 2 diabetes risk. Front. Endocrinol. 13, 965384.
Shih, Y.H., et al., 2021. Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: a birth cohort in the Faroe Islands. J Immunotoxicol 18, 85–92.
Staerke, N.B., et al., 2022. Cohort profile:the Danish national cohort study of effectiveness and safety of SARS-CoV-2 vaccines (ENFORCE). BMJ Open 12, e069065.
Sunderland, E.M., et al., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Expo. Sci. Environ. Epidemiol. 29, 131–147.
The Environmental Working Group (EWG), 2023. 'Forever Chemicals' Found in Wildlife Around the World, vol. 2023. https://www.ewg.org/interactive-maps/pfas_in_wildlife/map/.
Timmermann, C.A.G., et al., 2020. Serum perfluoroalkyl substances, vaccine responses, and morbidity in a cohort of Guinea-bissau children. Environ. Health Perspect. 128, 87002.
Timmermann, C.A.G., et al., 2022. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7-12 years exposed to marine pollutants, a cross sectional study. Environ. Res. 203, 111712.
Uhl, M., et al., 2023. PFASs: what can we learn from the European Human Biomonitoring Initiative HBM4EU. Int. J. Hyg Environ. Health 250, 114168.
Valvi, D., et al., 2021. Life-course exposure to perfluoroalkyl substances in relation to markers of glucose homeostasis in early adulthood. J. Clin. Endocrinol. Metab. 106, 2495–2504.
Vogel, G., 2020. Sweden's gamble. Science 370, 159–163.
Zhang, X., et al., 2022. Effects of exposure to per- and polyfluoroalkyl substances on vaccine antibodies: a systematic review and meta-analysis based on epidemiological studies. Environ Pollut 306, 119442.