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Liver and cardiometabolic markers and conditions in a cross-sectional study of three Australian communities living with environmental per- and polyfluoroalkyl substances contamination

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ABSTRACT

Background: Per- and polyfluoroalkyl substances (PFAS) have been associated with higher cholesterol and liver function markers in some studies, but the evidence for specific cardiometabolic conditions has been inconclusive.

Objectives: We quantified the associations of single and combined PFAS with cardiometabolic markers and conditions in a cross-sectional study of three Australian communities with PFAS-contaminated water from the historical use of aqueous film-forming foam in firefighting activities, and three comparison communities.

Methods: Participants gave blood samples for measurement of nine PFAS, four lipids, six liver function markers, and completed a survey on sociodemographic characteristics and eight cardiometabolic conditions. We estimated differences in mean biomarker concentrations per doubling in single PFAS concentrations (linear regression) and per interquartile range increase in the PFAS mixture (Bayesian kernel machine regression). We estimated prevalence ratios of biomarker concentrations outside reference limits and self-reported cardiometabolic conditions (Poisson regression).

Results: We recruited 881 adults in exposed communities and 801 in comparison communities. We observed higher mean total cholesterol with higher single and mixture PFAS concentrations in blood serum (e.g., 0.18 mmol/L, 95% credible interval −0.06 to 0.42, higher total cholesterol concentrations with an interquartile range increase in all PFAS concentrations in Williamtown, New South Wales), with varying certainty across communities and PFAS. There was less consistency in direction of associations for liver function markers. Serum perfluorooctanoic acid (PFOA) concentrations were positively associated with the prevalence of self-reported hypercholesterolemia in one of three communities, but PFAS concentrations were not associated with self-reported type II diabetes, liver disease, or cardiovascular disease.

Discussion: Our study is one of few that has simultaneously quantified the associations of blood PFAS concentrations with multiple biomarkers and cardiometabolic conditions in multiple communities. Our findings for

Abbreviations: AFFF, aqueous film-forming foam; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PFAS, per- and polyfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFDA, perfluorodecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PPARα, proliferator-activated receptor alpha; PPARγ, proliferator-activated receptor gamma; 6:2 FTS, 6:2 fluorotelomer sulfonic acid.

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total cholesterol were consistent with previous studies; however, substantial uncertainty in our estimates and the cross-sectional design limit causal inference.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic fluorinated chemicals with broad application in industry and consumer products because of their chemical stability and hydro- and lipophobic properties (Qi et al., 2020; Armstrong and Guo, 2019; Buck et al., 2011). They are commonly detected in humans because of their high resistance to degradation in the environment, ability to biomagnify in the food chain, and long biological half-lives, estimated to be 2–6 years for perfluorooctane sulfonic acid (PFOS) and 2–3 years for perfluorooctanoic acid (PFOA) (Qi et al., 2020; Li et al., 2018, 2022; Xu et al., 2020). PFAS are not metabolised in the human liver but bind to serum proteins, accumulating in tissues (e.g., liver tissue and bone for PFOA and PFOS), with eventual elimination in urine and bile, along with menstruation, placental transfer, and breast milk in women of reproductive age (Brase et al., 2021; Pérez et al., 2013).

Humans are exposed to different mixtures of PFAS, depending on their pattern of consumer product use and proximity to pollutant point sources. In Australia, the water supplies in some communities located near military bases have been contaminated by PFAS from the historical use of aqueous film-forming foam (AFFF) to extinguish aviation fires. We reported that people in these communities have higher mean serum concentrations of PFOS and perfluorohexane sulfonic acid (PFHxS), compared to people in communities without local environmental PFAS contamination (Smurthwaite et al., 2021). This is of concern because PFAS exposure may play a role in the development and progression of non-alcoholic fatty liver disease and cardiovascular disease (Armstrong and Guo, 2019; Meneguzzi et al., 2021; Costello et al., 2022), and environmental factors are thought to be significant contributors to the increasing global prevalence of these health conditions (Bhatnagar, 2006, 2017; Münzel et al., 2021).

Animal studies have shown that PFAS are hepatotoxic and can activate the peroxisome proliferator-activated receptor (PPAR) group of nuclear receptors, which regulate metabolism and energy homeostasis (Brase et al., 2021; Costello et al., 2022; Ojo et al., 2021). Peroxisome proliferator-activated receptor alpha (PPARα) activation is believed to be the primary mechanism for PFAS effects on lipids and the liver in rodents (Costello et al., 2022; Andersen et al., 2021; Das et al., 2017). While cell-culture studies suggest that PFAS also activates human PPARα, it is activated to a lesser extent than rodent PPARα (Costello et al., 2022; Andersen et al., 2021; Maloney and Waxman, 1999; Nakamura et al., 2009; Rosen et al., 2013; Filgo et al., 2014). PFAS have also been observed to affect lipids in PPARα-null mice, though the role of other nuclear receptors (e.g., PPARγ and pregnane X) is less well understood (Costello et al., 2022; Andersen et al., 2021; Das et al., 2017; Bijland et al., 2011). Higher PFAS levels have been associated with hepatomegaly, fatty liver, and markers of liver injury in rodents, and increased serum cholesterol in mice with humanised-PPARα (Costello et al., 2022; Andersen et al., 2021; Das et al., 2017; Wan et al., 2012). In addition, experimental studies exposing platelets to PFAS suggest that PFAS may directly activate platelets to promote thrombus formation (Meneguzzi et al., 2021).

In epidemiological studies, PFAS have been widely associated with elevated serum total- and low density lipoprotein (LDL) cholesterol, which are established risk factors for cardiovascular disease and type II diabetes, and elevated liver enzymes, such as alanine aminotransferase (ALT), which is a marker for non-alcoholic fatty liver disease (Costello et al., 2022; Andersen et al., 2021; Sunderland et al., 2019; Fenton et al., 2021; Lind and Lind, 2020; Fragki et al., 2021). However, the strength of evidence varies for different PFAS, with the strongest evidence for PFOA, and less compelling evidence for PFOS and PFHxS (Costello et al., 2022; Andersen et al., 2021).

Some of the inconsistencies in epidemiological findings may be explained by the differing exposure profiles between study populations and the potential for non-linearity in exposure–response associations (Andersen et al., 2021; Fenton et al., 2021; Lin et al., 2019). Studies have suggested that PFOA and PFOS serum concentrations have a supra-linear relationship with cholesterol concentrations; that is, a much steeper (increasing) slope at background PFAS concentrations (those typically observed in general population biomonitoring studies) than at higher PFAS concentrations (e.g., those usually observed in occupational studies), where the association with cholesterol plateaus (Andersen et al., 2021; Fenton et al., 2021; Steenland et al., 2009; Li et al., 2020). Depending on the exposure range, associations observed in epidemiological studies may reflect only a part of this overall relationship.

Serum concentrations of different PFAS can be highly correlated because of their common sources and long clearance times. It is not known whether associations seen for one PFAS are confounded by other co-occurring, and thus correlated, PFAS. Several reviews have identified the assessment of joint associations for PFAS mixtures, and the potential for interaction between PFAS, as a substantial research gap (Costello et al., 2022; Ojo et al., 2021; Rosato et al., 2022). In addition, biomarkers vary in their sensitivity and specificity, so there is a need to measure associations between PFAS, biomarkers and related health conditions concurrently.

We conducted a cross-sectional study of cardiometabolic outcomes and PFAS exposure in three Australian communities with known environmental PFAS contamination and three comparison communities. Our aims were to: (1) quantify associations between serum concentrations of single PFAS and the joint association of the PFAS mixture with serum lipids, including both total cholesterol and specific lipoproteins, and liver function markers, such as ALT; (2) assess non-linearity in exposure–response associations and statistical interaction between individual PFAS; and (3) quantify associations between blood serum PFAS concentrations and the prevalence of cardiometabolic disease, including several cardiovascular conditions, fatty liver disease, and type II diabetes.

2. Methods

2.1. Study design and population

The PFAS Health Study included a cross-sectional study of three Australian communities with known environmental PFAS contamination from the historical use of AFFF (Katherine in Northern Territory (NT), Oakey in Queensland (Qld), and Williamtown in New South Wales (NSW)), collectively known as 'PFAS Management Areas', and three comparison communities without known environmental PFAS contamination (Alice Springs in NT, Dalby in Qld, and Kiama and Shellharbour in NSW) (Fig. 1) (NHMRC and NRMMC, 2021). Comparison communities were selected to be similar to exposed communities based on area-level sociodemographic characteristics (Index of Relative Socio-economic Advantage and Disadvantage) (Australian Bureau of Statistics, 2016a), remoteness (Accessibility and Remoteness Index of Australia) (Australian Bureau of Statistics, 2016b), proportion of people in the community who identify as Aboriginal and Torres Strait Islander, population size, and access to pathology services for blood collection.

2.2. Recruitment

Participants from the exposed communities were recruited through

the Australian Government Voluntary Blood Testing Program for PFAS in 2019, which provided free blood tests for PFAS to people who live or work near PFAS Management Areas (Smurthwaite et al., 2021). Following blood collection, participants were invited to complete a survey and consent was sought for measurement of lipids and markers of liver function in blood serum. Ten thousand residents (≥16 years old) in each comparison community were randomly sampled from the Medicare Enrolment File (Australia's universal health insurance database, which provides virtually complete coverage of the general population) and invited to participate in the PFAS Health Study in 2020 (Smurthwaite et al., 2021; Lazarevic et al., 2021).

2.3. Data collection

2.3.1. Cross-sectional survey

Survey design and data collection are detailed elsewhere (Lazarevic et al., 2021). Briefly, participants were invited to complete an online or paper survey; the participant information sheet, consent form, and survey are available on the PFAS Health Study website (https://rsph.anu.edu.au/research/projects/pfas-health-study). Completions were accepted until January 2021 with no further follow-up or reminders. The survey collected information on PFAS exposure history, health conditions, and sociodemographic characteristics.

2.3.2. Blood collection and chemical analysis

Non-fasting blood samples were collected at pathology centres in a single BD vacutainer Serum Separator Tube, stored at 2–8 °C, and sent to a commercial pathology laboratory in Brisbane, Australia, for analysis. PFAS were extracted from serum samples with a solvent for protein precipitation via ultrasonication, followed by vortex extraction, centrifugation, and filtration. The samples were analysed using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Routine quality control and calibration was conducted and each sample was extracted and analysed in duplicate. Procedural blanks and standardised reference material (NIST SRM, 1957) were analysed together with each batch of samples. Further details are provided elsewhere (Smurthwaite et al., 2021).

Samples were analysed for nine common PFAS: PFOA, PFOS, PFHxS, perfluorobutane sulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and 6:2 fluorotelomer sulfonic acid (6:2 FTS). For each PFAS, we measured the total concentration in serum. For PFOS, we also measured linear and branched (1-methyl, other-methyl, and di-methyl) concentrations separately.

Serum lipids and biochemical markers of liver function were analysed according to standard Australian pathology testing protocols. Tests were conducted in batches over the same time period, using the same test instrument for samples from exposed and comparison communities.

2.4. Outcomes

2.4.1. Biochemical markers

We analysed biochemical markers as both continuous and binary outcomes. Binary outcomes were categorised as 'adverse' or not, with adverse defined as either 'high/elevated' (above the upper reference interval limit) or 'low' (below the lower reference interval limit), depending on the biomarker. To categorise biomarker values, we used age- and sex-specific population reference intervals that are used in clinical practice in Australia to identify and monitor cardiometabolic conditions (provided by a major Australian pathology provider and shown in Supplemental Table S1).

Lipid biomarkers included total cholesterol (millimoles per litre (mmol/L)), high-density lipoprotein (HDL) cholesterol (mmol/L), low-density lipoprotein (LDL) cholesterol (mmol/L), and triglycerides (mmol/L), as well as the total:HDL cholesterol ratio. We defined high total cholesterol as above 5.5 mmol/L, low HDL cholesterol as below 0.9 mmol/L for males and 1.1 mmol/L for females, high LDL cholesterol as above 4.0 mmol/L, high total:HDL cholesterol ratio as above 4.5, and high triglycerides as above 2.0 mmol/L.

Liver biomarkers included ALT (units per litre (U/L)), aspartate transaminase (AST) (U/L), gamma glutamyl transferase (GGT) (U/L), alkaline phosphatase (ALP) (U/L), serum albumin (grams per litre (g/L)), and total protein (g/L). We defined high ALT as above 40 U/L for males and 30 U/L for females, high AST as above 40 U/L for males and 35 U/L for females, and high GGT as above 40 U/L for males under 18 years of age, above 50 U/L for males 18 years and over, and above 30 U/L for females. High ALP and low serum albumin were defined by age and sex categories as described in Supplemental Table S1. We excluded binary total protein from our analyses as the other markers measured are more sensitive markers of liver function.

2.4.2. Self-reported health conditions

In the survey, participants were asked whether they had ever been diagnosed with any of four cardiovascular conditions (heart attack, high blood pressure, stroke, and hypercholesterolaemia), or three liver conditions (non-infectious hepatitis, fatty liver disease, and liver cirrhosis), or type II diabetes. We analysed health conditions separately, except those with fewer than 10 cases in all exposed communities. To allow analysis of these rarer health outcomes, we also analysed the combined outcomes 'any cardiovascular disease' (excluding hypercholesterolaemia) and 'any liver disease'. Self-reported health outcomes were analysed as binary variables (i.e., ever diagnosed or never

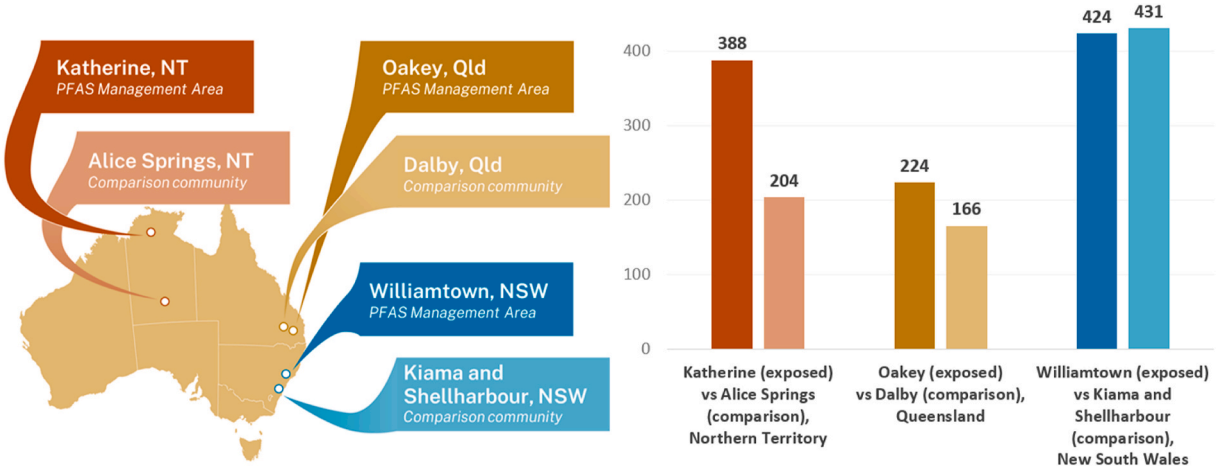

Fig. 1. Location and number of participants in exposed and comparison communities.

diagnosed with a health condition).

2.5. Exposures

We analysed blood serum concentrations of PFOS, PFOA, and PFHxS, which were detected in blood serum in >98% of participants. We did not analyse the other PFAS, which were detected in <62% of participants (0%–10% for PFHxA, PFHpA, PFDA, PFBS, and 6:2 FTS; and 62% for PFNA). Following convention, in the main analysis, we replaced PFAS values below the limit of quantification with the limit divided by the square root of two. We addressed the potential bias induced by this single imputation in a sensitivity analysis (Harel et al., 2014). We log-transformed (base 2) blood serum concentrations of PFAS to express effects per doubling in PFAS serum concentrations in analyses of single PFAS.

2.6. Statistical analysis

2.6.1. Associations between individual PFAS, biomarker concentrations and self-reported health conditions

Per doubling in PFAS serum concentrations, we estimated: (1) differences in mean biomarker concentrations; (2) prevalence ratios of 'adverse' biomarker concentrations; and (3) prevalence ratios of self-reported health conditions. We used linear regression models to estimate differences and modified Poisson regression models with log link and robust error variance to estimate prevalence ratios. Models were estimated via generalised estimating equations using an exchangeable correlation structure, to account for clustering of participants within households. When convergence could not be achieved using an exchangeable correlation structure, we used an independence correlation structure and cluster-robust standard errors. Linearity of relationships between outcomes and continuous covariates (including PFAS serum concentrations) was assessed using univariable generalised additive models. Age was subsequently modelled using a restricted cubic spline with three knots.

We estimated associations separately for each exposed community and comparison community pair. Models included an interaction term between PFAS and community membership so that associations were estimated separately for exposed and comparison communities. We did this as exposed and comparison communities were exposed to different mixtures of PFAS and were sampled several years apart. We report the strength of associations estimated for each exposed community and whether they were significantly different to the associations estimated in the corresponding comparison community, at a 5% significance level.

We adjusted the measures of association by variables thought to affect both the exposure and outcomes. In the primary analysis, we adjusted for sociodemographic variables: sex, age, education level (combined into three categories: bachelor degree level and higher, certificate or diploma, and high school or lower), and gross household annual income (five categories: ≤AU$25,999, AU$26,000–$64,999, AU $65,000–$129,999, AU$130,000–$233,999, ≥AU$234,000, modelled as an ordinal variable using category midpoints in the middle categories, and upper and lower limits in the lowest and highest categories, respectively).

In a sensitivity analysis, we additionally adjusted for the estimated glomerular filtration rate (eGFR; a measure of kidney function calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula based on age, sex, and serum creatinine concentrations) (Levey et al., 2009) and variables that may affect kidney function, including smoking status (combined into two categories due to low prevalence of current smokers: never and ever) and alcohol consumption (categorised according to Australian National Health and Medical Research Council guidelines (NHMRC, 2020): none, within guideline (≤10 standard drinks per week), exceeds guideline (>10 standard drinks per week)), on the basis that impaired kidney function may affect the renal excretion of PFAS and thus PFAS serum concentrations (Jain and Ducatman, 2019a). We did not adjust for body mass index (because it may be on the causal pathway between PFAS exposure and health outcomes), or physical activity (because it is unlikely to affect PFAS concentrations independently of body mass index) (Inoue et al., 2020).

We conducted a series of additional sensitivity analyses, in which we: (1) excluded exposed participants who were currently residing in comparison areas; (2) excluded exposed participants who had not resided in the exposed areas in the 10 years prior to survey completion and past workers, because their PFAS serum concentrations at the time of blood collection may not be reflective of their long-term PFAS exposure levels; (3) assessed the impact of missing values in confounder variables using multiple imputation by chained equations; (4) treated PFAS concentrations below the limit of quantification as censored values that we imputed using multiple imputation by chained equations; (5) excluded co-morbidities (including cancer, cardiovascular, autoimmune, liver, kidney, and thyroid conditions) diagnosed in the five years prior to blood collection in case treatment for these conditions affected biomarker concentrations; and (6) calculated E-values, which provide the minimum strength of the relationship between outcomes and an unmeasured confounder that are required to explain away our observed associations (VanderWeele and Ding, 2017).

2.6.2. Associations between the PFAS mixture and biomarker concentrations

Bayesian kernel machine regression is a method for analyses of the health effects of exposure mixtures that flexibly estimates a multivariate exposure–response function, allowing for non-linearity and interaction between the exposures (Bobb et al., 2014). We used Bayesian kernel machine regression to: (1) estimate the joint associations of the PFAS mixture on biomarker concentrations; (2) estimate the association of each PFAS with biomarker concentrations while controlling for other PFAS and confounders; (3) assess interaction between PFAS; and (4) assess non-linearity in exposure–response associations (Bobb et al., 2014). The single and joint associations are given by summary statistics of the estimated multivariate exposure-response function; e.g., the joint association is quantified as the difference in the mean outcome when all exposures are set at particular percentiles versus all at their 25th percentile (Bobb et al., 2014).

We did this for continuous biomarker concentrations using a Gaussian family model with identity link. We assumed that the prior probability of selection was 0.5 for any exposure. For the error variance, we used a diffuse inverse *Gamma* (0.001,0.001) prior. For parameters that use a Metropolis-Hastings sampler, we ran a range of different models and selected parameters that gave good acceptance rates (around 20–40%) and achieved convergence. For λ, we used a *Gamma* (4,2) prior, with mean and standard deviation of 2, and a proposal density standard deviation of 8 for lipids and 4 for liver markers. For r, the parameter that controls smoothing, we used a *Uniform* (0.001,10) prior, after running frequentist kernel machine regression to examine the likely values that this parameter may take (Bobb et al., 2014), and we used 0.01 for the standard deviation of the random walk proposal density. We ran the Markov chain Monte Carlo sampler for 50,000 iterations and used the last 20,000 iterations to generate estimates after assessing convergence. Analyses were conducted in Stata/SE v16.1 (StataCorp LLC, College Station, TX) and R v3.4.3 (R Core Team, 2021), using the *bkmr* package, v0.2.0 (Bobb et al., 2014).

3. Results

3.1. Participation

Participation rates have been described in detail previously (Smurthwaite et al., 2021; Lazarevic et al., 2021). In the comparison communities, 2.9% (877/30,000) of adult residents randomly sampled from the Medicare Enrolment File completed the survey. Nine percent (76/877) of these participants were re-classified as exposed participants

Table 1
Sociodemographic characteristics and PFAS serum concentrations of adult survey participants from PFAS Management Areas, 2016–2020, and comparison communities, 2020.

	Katherine (NT)	Alice Springs (NT)	Oakey (Qld)	Dalby (Qld)	Williamtown (NSW)	Kiama and Shellharbour (NSW)
Characteristic	Exposed N (%)	Comparison N (%)	Exposed N (%)	Comparison N (%)	Exposed N (%)	Comparison N (%)
Total sample[a]	388	204	224	166	424	431
Sex						
Male	199 (51%)	78 (38%)	156 (70%)	72 (43%)	278 (66%)	186 (43%)
Female	189 (49%)	126 (62%)	68 (30%)	94 (57%)	146 (34%)	245 (57%)
Age (mean years)	51	55	54	57	54	61
Age (years)						
16 to 30	30 (8%)	16 (8%)	11 (5%)	7 (4%)	25 (6%)	21 (5%)
31 to 45	104 (27%)	34 (17%)	40 (18%)	33 (20%)	88 (21%)	48 (11%)
46 to 60	130 (34%)	71 (35%)	98 (44%)	50 (30%)	125 (29%)	99 (23%)
≥61	124 (32%)	83 (41%)	75 (33%)	76 (46%)	186 (44%)	263 (61%)
Smoking status						
Never	232 (60%)	127 (62%)	135 (60%)	118 (71%)	257 (61%)	298 (69%)
Past	106 (27%)	66 (32%)	69 (31%)	39 (23%)	124 (29%)	118 (27%)
Current	34 (9%)	10 (5%)	14 (6%)	9 (5%)	29 (7%)	14 (3%)
Missing	16 (4%)	1 (0%)	6 (3%)	0 (0%)	14 (3%)	1 (0%)
Alcohol consumption (NHMRC, 2020 guidelines)						
No alcohol	101 (26%)	53 (26%)	61 (27%)	67 (40%)	103 (24%)	105 (24%)
Within guideline (≤10 standard drinks per week)	197 (51%)	108 (53%)	102 (46%)	71 (43%)	228 (54%)	248 (58%)
Exceeds guideline (>10 standard drinks per week)	51 (13%)	36 (18%)	39 (17%)	23 (14%)	65 (15%)	63 (15%)
Missing	39 (10%)	7 (3%)	22 (10%)	5 (3%)	28 (7%)	15 (3%)
Level of education						
Bachelor degree or higher	142 (37%)	109 (53%)	73 (33%)	50 (30%)	126 (30%)	188 (44%)
Certificate or diploma	181 (47%)	72 (35%)	115 (51%)	66 (40%)	222 (52%)	181 (42%)
High school certificate or lower	44 (11%)	20 (10%)	23 (10%)	43 (26%)	56 (13%)	52 (12%)
Missing	21 (5%)	3 (1%)	13 (6%)	7 (4%)	20 (5%)	10 (2%)
Gross household annual income (AUD)						
≤$25,999	22 (6%)	15 (7%)	16 (7%)	32 (19%)	33 (8%)	45 (10%)
$26,000 to $64,999	43 (11%)	21 (10%)	33 (15%)	41 (25%)	62 (15%)	108 (25%)
$65,000 to $129,999	149 (38%)	83 (41%)	82 (37%)	46 (28%)	142 (33%)	124 (29%)
$130,000 to $233,999	79 (20%)	58 (28%)	41 (18%)	23 (14%)	62 (15%)	64 (15%)
≥$234,000	10 (3%)	7 (3%)	5 (2%)	2 (1%)	9 (2%)	15 (3%)
Missing	85 (22%)	20 (10%)	47 (21%)	22 (13%)	116 (27%)	75 (17%)
PFAS serum concentrations (median (25th percentile, 75th percentile))[b]						
PFOS total (ng/mL)	5.2 (3.2,8.4)	2.7 (1.5,4.5)	5.7 (3.4,9.9)	3.5 (1.8,5.1)	5.3 (3.2,9.0)	3.6 (2.2,5.8)
PFOS branched isomers (ng/mL)	2.4 (1.4,3.9)	1.3 (0.8,2.3)	3.0 (1.7,5.0)	1.9 (1.0,2.9)	2.6 (1.4,4.1)	1.8 (1.0,2.8)
PFOA (ng/mL)	1.5 (1.0,2.2)	1.2 (0.8,1.9)	1.8 (1.2,2.6)	1.5 (0.9,2.1)	1.8 (1.2,2.4)	1.3 (0.9,2.0)
PFHxS (ng/mL)	3.7 (1.7,7.3)	0.8 (0.4,1.3)	2.8 (1.4,5.8)	1.3 (0.7,2.2)	3.2 (1.6,6.1)	1.1 (0.6,1.7)

N: sample size; PFOS: perfluorooctane sulfonic acid; PFOA: perfluorooctanoic acid; PFHxS: perfluorohexane sulfonic acid.

[a] In exposed communities, the total sample was defined as ever living or working in the PFAS Management Area, including participants who have lived or worked across multiple PFAS Management Areas.

[b] Displayed are summary statistics of PFAS serum concentrations for the subset of PFAS Health Study participants who responded to the cross-sectional survey, with sample sizes shown in Supplemental Table S4; PFAS serum concentrations of all individuals who participated in the PFAS Health Study are available in Smurthwaite et al. (2021).

based on their residential and work history. Of the 801 comparison participants who completed the survey, 692 consented to testing of their blood sample for cardiometabolic biomarkers. The sample of 801 comparison participants included 204 participants in Alice Springs (NT), 166 participants in Dalby (Qld), and 431 participants in Kiama and Shellharbour (NSW) (Fig. 1).

In the exposed communities, 881 adults completed the survey. This included 33% (779/2326) of the adult participants who provided a blood sample for the PFAS Health Study in 2016–2019, a further 26 participants recruited for the survey only, and the 76 comparison participants who were re-classified as exposed. Of the 881 exposed participants who completed the survey, 832 consented to testing of their blood sample for cardiometabolic biomarkers. The sample included 388 participants in Katherine (NT), 224 in Oakey (Qld), and 424 in Williamtown (NSW), which includes 144 participants who resided or worked in multiple exposed communities (Fig. 1).

3.2. Participant characteristics

Participants in exposed communities were slightly younger on average, with a higher proportion of males, than participants in comparison communities (Table 1).

Across both exposed and comparison communities, an adverse lipid profile was common (over 30% with hypercholesterolemia), and the most common health conditions reported were high blood pressure and hypercholesterolemia (Supplemental Tables S2, S3a, and S3b). Abnormal GGT was the most common liver abnormality across all communities (Supplemental Table S2). Few individuals presented with low serum albumin, stroke, hepatitis (non-infectious), or cirrhosis of the liver (Supplemental Table S2), so we did not analyse these outcomes separately.

Participants in exposed communities had higher median blood serum concentrations of PFOS and PFHxS, and to a lesser degree PFOA, than participants in comparison communities (Table 1 and Supplemental Table S4) (Smurthwaite et al., 2021).

3.3. Serum lipids

Estimated differences in mean lipid biomarker concentrations were close to zero and mostly not in a consistent direction across communities; however, within participants in Williamtown (NSW), mean total cholesterol, LDL cholesterol and the total:HDL cholesterol ratio were higher per doubling in serum concentrations of some PFAS (e.g., total cholesterol (PFOS total) difference = 0.11 mmol/L, 95% confidence interval (CI) 0.02 to 0.19) (Fig. 2 and Supplemental Tables S5a–c). When we considered all three PFAS together (i.e., PFOS, PFOA, and PFHxS), total cholesterol and LDL cholesterol were elevated in all communities, most strongly in participants from Williamtown (0.18 mmol/L, 95% credible interval (CrI) −0.06 to 0.42, higher total cholesterol concentrations with an interquartile range increase in all PFAS concentrations); however, associations near zero and negative associations were also consistent with the model and data (Fig. 3 and Supplemental Table S6). There was less certainty in the direction of association between the mixture of all three PFAS and HDL cholesterol, total:HDL cholesterol ratio, and triglycerides. There was no evidence of interaction between PFAS in their associations with lipids (Supplemental Fig. S1) or non-linearity in PFAS–lipid associations (Supplemental Figs. S2a–e).

In participants from Williamtown, we also observed higher prevalence of elevated total cholesterol per doubling in PFAS serum concentrations (e.g., total cholesterol (PFOS total) PR = 1.13, 95% CI 1.02 to 1.26; Fig. 4 and Supplemental Tables S7a–c). This was also the case for PFOA and both elevated LDL cholesterol (PR = 1.36, 95% CI 1.03 to 1.80) and the ratio of total cholesterol to HDL cholesterol (PR = 1.26, 95% CI 1.00 to 1.60). The evidence for positive associations was substantially weaker for the remaining PFAS in Williamtown, and between all serum PFAS and lipid concentrations in participants from Katherine (NT) and Oakey (Qld) (e.g., high LDL cholesterol: Katherine (PFOA) PR = 1.10, 95% CI 0.72 to 1.67; Oakey (PFOA) PR = 0.89, 95% CI 0.66 to 1.22). There were no significant differences between estimates of associations in exposed communities compared to the corresponding comparison communities, with one exception (PFOA and the total:HDL cholesterol ratio in Williamtown versus Kiama and Shellharbour) (Supplemental Tables S5a–c and Supplemental Tables S7a–c). The findings from the analyses of lipids did not change markedly in sensitivity analyses (Supplemental Tables S5a–c and Supplemental Tables S7a–c).

3.4. Liver function biomarkers

Differences in mean ALT, AST, ALP, GGT, total protein and serum albumin per doubling in PFAS serum concentrations were small, imprecisely estimated and largely uninformative as to the presence or absence of associations (Fig. 5 and Supplemental Tables S5a–c). When we considered all three PFAS together, there was little certainty in the direction of associations and little consistency across communities (Fig. 3 and Supplemental Table S6). Serum albumin was an exception with higher PFAS-mixture concentrations in participants from Oakey (Qld); however, the associations were weak (i.e., we estimated 0.52 g/L, 95% CrI −0.04 to 1.08, higher serum albumin concentrations with an interquartile range increase in all PFAS concentrations) and not supported by estimates in Williamtown (NSW) and Katherine (NT). There was no evidence of interaction between PFAS in their associations with liver markers (Supplemental Fig. S1) or non-linearity in PFAS–liver marker associations (Supplemental Figs. S2f–k).

The estimated prevalence ratios of elevated liver function biomarkers per doubling in PFAS serum concentrations were mostly inconsistent in direction and magnitude across communities (Fig. 6 and Supplemental Tables S7a–c). The evidence suggested higher prevalence

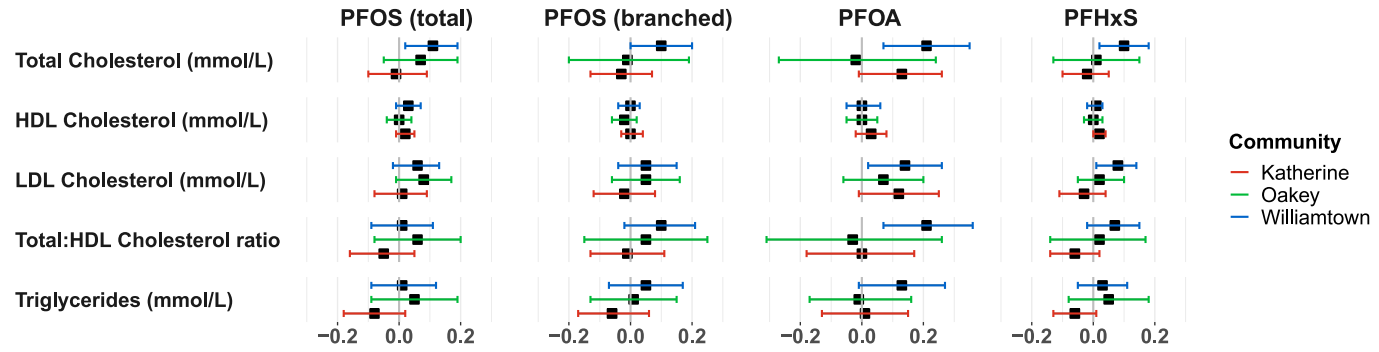

Fig. 2. Forest plot showing adjusted differences in mean lipid concentrations per doubling in PFAS serum concentrations in participants from PFAS Management Areas, 2016–2020. Differences were adjusted for age, sex, level of education, and gross household annual income.

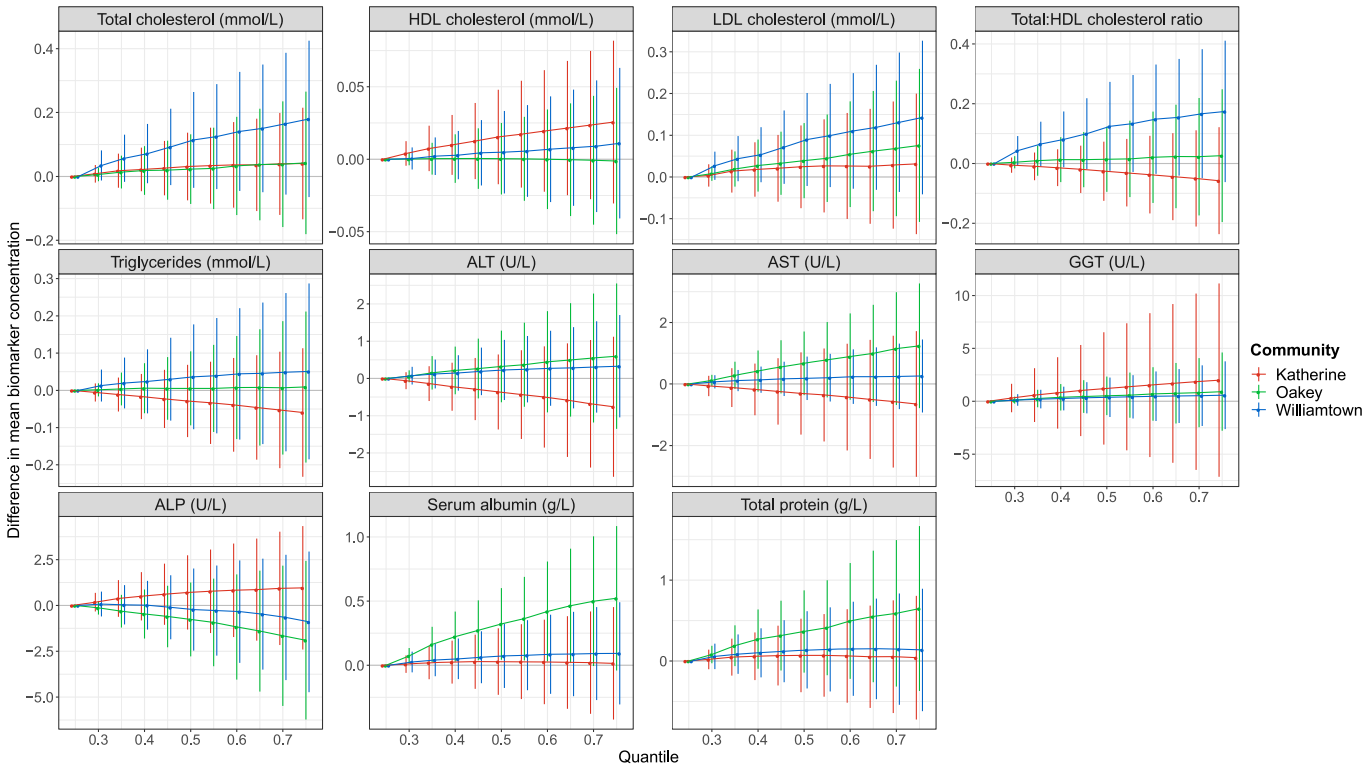

Fig. 3. Estimated joint associations of the three PFAS together, showing the estimated difference (and 95% credible intervals) in mean biomarker concentrations when all exposures are at specified percentiles versus all exposures at their 25th percentiles.

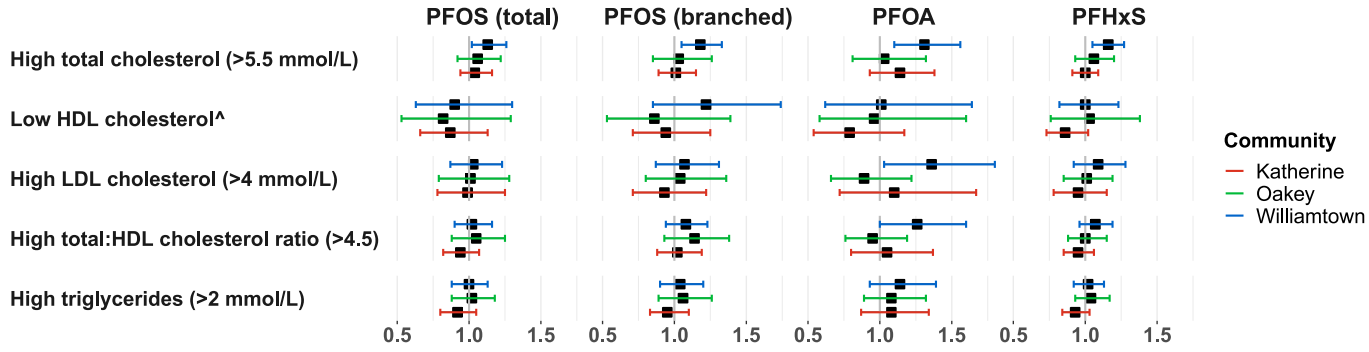

Fig. 4. Forest plot showing adjusted prevalence ratios of adverse lipid concentrations per doubling in PFAS serum concentrations in participants from PFAS Management Areas, 2016–2020. Prevalence ratios were adjusted for age, sex, level of education, and gross household annual income. ^ Reference intervals vary by sex and age.

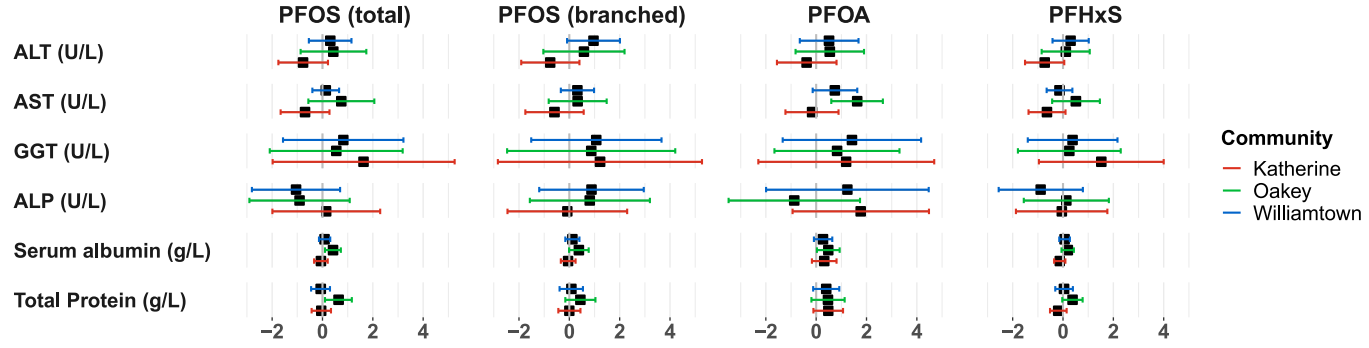

Fig. 5. Forest plot showing adjusted differences in mean liver function marker concentrations per doubling in PFAS serum concentrations in participants from PFAS Management Areas, 2016–2020. Differences were adjusted for age, sex, level of education, and gross household annual income.

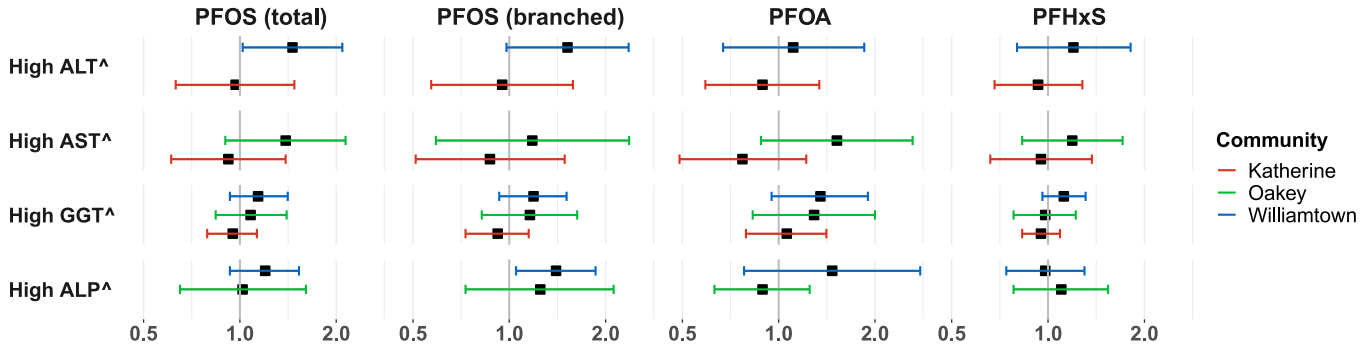

Fig. 6. Forest plot showing adjusted prevalence ratios of adverse liver function marker concentrations per doubling in PFAS serum concentrations in participants from PFAS Management Areas, 2016–2020. Prevalence ratios were adjusted for age, sex, level of education, and gross household annual income. Some associations are missing due to non-convergence. A base-2 log scale is used on the *x*-axis. ^ Reference intervals vary by sex and age.

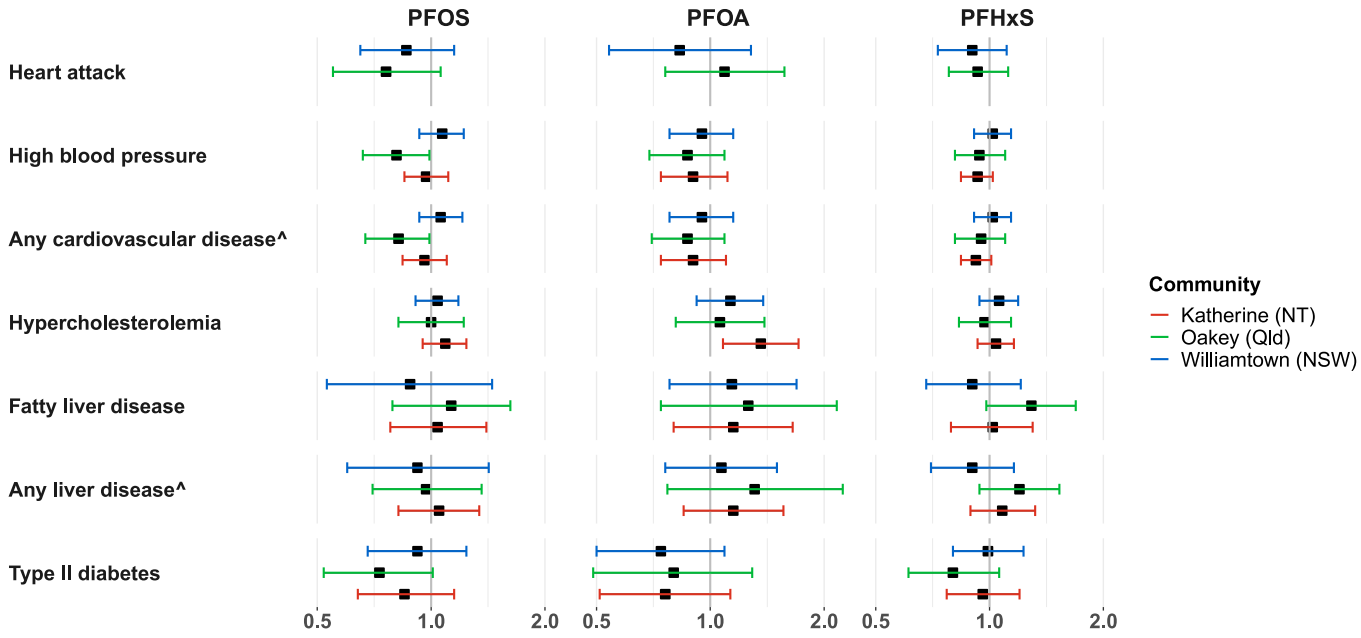

Fig. 7. Forest plot showing adjusted prevalence ratios of self-reported health outcomes per doubling in PFAS serum concentrations in participants from PFAS Management Areas, 2016–2020. Prevalence ratios were adjusted for age, sex, level of education, and gross household annual income. Some associations are missing due to non-convergence. A base-2 log scale is used on the *x*-axis. ^ Any cardiovascular disease includes heart attack, high blood pressure, and stroke. Any liver disease includes non-infectious hepatitis, fatty liver disease, and cirrhosis of the liver.

of elevated ALT and ALP in participants from Williamtown with higher serum PFOS concentrations (e.g., ALT (PFOS total) PR = 1.46, 95% CI1.02 to 2.09); however, the prevalence ratios in Katherine and Oakey gave little support to these observations. There were no significant differences between estimates of associations in exposed communities compared to the corresponding comparison communities, with few exceptions (e.g., elevated AST in participants from Oakey compared with Dalby; Supplemental Tables S5a–c and Supplemental Tables S7a–c). The findings from the analyses of liver function biomarkers did not change markedly in sensitivity analyses (Supplemental Tables S5a–c and Supplemental Tables S7a–c). An exception was when we imputed missing values in confounders, most prevalence ratios for liver function biomarkers in Williamtown were attenuated (Supplemental Table S7c).

3.5. Self-reported health conditions

We observed higher prevalence of self-reported hypercholesterolaemia per doubling in PFOA serum concentrations (Fig. 7 and Supplemental Tables S8a–c). The evidence was strongest in Katherine (NT) (PR = 1.36, 95% CI 1.08 to 1.71), and considerably weaker in Oakey (Qld) (PR = 1.06, 95% CI 0.81 to 1.39) and Williamtown (NSW) (PR = 1.13, 95% CI 0.92 to 1.38). In contrast with the observations for PFOA, there was no material difference in prevalence of hypercholesterolaemia per doubling in PFOS and PFHxS serum concentrations in exposed communities (e.g., PFHxS: Katherine, PR = 1.04, 95% CI 0.93 to 1.16; Oakey, PR = 0.97, 95% CI 0.83 to 1.14; Williamtown, PR = 1.06, 95% CI 0.94 to 1.19). This was also the case for the prevalence of self-reported heart attacks, high blood pressure, and any cardiovascular disease (e.g., any cardiovascular disease and PFOS: Katherine, PR = 0.96, 95% CI 0.84 to 1.10; Oakey, PR = 0.82, 95% CI 0.67 to 0.99; Williamtown, PR = 1.06, 95% CI 0.93 to 1.21).

There was no clear evidence of associations between PFAS serum concentrations and self-reported liver disease (Fig. 7 and Supplemental Tables S8a–c). While some prevalence ratio estimates were in a consistent positive direction across exposed communities, they were mostly small in magnitude and uninformative with regard to the presence or absence of associations (e.g., any liver disease and PFOA: Katherine, PR = 1.15, 95% CI 0.85 to 1.56; Oakey, PR = 1.31, 95% CI 0.77 to 2.24; Williamtown, PR = 1.07, 95% CI 0.76 to 1.50).

There was no clear evidence of associations between PFAS serum

concentrations and self-reported type II diabetes (e.g., PFOS: Katherine, PR = 0.85, 95% CI 0.64 to 1.15; Oakey, PR = 0.73, 95% CI 0.52 to 1.01; Williamtown, PR = 0.92, 95% CI 0.68 to 1.24) (Fig. 7 and Supplemental Tables S8a–c). The findings from the analyses of self-reported conditions were not markedly changed in sensitivity analyses (Supplemental Tables S8a–c).

4. Discussion

In this cross-sectional study of six Australian communities, three with known environmental PFAS-contamination and three comparison communities, we found that higher concentrations of a mixture of three PFAS were associated with higher mean total cholesterol concentrations in one community, but there was variation in the direction and magnitude of associations among exposed communities. This was also the case in models of total cholesterol levels in relation to individual PFAS, both when estimating differences in means and prevalence of elevated levels, with the strongest associations observed for PFOA, which was not elevated in exposed communities relative to comparison communities (Smurthwaite et al., 2021). Similarly, single PFAS and the PFAS-mixture were not consistently associated with biochemical markers of liver function. We also found that PFOA was associated with higher prevalence of self-reported hypercholesterolemia in one of three communities, but PFAS serum concentrations were not associated with self-reported type II diabetes, liver disease, or cardiovascular disease.

4.1. Lipids and cardiovascular disease

Our findings are consistent with a substantial body of evidence suggesting that exposure to PFAS is associated with abnormal lipid profiles, particularly elevated total cholesterol levels (Sunderland et al., 2019; Fenton et al., 2021; Lind and Lind, 2020; Fragki et al., 2021). Both cross-sectional and cohort studies have reported positive associations between PFAS (particularly PFOA) and elevated total cholesterol levels: in general population studies at 'background' exposure levels (Eriksen et al., 2013; He et al., 2018; Liu et al., 2018), in communities exposed to PFAS-contaminated water (Steenland et al., 2009; Li et al., 2020; Winquist and Steenland, 2014), and in occupational studies (Costa et al., 2009; Sakr et al., 2007a, 2007b). However, several small studies have reported no association (Chen et al., 2019; Donat-Vargas et al., 2019), including at higher exposure levels among workers (Rotander et al., 2015; Olsen et al., 2003).

For specific lipoproteins and triglycerides, the evidence is less consistent: positive findings have been reported in large studies of exposed communities (Steenland et al., 2009; Li et al., 2020), but no associations were reported in several occupational studies (Sakr et al., 2007a, 2007b; Rotander et al., 2015). In studies of PFAS mixtures, a mixture of four PFAS (PFOA, PFOS, PFHxS, and PFNA) was positively associated with serum total-, LDL-, and HDL-cholesterol, but not with triglycerides, in a cross-sectional analysis of the U.S. National Health and Nutrition Examination Survey (NHANES) (Fan et al., 2020), while a case-control study of the general population in China, with substantially higher median PFOA and PFOS concentrations, found that a mixture of seven PFAS (PFOA, PFOS, PFHxS, PFNA, PFDA, perfluoroundecanoic acid (PFUnDA), and 6:2 chlorinated polyfluoroalkyl ether sulfonic acid (6:2 Cl-PFESA)) was not associated with the same biomarkers, even though single PFAS were associated with serum LDL-cholesterol concentrations (Han et al., 2021).

Mechanistic studies have demonstrated that PFAS have the ability to perturb cholesterol homeostasis (Lind and Lind, 2020; Fragki et al., 2021). It is recognised that PFAS can activate PPARα and other nuclear receptors, affect fatty acid oxidation enzymes, and affect the expression of genes involved in cholesterol transport (Lind and Lind, 2020; Fragki et al., 2021; Fletcher et al., 2013); however, exposure concentrations may need to be higher than is seen in epidemiological studies to involve these pathways (Andersen et al., 2021). A human clinical trial involving extreme PFOA doses (serum concentrations >10,000 times higher than the maximum observed in the PFAS Health Study) found reduced, rather than increased, serum cholesterol levels (Andersen et al., 2021; Convertino et al., 2018). At the lower serum PFAS concentrations in our study, we found no evidence of non-linear associations with serum lipids.

Whether the associations observed in epidemiological studies reflect a causal relationship between PFAS and cholesterol is therefore not yet known (Andersen et al., 2021). Several alternative explanations have been proposed: (1) reverse causation is a possibility if PFAS bind to the lipid fractions of blood serum, rather than solely plasma proteins (Andersen et al., 2021; Butenhoff et al., 2012); (2) uncontrolled confounding by thyroid and kidney disease, which may be accompanied by elevated serum cholesterol, is also a possibility, though we found no suggestion of this in sensitivity analyses; and (3) uncontrolled confounding by diet and the enterohepatic cycling process of PFAS and bile acids (Fragki et al., 2021). In particular, low dietary fibre has been associated with higher plasma PFAS concentrations (Lin et al., 2020).

The differences in mean total cholesterol concentrations that we found were small at an individual level (ranging 0.04–0.18 mmol/L per interquartile range increase in the PFAS mixture), but may correspond to increases in cardiovascular disease burden at a population level. However, PFAS serum concentrations were not clearly associated with increased prevalence of self-reported cardiovascular outcomes (including heart attack, high blood pressure, and stroke) in our study. Previous evidence for relationships between PFAS and specific cardiovascular diseases has generally been inconclusive (Sunderland et al., 2019; Fragki et al., 2021). Cross-sectional associations of PFAS with hypertension and cardiovascular disease prevalence have been reported in a representative sample of the U.S. population in the NHANES (Huang et al., 2018; Shankar et al., 2012; Min et al., 2012). However, the findings of prospective cohort studies and case-control studies, which offer better-quality evidence, have been inconsistent (Sunderland et al., 2019). For example, studies of Italian and US communities exposed to PFAS-contaminated drinking water have reported either positive associations (Pitter et al., 2020) or no associations (Winquist and Steenland, 2014) with the risk of hypertension and cardiovascular disease. No associations with increased risk of myocardial infarction or stroke were reported in a recent nested case-control study of two Swedish population-based cohorts, despite positive associations between plasma PFAS and increased cholesterol levels (Schillemans et al., 2022).

4.2. Liver function markers and conditions

We observed higher prevalence of ALT and ALP in one of three communities (Williamtown, NSW), which was not supported by estimates in the other two communities. These findings were based on few cases, whose liver enzymes were only mildly elevated (ranging 1.02 to 1.60 times the upper reference interval limit, with values less than five times the upper limit are considered mild) (Oh et al., 2017). Studies suggest that 10 percent of people in the general adult population have elevated ALT levels, but only 5% of these individuals have liver disease (Oh et al., 2017; Lilford et al., 2013). For almost all cases of elevated ALP, we did not see a concurrent elevation in GGT, suggesting that liver disease is not the cause. We also observed higher serum albumin with higher single PFAS and PFAS mixture concentrations in one community (Oakey, Qld); however, lower, rather than higher, serum albumin is indicative of liver damage. Elevations in some liver biomarkers (especially ALP and serum albumin) can reflect acute alcohol consumption, cancer, or alcoholic fatty liver disease (Costello et al., 2022).

Mechanistic studies suggest that PFAS exposure may contribute to the development and progression of non-alcoholic fatty liver disease and toxicant-associated fatty liver disease (Armstrong and Guo, 2019). Despite this, epidemiological studies have inconsistently linked PFAS exposure to biomarkers of liver function and there is a paucity of studies that link PFAS directly to clinically diagnosed liver disease (Fenton

et al., 2021; Lind and Lind, 2020). Positive associations have been primarily reported for PFOA and PFOS with elevated liver enzymes, especially ALT, in population-based cross-sectional studies and cohort studies in the USA (Jain and Ducatman, 2019b), Sweden (Salihovic et al., 2018), and for both single PFAS and PFAS-mixtures in China (Nian et al., 2019; Liu et al., 2022), in cross-sectional studies of communities exposed to PFAS-contaminated water (Darrow et al., 2016; Gallo et al., 2012), and in occupational studies (Costa et al., 2009). The findings have been inconsistent across specific PFAS and liver enzymes, and lack of associations have also been reported (Costa et al., 2009; Salihovic et al., 2018; Darrow et al., 2016; Gallo et al., 2012; Rantakokko et al., 2015). A recent meta-analysis concluded that there was sufficient evidence for an association with markers of liver injury for PFOA and PFOS, but not PFHxS; however, the finding for PFOS was based on cross-sectional studies (Costello et al., 2022).

We found no associations between the PFAS mixture and liver enzymes. To our knowledge, only one other study has considered PFAS mixtures in the context of liver function in adults: a cross-sectional study in China, with higher median PFAS concentrations than in our study, reported that a mixture of 13 PFAS (PFOS, PFOA, PFHxS, PFNA, PFDA, PFHpA, PFHxA, perfluoroheptanesulfonic acid (PFHpS), perfluorododecanoic acid (PFDoDA), PFUnDA, perfluorotridecanoic acid (PFTrDA), and two chlorinated polyfluoroalkyl ether sulfonic acids, 6:2 Cl-PFESA and 8:2 Cl-PFESA) was positively associated with differences in mean ALT, GGT, and AST serum concentrations, but not ALP, and with the odds of abnormal liver function (defined as at least one biomarker above the upper reference limit); the latter association was driven by PFOS, which was at ~2.5 times the median concentration observed in our study (Liu et al., 2022).

In the absence of clinical symptoms, biomarker values outside of reference intervals are not necessarily indicative of disease. Among studies that directly assessed liver disease, PFAS have been associated with mortality due to cirrhosis of the liver in one of two cohort studies of highly exposed workers (Girardi and Merler, 2019; Lundin et al., 2009). Our findings are consistent with two cross-sectional studies of communities with PFAS-contaminated water in reporting no associations with liver disease (Darrow et al., 2016; Emmett et al., 2006).

4.3. Diabetes

PFAS serum concentrations were not associated with the prevalence of self-reported type II diabetes in this study. Evidence for associations between PFAS exposure and type II diabetes have been inconclusive (Qi et al., 2020; Fenton et al., 2021). Consistent with our findings, studies assessing relationships between PFAS exposure and type II diabetes have reported no association in cross-sectional studies (Salihovic et al., 2018; Nian et al., 2019), nor in a retrospective cohort study (Karnes et al., 2014). Several studies have reported positive cross-sectional associations with exposure to PFOS (Su et al., 2016) and PFOA (He et al., 2018), as well as positive associations in prospective studies of U.S. women (Sun et al., 2018) and highly exposed workers (Steenland et al., 2015). However, inverse associations (Su et al., 2016; Conway et al., 2016) and non-linear associations (Mancini et al., 2018) have also been reported.

The evidence has also been largely inconclusive among studies assessing markers of diabetes risk, including blood insulin and fasting glucose levels (Qi et al., 2020; Fenton et al., 2021). However, in a cross-sectional study of young adults in California, a mixture of three PFAS (PFOA, PFOS, and PFHxS) was found to be associated with higher 30-min glucose levels in oral glucose tolerance testing, and a targeted metabolomics analysis suggested increased lipolysis and beta-oxidation as the mechanism linking PFAS to impaired glucose metabolism (Chen et al., 2020).

4.4. Strengths and limitations

Our study simultaneously quantified the single and joint associations of a mixture of PFAS on multiple cardiometabolic markers and underlying health conditions in several communities living with PFAS-contaminated water and land. However, we used a cross-sectional design, which precludes causal inference. We related self-reported lifetime history of particular health conditions to PFAS concentrations in serum measured at the time of blood collection, 2016–2020 (Smurthwaite et al., 2021). Exposure measurement, therefore, occurred after disease onset, and we were not able to take into account the extent to which participants were exposed to PFAS before disease onset. We measured exposure at a single time point, which does not reflect variation in PFAS serum concentrations over time, is an imperfect measure of cumulative exposure levels, and may not reflect exposure levels at pertinent times for disease development. While PFAS have biological half-lives of several years, serum concentrations of PFOA, PFOS, and PFHxS have been decreasing in the Australian population since 2002 (Toms et al., 2019). However, in sensitivity analyses we restricted our sample to participants who resided in the exposed communities in the 10 years prior to the survey (also five and 15 years), with negligible change (results not shown for five and 15 years).

Participants in comparison communities were randomly sampled; however, participants in exposed communities were self-selected through their participation in the Australian Government Voluntary Blood Testing Program for PFAS. Awareness of exposure status (e.g., due to occupational use of firefighting foam or bore water consumption) and the perception that PFAS adversely affects health may have influenced participation, leading to a potential selection bias.

Outcome misclassification is a possibility for outcomes that were based on self-report and not validated in medical records. We assessed the sensitivity of our results to assumptions on the relationships between the outcomes, exposures, and confounders. Our findings may be explained by residual confounding due to the coarse resolution of some self-reported confounder data, or uncontrolled confounding (e.g., by dietary factors). In particular, higher consumption of fish, fruit, and vegetables may be beneficial for cardiometabolic health (Micha et al., 2017), but local produce and wild-caught fish are a known source of PFAS in the contaminated areas (AECOM, 2017b; Coffey Services, 2018; AECOM, 2017a). We calculated E-values (VanderWeele and Ding, 2017), which suggested that relatively small associations between the outcomes and uncontrolled confounders (e.g., for PFAS and total cholesterol in Williamtown, NSW, PRs between 1.51 and 1.95; Supplemental Tables S7a–c and S8a–c) could nullify our findings.

We performed numerous analyses without correcting for multiple testing, therefore some of the associations that we observed may have been due to chance. Our sample size was relatively small, which limited statistical power. We did not analyse the three exposed communities together due to considerable differences in exposure pathways (e.g., contamination of bore (ground) water versus municipal water), potential exposure to different mixtures of environmental chemicals, access to healthcare between communities, and a complex data structure (e.g., some individuals were part of families residing in one community but worked in multiple communities). We therefore analysed each community separately and took into account clustering at the household level.

5. Conclusions

In this cross-sectional analysis of PFAS exposure, serum lipids, and biomarkers of liver function in three Australian communities with known environmental PFAS contamination, we found some evidence for associations between PFAS and elevated cholesterol levels that are consistent with previous studies. PFAS serum concentrations were not clearly associated with liver function markers, or with self-reported history of type II diabetes, cardiovascular conditions, and liver conditions. Despite the similarity in PFAS-exposure profiles between these communities, there was little consistency in magnitude and/or direction of associations. However, we had low statistical power for detecting

small associations and the cross-sectional design of the study limited causal inference.

Credit author statement

NL: conceptualisation, investigation, methodology, software, formal analysis, visualisation, writing – original draft, writing – review and editing. KSS: conceptualisation, data curation, investigation, methodology, software, writing – review and editing. CD: conceptualisation, methodology, writing – review and editing. RML: conceptualisation, investigation, writing – review and editing. BA: conceptualisation, writing – review and editing. ACAC: conceptualisation, writing – review and editing. SMT: conceptualisation, data curation, project administration, writing – review and editing. IG: data curation, software. RH: data curation, investigation, writing – review and editing. HL: conceptualisation, writing – review and editing. JM: conceptualisation, writing – review and editing. JB: conceptualisation, writing – review and editing. SN: writing – review and editing. JL: conceptualisation, writing – review and editing. AL: conceptualisation, writing – review and editing. BAL: writing – review and editing. RJK: conceptualisation, supervision, writing – review and editing, funding acquisition. MDK: conceptualisation, supervision, data curation, investigation, writing – review and editing, funding acquisition.

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Ethics approval

The study was approved by the Northern Territory Department of Health and the Menzies School of Health Research Human Research Ethics Committee (protocol 2018–3130) and the ANU Human Research Ethics Committee (protocol 2016/707) in an initial ethics submission in 2016 and a series of amendments from 2017 to 2020.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Professor Martyn Kirk worked part-time for the Australian Government Department of Health between 2020 and 2022 on the Australian national COVID-19 response.

Data availability

Researchers interested in using aggregated data for the purposes of public health research should contact the PFAS Health Study team.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115621.

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