Journal of Nutrition & Intermediary Metabolism 18 (2019) 100102

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

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

Polyunsaturated fatty acid intake and lung function in a regional Australian population: A cross-sectional study with a nested case-control analysis

Ashley S. Fulton[a,1], Katherine L. Baldock[b], Alison M. Coates[a], Marie T. Williams[a], Peter R.C. Howe[a,c,2], Matthew T. Haren[d,3], Manohar L. Garg[c], Alison M. Hill[a,e,*]

[a] Alliance for Research in Exercise, Nutrition and Activity, Sansom Institute for Health Research, School of Health Sciences, University of South Australia, Adelaide, South Australia, 5000, Australia
[b] School of Health Sciences, University of South Australia, Adelaide, South Australia, 5000, Australia
[c] School of Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, New South Wales, 2308, Australia
[d] Sansom Institute for Health Research, Division of Health Sciences, University of South Australia, Adelaide, SA, Australia
[e] School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, 5000, Australia

HIGHLIGHTS

- Fried fish, but not other fish or PUFA intake, was negatively associated with lung function (FEV_1% predicted).
- Cases and controls had similar fish and PUFA intakes, and PUFA levels in phospholipids.
- In case-control analyses, COPD risk was associated with total LCn-3PUFA levels in phospholipids.
- Overall, n-3PUFA intake and status are not determinants of improved lung function in this regional Australian population.

ARTICLE INFO

Keywords:
Lung function
Chronic obstructive pulmonary disease
COPD
Polyunsaturated fatty acid
PUFA

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a progressive disease of the airways, underpinned by inflammation and worsening lung function. Omega-3 polyunsaturated fatty acids (n-3PUFA) can modulate inflammatory mechanisms and may therefore impact lung function in people with COPD. This observational, cross-sectional study of 577 adults in the Whyalla Intergenerational Study of Health (WISH), conducted during 2008–09 in regional South Australia, explored associations between fish and PUFA intakes (from food frequency questionnaires) and lung function (spirometry). It also included a nested case-control study which compared fish and PUFA intakes and plasma phospholipid PUFA levels between 40 people with COPD and 80 age-sex matched controls. In the whole population, linear regression models adjusted for age, sex, smoking status and education demonstrated a weak negative association between lung function (FEV_1% predicted) and consumption of fried fish (OR -0.12, 95% CI -0.22, −0.01, P = 0.026) but not fish prepared by other cooking methods or estimated intakes of PUFA. There was no association between fish or PUFA intakes and COPD risk. Compared to age and sex matched controls, cases had poorer lung function and a higher rate of smoking prevalence but did not differ in their intakes of fish or PUFA or their PUFA levels in plasma phospholipids. In this sub-population, we found a marginally significant association between COPD risk and total long chain n-3PUFA levels in plasma phospholipids (OR 1.22 95% CI 1.00–1.49, P = 0.046). Given the relatively small number of cases in this analysis, this finding should be interpreted with caution, especially given the lack of association with other markers of n-3PUFA intake or status. Taken together, our data suggest that n-3PUFA intake and status are not determinants of improved lung function in this regional Australian population.

* Corresponding author. School of Pharmacy & Medical Sciences, University of South Australia Adelaide, South Australia, 5000, Australia.
E-mail addresses: ashley.fulton@mymail.unisa.edu.au (A.S. Fulton), katherine.baldock@unisa.edu.au (K.L. Baldock), alison.coates@unisa.edu.au (A.M. Coates), marie.williams@unisa.edu.au (M.T. Williams), peter.howe@newcastle.edu.au (P.R.C. Howe), matthew.haren@gmail.com (M.T. Haren), manohar.garg@newcastle.edu.au (M.L. Garg), alison.hill@unisa.edu.au (A.M. Hill).
[1] Present address: Joanna Briggs Institute, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, Australia.
[2] Present address: University of Southern Queensland, Institute for Resilient Regions, Springfield Central, Queensland 4300, Australia.
[3] Present address: Test Kitchen SA Pty Ltd, Adelaide, Australia.

https://doi.org/10.1016/j.jnim.2019.100102
Received 15 July 2019; Received in revised form 9 October 2019; Accepted 15 October 2019
Available online 17 October 2019

Abbreviations

AA	arachidonic acid
ALA	alpha linoleic acid
BMI	body mass index
CI	confidence interval
COPD	chronic obstructive pulmonary disease
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FEV1	Forced expiratory volume in 1 s
FVC	forced vital capacity
g	grams
kJ	kilojoule
LA	linoleic acid
LCn-3PUFA	long chain omega-3 polyunsaturated fatty acids
mg	milligrams
n-3	omega-3
n-6	omega-6
OR	odds ratio
PUFA	polyunsaturated fatty acids
SPMs	specialised pro-resolving mediators
Total E	Total energy
WISH	Whyalla Intergenerational Study of Health
ΣLCn3PUFA	=EPA + DPA + DHA

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive disease of the airways, characterised by airway obstruction that is not fully reversible [1]. Symptoms of COPD (such as breathlessness, cough and chest tightness) worsen as lung function declines and frequency of exacerbations increases [1]. Inflammation and narrowing of the airway restricts airflow leading to decreased lung function and destruction of lung tissue, contributing to airflow limitation and gas transfer impairment [1].

Polyunsaturated fatty acids (PUFA) play vital roles in many systems in the body including inflammatory processes [2]. Metabolism of omega-6 (n-6) and omega-3 (n-3) PUFA results in pro-inflammatory and anti-inflammatory eicosanoid production [2] respectively. The long chain omega-3 polyunsaturated fatty acids (LCn-3PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found predominately in marine sources such as fish and krill [3,4], also produce specialised pro-resolving mediators (SPMs), viz. lipoxins, resolvins, protectins and maresins, that can act to resolve inflammation [5,6].

Despite a strong biological rationale, epidemiologic and interventional data demonstrating potential benefit of LCn-3PUFA supplementation in inflammatory conditions is limited and often conflicting [2,3,7–9]. In asthma, a number of studies have shown that LCn-3PUFA (in particular DHA) are able to reduce eosinophilic infiltration (an important modulator of the inflammatory response in asthma) and leukocytes in the lungs, thereby reducing inflammation and improving lung function [10]. In healthy people, the airway mucosa contains DHA, which facilitates the production of SPMs in the lung when an acute inflammatory response is required [6]. In people with chronic lung diseases such as asthma and cystic fibrosis the levels of mucosal DHA levels are decreased [11]. This reduction in mucosal DHA has detrimental effects on the production of SPMs, with several studies finding deficiencies in SPMs in asthma [6,12–14].

There is far less evidence for an effect of LCn-3PUFA supplementation in COPD than in asthma. Hsiao et al. [15] found that when lung tissue from patients with COPD was treated with Resolvin D1, chronic inflammation was effectively controlled. Mice exposed to long-term cigarette smoke were at lower risk of developing smoke-induced emphysema, and had a reduction in inflammation, oxidative stress and cell death when treated with Resolvin D1 treatment [15]. In a large (n= > 120,000 participants) cross sectional study, Varraso et al. [8] found that higher fish intake (≥4 servings per week) was associated with lower risk of COPD diagnosis; however, this relationship was no longer significant when adjusted for overall dietary pattern. This suggests that the benefits are a result of an overall healthier diet, rather than fish intake independently. Two recent systematic reviews have evaluated whether LCn-3PUFA intake can benefit individuals with COPD [16,17]. Atlantis and Cochrane [16] found limited evidence to support the use of LCn-3PUFA supplementation to reduce chronic inflammation and improve functional capacity. Similarly, we concluded that there was insufficient evidence to establish whether a relationship exists between LCn-3PUFA intake and prevalence or severity of COPD [17].

Given the limited and conflicting evidence for a beneficial effect of PUFA on lung function, this study had two aims: firstly, to explore the associations between amount of self-reported fish and fatty acid intake and lung function (including COPD diagnosis) in a regional Australian population (cross-sectional study); and secondly, to compare the consumption of fish (self-reported g/day) and blood levels of PUFA (plasma phospholipids) between individuals meeting criteria for COPD and age-sex matched controls (case control study).

2. Materials and methods

This was a cross sectional study with a nested case control study using data collected as part of the Whyalla Intergenerational Study of Health (WISH) conducted in 2008–09 in the regional South Australian city of Whyalla. The original objectives of WISH were to describe the health behaviours, issues and exposures of individuals and families in Whyalla in relation to respiratory and metabolic fitness, mental well-being, aging and men's health [18]. Whyalla was of interest because of its higher prevalence of respiratory conditions compared to other regional centres in South Australia [19]. One potential contributor may have been the particulate matter associated with iron ore processing and steel manufacturing that occurred in Whyalla in the years preceding the study, although evidence for this is inconclusive [19].

Ethics approval for this project was granted by the University of South Australia Human Research Ethics Committee (project number 0000032145) and the Aboriginal Research Ethics Committee of South Australia. Permission to access the data for this study was obtained from the WISH data custodian prior to data analysis. Participants in the WISH provided written consent at the time of enrolment for their data to be stored and analysed for future potentially unrelated projects.

2.1. Study population

A random sample of houses (n = 2500) in Whyalla were selected from the residential housing database of the State Planning Authority [18]. Of the 2500 randomly selected houses, 722 people completed the clinical protocol. Further details on the methodology of WISH have been reported previously [18]. Those eligible for inclusion in this cross-sectional study if they were aged ≥18 years and had completed a food frequency questionnaire and tests of pulmonary function. The 2013 GOLD criteria for COPD severity were used to classify participants (Forced expiratory volume in 1 s/forced vital capacity $FEV_1/FVC < 0.70$) [20].

The case control study included those from whom blood samples were available for measurement of plasma phospholipid fatty acid levels. Those who had significant post bronchodilator reversibility (an increase in FEV_1 (L) of > 12% and 200 mls post short acting

bronchodilator) [21], were pregnant at the time of data collection or had a diagnosis of a disease that may alter the absorption of n-3PUFA (e.g. Crohn's disease) were excluded. Two controls were individually matched to each COPD case (1 case: 2 controls) by sex and age (years at time of testing, within two years, except one participant aged 88 years who was matched within 10 years due to lack of appropriate controls). Where more than two controls were possible matches for a single case, the control participants with the best lung function (highest FEV_1% predicted) were selected. Where possible, participants were matched for smoking status as tobacco smoking is an important risk factor for COPD.

2.2. Outcomes

A portable ultrasonic sensor spirometer (ndd Easy-one™) with a laptop computer using EasyWare 2008 software (ndd Medizintech AG, Zurish Switzerland) was used to determine post bronchodilator FEV_1 (L) and FVC in accordance with the American Thoracic Society Guidelines [22]. FEV_1 percent of predicted was determined by calculating the predicted FEV_1 (L) using prediction equations described by Gore et al. [23] (participant age (years) and height (m) to predict FEV_1). The FEV_1/FVC ratio was determined by dividing the post bronchodilator FEV_1 (L) by post bronchodilator FVC (L).

2.3. Exposures

All participants completed a Cancer Council of Victoria Dietary Questionnaire for Epidemiological studies (DQES) version 3.0 or 3.1. Select data from the DQES were extracted: total energy (kJ/day), macronutrients (g/day of protein, carbohydrate, fat and alcohol), frequency of fish intake, g/day of fish and g/day of the following individual fatty acids: alpha linoleic acid (ALA), linoleic acid (LA), arachidonic acid (AA), EPA, docosapentaenoic acid (DPA), and DHA. The DQES has been shown to have good agreement with nutrient estimates from 24 h recalls (validation coefficients range 0.246–0.83), and these are comparable to other food frequency questionnaires [24]. Fatty acid values for earlier versions of the DQES have been validated in a sample of 4439 by Hodge et al. [25] who reported crude (uncorrected) correlation coefficients in the range of 0.18–0.40 for EPA, DHA and total n-3 FA,. In a comparatively smaller sample (n = 40) Zhang and Downie [26] compared estimates from the DQES version 3.2 against fatty acid profiles obtained from dried blood spot testing. They reported correlation coefficients of 0.12–0.40 for EPA, DPA, DHA, and total LC n-3 PUFA.

Fatty acid levels were measured in plasma phospholipids of the case-control subgroup only. Lipids were extracted from plasma according to Folch et al. [27], spotted onto thin layer chromatography plates and developed in a solvent system comprising of hexane:diethyl ether:acetic acid (85:15:1, by volume) for the separation of phospholipids from other lipid fractions. Phospholipid containing spots were scraped off the plates, trans-methylated and analysed by gas chromatography to determine fatty acid composition, expressed as a percentage of total fatty acids, as detailed by Garg et al. [28]. Only fatty acids with area > 0.1% were reported. Plasma phospholipids are a reliable marker of fatty acid levels in tissues, indicating intake for the previous two to three weeks [29], with reliability correlation coefficients for n-3 and n-6 fatty acids ranging from 0.62 to 0.72 [30]. LCn-3PUFA in plasma phospholipids also correlate well with dietary intake of fatty fish [31,32] and fish oil supplementation [33,34].

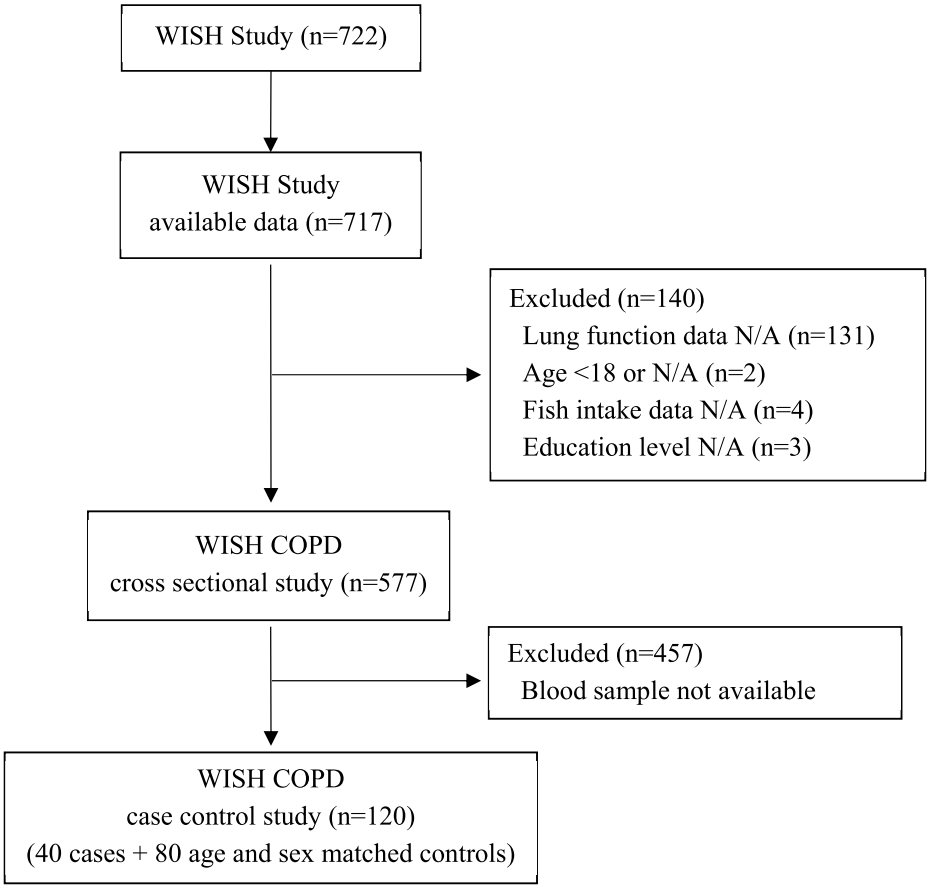

Fig. 1. Flow diagram of sample selection and exclusion process for both the cross sectional and case-control studies.

Table 1
Participant characteristics for the cross-sectional study, cases and controls.

	Cross-sectional (n = 577)	Cases (n = 40)	Controls (n = 80)
Age, years	49.2 ± 15.7	61.7 ± 13.1	60.5 ± 13.0
Sex M:F, n	244 : 333	15 : 25	32 : 48
BMI, kg/m^2	28.8 ± 6.3	28.5 ± 6.4	30.5 ± 5.7
COPD, n (%)	62 (10.7)		
Lung Function			
FEV$_1$/FVC	0.79 ± 0.08	0.63 ± 0.08	0.83 ± 0.05*
FEV$_1$% Predicted	92.6 ± 16.5	68.7 ± 18.1	97.4 ± 15.8*
Smoking status, n (%)			
Never smoked	250 (43.3)	7 (17.5)	43 (53.8)*
Ex-smoker	197 (34.1)	18 (45.0)	28 (35.0)
Currently smokes	130 (22.5)	15 (37.5)	9 (11.3)*
Bachelor degree obtained, n (%)	88 (15.3)	1 (2.5)	11 (13.8)

Data are mean ± SD.
*Significantly different to cases, P < 0.001.
BMI, body mass index; FEV$_1$/FVC: forced expiratory volume in 1 s/forced vital capacity; FEV$_1$% predicted: forced expiratory volume in 1 s percent of predicted.

2.4. Descriptive variables (including covariates)

Demographic information (age, education, and smoking status) was collected via a self-reported survey. Height was determined to within 1 mm using a wall stadiometer (Surgical and Medical Products No. 26SM, Mentone Education Supplies, Melbourne, Australia) and body mass was measured to the nearest 100 g with an electronic scale (Tanita BF-681). Body mass index was calculated using the formula mass (kg)/height (m)^2

2.5. Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) (Version 25. Chicago, SPSS Inc.), Statistical significance was set at $\alpha < 0.05$. The normality of the data was visually inspected and evaluated using the Shapiro Wilk and Kolmogorov-Smirnov tests.

The primary outcomes for the cross-sectional study were FEV$_1$/FVC (< 0.7 classified as COPD diagnosis) and FEV$_1$% predicted (an indicator of COPD severity). Linear regression analysis was used to examine the associations between fish consumption (g/day), fatty acid intake (g or mg/day) and the two continuous normally distributed lung function measures (FEV$_1$% predicted and FEV$_1$/FVC). Logistic regression was used to examine associations between fish consumption (g/day), fatty acid intake (g or mg/day) and COPD diagnosis (COPD risk). All models were adjusted for standard covariates known to impact COPD risk and dietary intake: age, sex, education (whether a bachelor's degree was obtained or not), and smoking status (never, ex-smoker and current smoker).

Differences in lung function, self-reported fish (g/day) and individual fatty acid (g/day) intake, and plasma phospholipid content of the following individual fatty acids LA, ALA, AA, EPA, DPA, DHA and sum of LCn-3PUFA (EPA + DPA + DHA) were assessed between cases and controls. Due to the non-normality of the data, Mann-Whitney U tests were used to determine differences between cases and controls for plasma phospholipid fatty acids, and consumption of fish, macronutrients and n-3 and n-6 PUFA. Independent samples t-tests were used to determine differences between cases and controls in the mean values for FEV$_1$/FVC, and FEV$_1$% predicted. Differences between categorical variables were assessed using the Chi-square test for independence. As associations between fish consumption (g/day), fatty acid intake (g or mg/day) and COPD diagnosis (COPD risk) were assessed in the larger population, in the case-control analysis we explored associations between plasma phospholipid fatty acid levels (measured in this subsample only) and COPD status (COPD risk) using logistic regression. Models were adjusted for age, sex, education and smoking status. Spearman's correlations were used to determine the relationship between fish and fatty acid intake (from FFQ) and fatty acid levels in plasma phospholipids.

3. Results

Of the 722 participants who completed the WISH clinical protocol, data were available for 717 (99.3%) adult participants. Of those 717 participants, 131 (18.3%) were excluded due to missing pulmonary function data, one because age could not be determined (0.14%), and one because they were less than 18 years of age (0.14%), leaving 584 (81.5%) eligible participants. Of those 584 participants, seven (1.2%) were excluded due to missing fish intake or education data. A total of 577 participants were included in the cross-sectional study. As blood samples were available from a proportion of these, 120 were included in the case control study: 40 with COPD (as indicated by FEV$_1$/FVC < 0.7) and 80 matched controls (see Fig. 1).

3.1. Participant characteristics

Participant characteristics for both the cross-sectional study and the case-control study are presented in Table 1. The mean age of participants in the cross-sectional study was younger than that of the cases or controls. A similar proportion of cases and controls were male (37.5% vs. 40% respectively), with no significant differences in age, indicating effective matching by these characteristics. As expected, the COPD group had significantly impaired lung function compared to controls (both FEV$_1$/FVC and FEV$_1$% predicted). A greater proportion of cases were current smokers, and fewer had never smoked.

3.2. Background diet, fish and omega-3 supplement consumption

Total energy, macronutrients (protein, carbohydrate and fat), alcohol and percent of total energy from individual macronutrients were similar between the three groups (Table 2). Total fish consumption in the cross-sectional study population was low, median daily consumption was ~27 g (Table 2). A large proportion of the population reported consuming fish less than once per week, including fried fish (76%), steamed, grilled or baked fish (79%) and tinned fish (65%) (Table S1), however ~30% reported consuming fish (of any type) two times per week. Most participants reported that they never consumed cod liver oil (96.4%) or fish oil supplements (74.5%, Table S1).; When combined, ~17% of the population reported consuming n-3 rich oil (from fish oil or cod liver oil) at least daily.

There were no differences between cases and controls for any dietary intake variables (Table 2), including frequency of fish consumption (Table S1). Although not statistically significant, estimated intake of total fish was higher in controls and likely contributed to the higher intake of EPA and DHA, as well as the sum of LCn-3PUFA (Table 2). Plasma phospholipid fatty acid levels were similar for cases and controls (Table 2).

3.3. Cross sectional study

FEV$_1$% predicted was inversely associated with intake of fried fish (P = 0.026) but was not related to intake of other types of fish or fatty acids (Table 3). To explain the relationship between fried fish and lung function, an additional analysis including SFA in the model was conducted, but this did not change the relationship substantially from that presented in Table 3 (B value −0.12, 95% CI -0.23, −0.02, P = 0.024). There were no statistically significant relationships between FEV$_1$/FVC or COPD risk (defined as FEV$_1$/FVC < 0.7) and fish or fatty acid

Table 2
Dietary intake and plasma phospholipid fatty acid levels for the cross-sectional study, cases and controls.

	Cross-sectional (n = 577)	Cases (n = 40)	Controls (n = 80)
Total energy (kJ/day)	8549.6 (3578.3)	8483.7 (2634.7)	8408.5 (3603.7)
Macronutrients, g/day			
Protein	94.7 (38.3), 18.7% total E	93.2 (34.9), 18.0% total E	99.6 (38.6), 18.2% total E
Carbohydrate	188.8 (80.8), 37.1% total E	192.0 (76.2), 38.0% total E	186.9 (63.4), 36.9% total E
Fat	86.7 (41.9), 38.0% total E	88.4 (32.5), 39.3% total E	89.6 (43.5), 38.0% total E
Saturated fat	30.2 (15.1)	29.2 (15.9)	30.8 (13.7)
Monounsaturated fat	36.0 (17.6)	36.4 (12.3)	39.3 (18.3)
Polyunsaturated fat	15.4 (9.0)	15.2 (8.4)	15.5 (9.0)
Alcohol	3.6 (15.5), 1.3% total E	1.6 (15.5), 0.6% total E	2.9 (14.0), 1.0% total E
Fish intake, g/day			
Fried	3.0 (7.6)	6.8 (18.1)	10.5 (18.1)
Steamed	4.0 (15.5)	4.2 (12.9)	4.1 (23.3)
Tinned	7.9 (13.6)	7.9 (15.9)	7.9 (13.6)
Total fish	27.0 (41.0)	20.2 (36.7)	29.0 (44.7)
Fatty acid intake, mg/day			
18:3n-3 ALA), g	1.8 (1.3)	1.7 (1.7)	1.8 (1.3)
18:2n-6 (LA), g	12.6 (8.0)	12.7 (7.6)	12.9 (7.4)
20:4n-6 (AA)	139.3 (87.8)	127.9 (86.4)	149.8 (83.8)
20:5n-3 (EPA)	71.7 (91.7)	56.3 (68.8)	78.6 (94.6)
22:5n-3 (DPA)	79.3 (62.8)	74.4 (51.4)	85.5 (76.2)
22:6n-3 (DHA)	127.4 (193.7)	93.8 (146.5)	130.9 (197.6)
ΣLCn3PUFA	272.5 (320.5)	230.9 (283.8)	315.0 (342.1)
Plasma phospholipid levels, % of total FA			
C18:3n-3		0.3 (0.1)	0.3 (0.1)
C18:2n-6		15.1 (5.1)	17.0 (3.9)
C20:4n-6		15.5 (12.1)	14.9 (11.2)
C20:5n-3		1.4 (0.9)	1.3 (0.9)
C22:5n-3		1.1 (0.3)	1.1 (0.3)
C22:6n-3		3.6 (1.1)	3.8 (1.8)
ΣLCn3PUFA		6.1 (2.2)	6.4 (2.6)

Data are median (IQR), % are medians.
Note g/day data not available for cod liver or fish oil supplements.
AA, arachidonic acid; ALA, alpha linoleic acid, DHA: docosaheaxaneoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; g, grams; kJ, kilojoule; LA, linolenic acid; LCn-3PUFA: long chain omega-3 polyunsaturated fatty acid; mg, milligrams; total E: total energy; ΣLCn3PUFA = EPA + DPA + DHA.

consumption (Table 3). Exclusion of smokers from the analysis did not substantially change the findings other than reduce the precision of the estimates due to a smaller sample size (n = 96).

Table 4
Association between COPD and plasma phospholipid fatty acid levels (n = 120).

Exposure	OR (95% CI)	P-value
C18:3n-3	4.26 (0.02, 895.46)	0.595
C18:2n-6	0.90 (0.77, 1.06)	0.214
C20:4n-6	1.00 (0.93, 1.08)	0.995
C20:5n-3	1.48 (1.00, 2.19)	0.050
C22:5n-3	0.94 (0.17, 5.33)	0.946
C22:6n-3	1.43 (1.00, 2.04)	0.051
ΣLCn3PUFA	1.22 (1.00, 1.49)	0.046

Adjusted for age, sex, smoking status & education.
CI, confidence interval; LCn-3PUFA: long chain omega-3 polyunsaturated fatty acid; ΣLCn3PUFA = EPA + DPA + DHA; OR, odds ratio.

3.4. Case control study

Table 4 summarises the results of the logistic regression analyses with respect to plasma phospholipid fatty acids. There were no associations between COPD risk and individual fatty acid levels in plasma phospholipids, however the sum of LCn-3PUFA was associated with an increased risk of COPD (P = 0.046).

The results of the correlation analyses between dietary intakes of fish (g/day) and fatty acid levels in plasma phospholipids are provided in Table 5. Plasma phospholipid levels of EPA and DHA were significantly and positively associated with tinned and total fish intake; DHA levels were also positively associated with intake of steamed, grilled or baked fish. The sum of plasma phospholipid LCn-3 PUFA (EPA, DPA and DHA) was positively correlated with the various self-reported intakes of fish, including steamed, grilled or baked fish, tinned and total fish (Table 3). Plasma phospholipid concentrations of EPA, DHA, and the sum of LCn-3 PUFA were significantly and positively associated with their respective estimated intake from FFQ (EPA r = 0.24, P = 0.009; DHA r = 0.31, P < 0.001; ΣLCn3PUFA r = 0.25, P = 0.007).

4. Discussion

This study had two aims, firstly to explore the relationships between measures of airway obstruction, fish and PUFA intake in a regional South Australian population at increased risk for respiratory disease and secondly, to compare consumption of fish and plasma phospholipid PUFA levels between people with COPD and age and sex matched controls. We found no compelling evidence for a relationship between lung function and intakes of either fish or PUFA in this population. Only

Table 3
Association between lung function (FEV_1% predicted and FEV_1/FVC), COPD and self-reported fish and fatty acid intake (n = 577).

Exposure	FEV_1% predicted B value (95% CI)	FEV_1/FVC B value (95% CI)	COPD OR (95% CI)
Fish intake			
Fried	−0.12 (−0.22, −0.01)	0.00 (0.0, 0.0)	1.00 (0.98, 1.02)
Steamed, grilled or baked	0.03 (−0.03, −0.1)	0.00 (0.0, 0.0)	0.99 (0.98, 1.01)
Tinned	0.04 (−0.02, 0.1)	0.00 (0.0, 0.0)	1.00 (0.99, 1.02)
Fatty acid intake			
18:3n-3 FFQ (ALA)	−0.35 (−1.54, 0.83)	0.00 (−0.01, 0.01)	0.94 (0.74, 1.2)
18:2n-6 FFQ (LA)	−0.01 (−0.2, 0.19)	0.00 (0.0, 0.0)	1.00 (0.96, 1.04)
20:4n-6 FFQ (AA)	0.01 (−0.0, 0.03)	0.00 (0.0, 0.0)	1.00 (0.99, 1.00)
20:5n-3 FFQ (EPA)	0.01 (0.0, 0.03)	0.00 (0.0, 0.0)	1.00 (1.00, 1.00)
22:5n-3 FFQ (DPA)	0.02 (0.0, 0.05)	0.00 (0.0, 0.0)	1.00 (0.99, 1.00)
22:6n-3 FFQ (DHA)	0.01 (0.0, 0.01)	0.00 (0.0, 0.0)	1.00 (1.00, 1.00)
ΣLCn-3PUFA	0.01 (0.0, 0.01)	0.00 (0.0, 0.0)	1.00 (1.00, 1.00)

Adjusted for age, sex, smoking status & education.
AA, arachidonic acid; ALA, alpha linoleic acid, CI, confidence interval; DHA: docosaheaxaneoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; FEV_1/FVC: forced expiratory volume in 1 s/forced vital capacity; FEV_1% predicted: forced expiratory volume in 1 s percent of predicted; LA, linolenic acid; LCn-3PUFA: long chain omega-3 polyunsaturated fatty acid; ΣLCn3PUFA = EPA + DPA + DHA; OR, odds ratio.

Table 5
Correlation results for self-reported fish intake and plasma phospholipid levels for cases and controls combined (n = 120)*.

Fish intake (FFQ)	C20:5n-3 (EPA)	C22:6n-3 (DHA)	ΣLCn3PUFA
Fried	−0.01 (0.938)	0.11 (0.247)	0.04 (0.648)
Steamed, grilled or baked	0.11 (0.226)	0.31 (0.001)	0.25 (0.007)
Tinned	0.21 (0.021)	0.23 (0.012)	0.24 (0.008)
Total fish	0.24 (0.008)	0.33 (0.000)	0.32 (0.000)

*Data are Spearman's rho (p-value).
DHA: docosahexaenoic acid; DPA: EPA: eicosapentaenoic acid; FFQ, food frequency questionnaire; LCn-3PUFA: long chain omega-3 polyunsaturated fatty acid; ΣLCn3PUFA = EPA + DPA + DHA.

one statistically significant association was found; fried fish consumption was inversely associated with FEV_1% predicted, however this is unlikely to be clinically meaningful (consumption of fried fish in the median quantile was less than once per month).

Few studies have investigated LCn-3PUFA status in people with COPD. A unique element of this study therefore was the inclusion of a biological marker of PUFA status (plasma phospholipid fatty acid levels) which allowed us to investigate associations with COPD risk in a sub-sample of cases and age and gender-matched controls. Consistent with our results, Wada et al. [35] reported no significant difference in levels of LCn-3PUFA (expressed as a percentage of total fatty acids) in people with COPD compared to the control group. Two larger cross-sectional studies have explored associations between serum fatty acid levels and lung health [36,37]. Shahar et al. [37] reported an OR of 0.62 for COPD between the 1st (0.78%–2.15% of total fatty acids) and the 4th (3.18%–8.88% of total fatty acids) quartile of DHA level (% of total fatty acids). No association was observed for EPA. Similarly, Kompauer et al. [36] reported significant positive associations between FEV_1% predicted, FVC and DHA (but not EPA) levels in serum phospholipids Interestingly, we observed no relationship with direct measures of lung function (FEV_1% predicted or FEV_1/FVC) and an increased risk of COPD with higher levels of total LCn-3PUFA in phospholipids. This contrasts with the proposed biological rationale for this study. One possible explanation is that a COPD diagnosis may lead to improved health behaviours including diet which may not have been effectively captured by FFQ. However, given the lack of significant associations with individual fatty acids or estimates of PUFA intake, it is likely that this is a type II error and should be interpreted with caution.

In this study, the average consumption of total LCn-3PUFA was 368 mg/day for the whole population. The median intake (273 mg/day) is 25% lower than the mean intake, indicating a skewed distribution. This is supported by the reported frequency by which fish and n-3PUFA rich oils were consumed, ~30% reported having fish at least twice per week and ~17% took supplements daily, however the majority were never or infrequent consumers. On average, this population consumed 36 g/day of fish, however the median intake was 27 g/day, which is similar to that reported in Australian adults (Females 24 g and Males 28 g of fish/seafood per day) [38]. LCn-3PUFA intake is also comparable to the Australian population (390 mg/day) [38]. Recent reports suggest that while LCn-3PUFA consumption in Australia has increased since 1995, only 20% of the adult population surveyed in 2011–12 met the National Health and Medical Research Council Suggested Dietary Targets (based on the 90th centile of intakes from the previous National Nutrition Survey 1995) and this was largely due to contributions from LCn-3PUFA supplements [38].

Intervention studies that report positive benefits of LCn-3PUFA in inflammatory diseases, such as cardiovascular disease, typically use higher doses of LCn-3PUFA, well above what is consumed in the typical Australian diet [38]. Calder et al. [7] suggests that more than 2 g/day LCn-3PUFA is required to elicit anti-inflammatory effects, particularly in conditions such as rheumatoid arthritis, which is recognised as an autoimmune inflammatory condition. A recent meta-analysis of randomised controlled trials reported that supplementation with n-3 PUFA (0.3–9.60 g/day; only two studies administered doses less than 2.2 g/day) significantly reduced levels of LTB_4 in patients with rheumatoid arthritis and improved markers of disease activity including early morning stiffness, pain and tenderness [39]. Comparatively fewer studies have evaluated changes in inflammatory markers following EPA/DHA supplementation alone in people with COPD [16,17,40]. This makes it difficult to ascertain the optimal dose for reducing inflammation in this condition. However, based on recommendations for cardiovascular disease and arthritis, it is likely that the level of consumption in this population is well below the required intake for general wellbeing, let alone enough to mediate anti-inflammatory actions in people with COPD [41].

Most participants reported that they never consumed fish or ate it less than once per week, and with only small numbers of participants in this study reporting (at least) daily consumption of cod liver oil (2%), fish oil supplements (16%) or fish (0.5%), it was not possible to explore with confidence the effect of higher frequencies of intake (e.g. ≥ four times per week vs < once per week) on lung function. Two large population studies have evaluated whether frequency of fish or n-3PUFA supplementation influences lung function, with contradictory outcomes. A cross sectional study by Ng et al. [42] in Singapore reported that after adjustment for multiple confounders (including other dietary variables) both dietary intake of fish at least three times per week and daily supplemental intake of n-3PUFA were positively and independently associated with pulmonary function (FEV_1 and FVC, respectively) in a sub-sample (n = 2478) of the Singapore Longitudinal Ageing study. Comparatively, while a large US-based cohort study (n = 120,175 from the Nurses' Health Study and Health Professionals Follow-Up Study) reported participants who ate fish ≥ 4 servings/week had a lower risk of COPD diagnosis compared with those who ate < 1 serving of fish per week (Hazard ratio 0.71; 95% CI 0.54, 0.94), this relationship was lost when adjusted for overall dietary pattern [8]. More recently, Lemoine and colleagues [43] reported no statistically significant effects of EPA + DHA intake on respiratory symptoms in a cohort of adults with COPD from the United States.

While there was a statistically significant negative association between FEV_1% predicted and fried fish consumption it is unlikely to be clinically meaningful. There are several likely explanations for this relationship. Firstly, the result is a random error due to multiplicity of the analysis and no relationship exists. Secondly, the results may be influenced by very low fried fish consumption in the population (52% consumed fried fish less than once per month, with a mean intake of ~10 g/day), meaning that it is difficult to determine if a true relationship exists. Thirdly, a significant relationship may exist and those with worse lung function consume more fried fish. The biological mechanism for this relationship is unclear but may relate to the potential for frying to induce lipid peroxidation and elevate reactive aldehydes [44] which at high levels may exacerbate oxidative stress and inflammation [45].

This study has several limitations. In this relatively small sample of free living adults in a specific geographic locale, there were a disproportionate number of participants who did not consume any fish or fish oil supplements. Airways obstruction and COPD diagnosis was based upon a single spirometric measure reviewed by a physician (FEV1/FVC < 0.70) consistent with criteria available at the time of undertaking the analysis [20]. Diagnostic criteria have since evolved to include symptoms [1]. Analysis of available data for frequency of self-report asthma and symptoms for this study (frequency of wheezing and coughing, sputum production and increasing breathlessness) revealed no differences between cases and controls.

Tobacco smoking is an important risk factor for COPD [1], therefore smoking history is a likely confounding factor. While attempts were made to match cases and controls by smoking status, this was challenging as most of the age and sex matched controls were non-smokers ('never' smoked). Smoking status was included in the statistical models

where possible to account for this difference. It is possible that a selection bias may have been introduced by removing participants with missing data, the most frequent being missing pulmonary function data (n = 131). Reasons for missing lung function data were not reported, but it is possible that people with lower function were unable to complete or declined the required spirometry manoeuvre. Other limitations include self-report of food intake data, which may be influenced by under-reporting [46,47]. Compared to other studies [30], we observed lower correlation coefficients between fatty acid/fish intake and fatty acid levels measured in plasma phospholipids. This may relate to the use of FFQ which capture dietary intake over a longer-term (12 months) rather than over the previous two to three weeks which is reflected in plasma phospholipids [29], It also suggests that at the time of data collection actual dietary intake of LCn-3PUFA was different to that reported, and captured by FFQ. Finally, we did not evaluate the overall 'healthfulness' of the diet, which as discussed in a recent review, may be a key determinant of COPD development and progression [40].

5. Conclusions

In this population at elevated risk for respiratory disease, the relationships between fish/PUFA intakes and lung function were unconvincing and not likely to be clinically meaningful. Furthermore, we observed no differences between individuals with COPD and controls for plasma phospholipid fatty acid levels or intakes of fish or PUFA. Future studies should include a larger number of participants with a wider range of LCn-3PUFA intakes in order to determine the likelihood of a true relationship.

Supplementary materials

The following are available; Table S1: Self-reported frequency of fish and supplement intake for the cross-sectional study, cases and controls, n (%).

Funding

This work was supported by the Premiers Science Research Fund through the South Australian Population Health Intergenerational Research (SAPHIRe) collaborative and the Department of Health and Ageing. The funding bodies played no role in the design of the study, data collection, analyses, or interpretation. ASF was supported by an APA scholarship.

CRediT authorship contribution statement

Ashley S. Fulton: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Katherine L. Baldock:** Formal analysis. **Alison M. Coates:** Conceptualization, Writing - review & editing. **Marie T. Williams:** Conceptualization, Writing - review & editing. **Peter R.C. Howe:** Conceptualization, Writing - review & editing. **Matthew T. Haren:** Investigation, Funding acquisition, Writing - review & editing. **Manohar L. Garg:** Investigation, Resources, Writing - review & editing. **Alison M. Hill:** Conceptualization, Methodology, Formal analysis, Writing - original draft.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

The investigators thank Professor Graham Giles of the Cancer Epidemiology Centre of the Cancer Council Victoria, for permission to use the Dietary Questionnaire for Epidemiological Studies: The Cancer Council Victoria, 2002.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnim.2019.100102.

References

[1] Global Initiative for Chronic Obstructive Lung Disease, Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Lung Disease, (2019) https://goldcopd.org/wp-content/uploads/2018/11/GOLD-2019-v1.7-FINAL-14Nov2018-WMS.pdf , Accessed date: 2 June 2019.
[2] P.C. Calder, n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases, Am. J. Clin. Nutr. 83 (6) (2006) 1505–1519S.
[3] R. Wall, R.P. Ross, G.F. Fitzgerald, C. Stanton, Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids, Nutr. Rev. 68 (5) (2010) 280–289.
[4] A.P. Simopoulos, Evolutionary Aspects of the Dietary Omega-6/Omega-3 Fatty Acid Ratio: Medical Implications, Evolutionary Thinking in Medicine: from Research to Policy and Practice Springer, (2016), pp. 119–134.
[5] C. Serhan, N. Chiang, T.E. Van Dyke, Resolving inflammation:dual anti-inflammatory and pro-resolution lipid mediators, Nat. Rev. Immunol. 8 (5) (2008) 349–361.
[6] M.G. Duvall, B.D. Levy, DHA-and EPA-derived resolvins, protectins, and maresins in airway inflammation, Eur. J. Pharmacol. 785 (2016) 144–155.
[7] P.C. Calder, Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br. J. Clin. Pharmacol. 75 (3) (2012) 645–662.
[8] R. Varraso, R.G. Barr, W.C. Willett, F.E. Speizer, C.A. Camargo, Fish intake and risk of chronic obstructive pulmonary disease in 2 large US cohorts, Am. J. Clin. Nutr. 101 (2) (2015) 354–361.
[9] A.M. Giudetti, R. Cagnazzo, Beneficial effects of n-3 PUFA on chronic airway inflammatory diseases, Prostaglandins Other Lipid Mediat. 99 (3–4) (2012) 57–67.
[10] C. Beermann, S. Neumann, D. Fußbroich, S. Zielen, R. Schubert, Combinations of distinct long-chain polyunsaturated fatty acid species for improved dietary treatment against allergic bronchial asthma, Nutrition 32 (11) (2016) 1165–1170.
[11] S.D. Freedman, P.G. Blanco, M.M. Zaman, J.C. Shea, M. Ollero, I.K. Hopper, D.A. Weed, A. Gelrud, M.M. Regan, M. Laposata, Association of cystic fibrosis with abnormalities in fatty acid metabolism, N. Engl. J. Med. 350 (6) (2004) 560–569.
[12] B.D. Levy, P. Kohli, K. Gotlinger, O. Haworth, S. Hong, S. Kazani, E. Israel, K.J. Haley, C.N. Serhan, Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness, J. Immunol. 178 (1) (2007) 496–502.
[13] J. Miyata, K. Fukunaga, R. Iwamoto, Y. Isobe, K. Niimi, R. Takamiya, T. Takihara, K. Tomomatsu, Y. Suzuki, T. Oguma, Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma, J. Allergy Clin. Immunol. 131 (2) (2013) 353–360.
[14] R.A. Hasan, E. O'Brien, P. Mancuso, Lipoxin A4 and 8-isoprostane in the exhaled breath condensate of children hospitalized for status asthmaticus, Pediatr. Crit. Care Med. 13 (2) (2012) 141–145.
[15] H.-M. Hsiao, T.H. Thatcher, R.A. Colas, C.N. Serhan, R.P. Phipps, P.J. Sime, Resolvin D1 reduces emphysema and chronic inflammation, Am. J. Pathol. 185 (12) (2015) 3189–3201.
[16] E. Atlantis, B. Cochrane, The association of dietary intake and supplementation of specific polyunsaturated fatty acids with inflammation and functional capacity in chronic obstructive pulmonary disease: a systematic review, Int. J. Evid. Based Healthc. 14 (2) (2016) 53–63.
[17] A.S. Fulton, A.M. Hill, M.T. Williams, P.R. Howe, A.M. Coates, Paucity of evidence for a relationship between long-chain omega-3 fatty acid intake and chronic obstructive pulmonary disease: a systematic review, Nutr. Rev. 73 (9) (2015) 612–623.
[18] M.T. Haren, G. Misan, T.-J. Paterson, R.E. Ruffin, J.F. Grant, J.D. Buckley, P.R. Howe, J. Newbury, A.W. Taylor, R.A. McDermott, Abdominal adiposity and obstructive airway disease: testing insulin resistance and sleep disordered breathing mechanisms, BMC Pulm. Med. 12 (1) (2012) 31.
[19] Government of South Australia, Whyalla Health Impact Study, Department of Health, Adelaide South Australia, 2007.
[20] J. Vestbo, S.S. Hurd, A.G. Agustí, P.W. Jones, C. Vogelmeier, A. Anzueto, P.J. Barnes, L.M. Fabbri, F.J. Martinez, M. Nishimura, R.A. Stockley, D.D. Sin, R. Rodriguez-Roisin, Global strategy for the diagnosis, management, and prevention of chronic obstructive, Pulmonary Disease 187 (4) (2013) 347–365.
[21] Global Initiative for Asthma, GINA Report, Global Strategy for Asthma Management and Prevention, (2019) https://ginasthma.org/wp-content/uploads/2019/06/GINA-2019-main-report-June-2019-wms.pdf , Accessed date: 15 June 2019.
[22] M. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, R. Crapo, P. Enright, C. van der Grinten, P. Gustafsson, Standardization of spirometry, 1994 update. American thoracic society, Am. J. Respir. Crit. Care Med. 152 (3) (1995) 1107–1136.
[23] C. Gore, A. Crockett, D. Pederson, M. Booth, A. Bauman, N. Owen, Spirometric standards for healthy adult lifetime nonsmokers in Australia, Eur. Respir. J. 8 (5) (1995) 773–782.
[24] J.K. Bassett, D.R. English, M.T. Fahey, A.B. Forbes, L.C. Gurrin, J.A. Simpson, M.T. Brinkman, G.G. Giles, A.M. Hodge, Validity and calibration of the FFQ used in the Melbourne collaborative cohort study, Public Health Nutr. 19 (13) (2016) 2357–2368.
[25] A.M. Hodge, J.A. Simpson, R.A. Gibson, A.J. Sinclair, M. Makrides, K. O'Dea, D.R. English, G.G. Giles, Plasma phospholipid fatty acid composition as a biomarker

of habitual dietary fat intake in an ethnically diverse cohort, Nutr. Metab. Cardiovasc. Dis. 17 (6) (2007) 415–426.
[26] A.C. Zhang, L.E. Downie, Preliminary validation of a food frequency questionnaire to assess long-chain omega-3 fatty acid intake in eye care practice, Nutrients 11 (4) (2019) 817.
[27] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1) (1957) 497–509.
[28] M.L. Garg, J. Leitch, R.J. Blake, R. Garg, Long-chain n − 3 polyunsaturated fatty acid incorporation into human atrium following fish oil supplementation, Lipids 41 (12) (2006) 1127–1132.
[29] L. Serra-Majem, M. Nissensohn, N.C. Øverby, K. Fekete, Dietary methods and biomarkers of omega 3 fatty acids: a systematic review, Br. J. Nutr. 107 (2012) S64–76.
[30] A. Zeleniuch-Jacquotte, V. Chajes, A. Van Kappel, E. Riboli, P. Toniolo, Reliability of fatty acid composition in human serum phospholipids, Eur. J. Clin. Nutr. 54 (5) (2000) 367–372.
[31] M. Kobayashi, S. Sasaki, T. Kawabata, K. Hasegawa, M. Akabane, S. Tsugane, Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged Japanese men, Eur. J. Clin. Nutr. 55 (8) (2001) 643–650.
[32] A. Philibert, C. Vanier, N. Abdelouahab, H.M. Chan, D. Mergler, Fish intake and serum fatty acid profiles from freshwater fish, Am. J. Clin. Nutr. 84 (6) (2006) 1299–1307.
[33] K.D. Stark, G. Mulvad, H.S. Pedersen, E.J. Park, E. Dewailly, B.J. Holub, Fatty acid compositions of serum phospholipids of postmenopausal women: a comparison between Greenland Inuit and Canadians before and after supplementation with fish oil, Nutrition 18 (7) (2002) 627–630.
[34] M. Laidlaw, B.J. Holub, Effects of supplementation with fish oil–derived n − 3 fatty acids and γ-linolenic acid on circulating plasma lipids and fatty acid profiles in women, Am. J. Clin. Nutr. 77 (1) (2003) 37–42.
[35] H. Wada, S.-i. Hagiwara, E. Saitoh, R. Ieki, Y. Yamamoto, I. Adcock, H. Goto, Up-regulation of blood arachidonate (20:4) levels in patients with chronic obstructive pulmonary disease, Biomarkers 17 (6) (2012) 520–523.
[36] I. Kompauer, H. Demmelmair, B. Koletzko, G. Bolte, J. Linseisen, J. Heinrich, Association of fatty acids in serum phospholipids with lung function and bronchial hyperresponsiveness in adults, Eur. J. Epidemiol. 23 (3) (2008) 175–190.
[37] E. Shahar, L.L. Boland, A.R. Folsom, M.s. Tockman, P.G. McGovern, J.H. Eckfeldt, Docosahexaenoic Acid and smoking-related chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 159 (6) (1999) 1780–1785.
[38] B.J. Meyer, Australians are not meeting the recommended intakes for omega-3 long chain polyunsaturated fatty acids: results of an analysis from the 2011–2012 national nutrition and physical activity Survey, Nutrients 8 (3) (2016) 111.
[39] A. Gioxari, A.C. Kaliora, F. Marantidou, D.P. Panagiotakos, Intake of ω-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: a systematic review and meta-analysis, Nutrition 45 (2018) 114–124 e4.
[40] E. Scoditti, M. Massaro, S. Garbarino, D.M. Toraldo, Role of diet in chronic obstructive pulmonary disease prevention and treatment, Nutrients 11 (6) (2019) 1357.
[41] P.C. Calder, Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br. J. Clin. Pharmacol. 75 (3) (2013) 645–662.
[42] T.P. Ng, M. Niti, K.B. Yap, W.C. Tan, Dietary and supplemental antioxidant and anti-inflammatory nutrient intakes and pulmonary function, Public Health Nutr. 17 (9) (2014) 2081–2086.
[43] C.M. Lemoine S, E.P. Brigham, H. Woo, C.K. Hanson, M.C. McCormack, A. Koch, N. Putcha, N.N. Hansel, Omega-3 fatty acid intake and prevalent respiratory symptoms among U.S. adults with COPD, BMC Pulm. Med. 19 (1) (2019) 97.
[44] K.S. Leung, J.-M. Galano, T. Durand, J.C.-Y. Lee, Profiling of omega-polyunsaturated fatty acids and their oxidized Products in salmon after different cooking methods, Antioxidants 7 (8) (2018) 96.
[45] L. Tao, Oxidation of polyunsaturated fatty acids and its impact on food quality and human health, Adv Food Technol Nutr Sci Open J 1 (6) (2015) 135–142.
[46] L. Gemming, Y. Jiang, B. Swinburn, J. Utter, C.N. Mhurchu, Under-reporting remains a key limitation of self-reported dietary intake: an analysis of the 2008/09 New Zealand Adult Nutrition Survey, Eur. J. Clin. Nutr. 68 (2) (2014) 259–264.
[47] L. Gemming, C.N. Mhurchu, Dietary under-reporting: what foods and which meals are typically under-reported, Eur. J. Clin. Nutr. 70 (5) (2016) 640–641.