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Sex-dependent association between erythrocyte *n*-3 PUFA and type 2 diabetes in older overweight people

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Abstract

The association between *n*-3 PUFA intake and type 2 diabetes (T2D) is unclear, and studies relating objective biomarkers of *n*-3 PUFA consumption to diabetic status remain limited. The aim of this study was to determine whether erythrocyte *n*-3 PUFA levels (*n*-3 index; *n*-3I) are associated with T2D in a cohort of older adults (*n* 608). To achieve this, the *n*-3I (erythrocyte %EPA + %DHA) was determined by GC and associated with fasting blood glucose; HbA1c; and plasma insulin. Insulin resistance (IR) was assessed using the homeostatic model assessment of insulin resistance (HOMA--IR). OR for T2D were calculated for each quartile of *n*-3I. In all, eighty-two type 2 diabetic (46·3 % female; 76·7 (SD 5·9) years) and 466 non-diabetic (57·9 % female; 77·8 (SD 7·1) years) individuals were included in the analysis. In overweight/obese (BMI ≥ 27 kg/m^2), the prevalence of T2D decreased across ascending *n*-3I quartiles: 1·0 (reference), 0·82 (95 % CI 0·31, 2·18), 0·56 (95 % CI 0·21, 1·52) and 0·22 (95 % CI 0·06, 0·82) ($P_{trend} = 0·015$). A similar but non-significant trend was seen in overweight men. After adjusting for BMI, no associations were found between *n*-3I and fasting blood glucose, HbA1c, insulin or HOMA-IR. In conclusion, higher erythrocyte *n*-3 PUFA status may be protective against the development of T2D in overweight women. Further research is warranted to determine whether dietary interventions that improve *n*-3 PUFA status can improve measures of IR, and to further elucidate sex-dependent differences.

Key words: *n*-3 Index: Type 2 diabetes: Obesity: PUFA: Insulin resistance

Obesity is strongly associated with the development of insulin resistance (IR) and subsequent progression to type 2 diabetes (T2D), a growing public health problem, with worldwide prevalence expected to more than double between 2000 and 2030[1]. Dietary consumption of *n*-3 PUFA, particularly marine-based EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), has been shown to be effective in controlling dyslipidaemias and inflammation in diabetics[2]; however, the role of *n*-3 PUFA in the prevention of T2D is unclear.

A Cochrane review reported that *n*-3 PUFA supplements did not affect glycaemic control in T2D; however, a non-significant reduction in fasting insulin levels was noted[3]. Prospective cohort studies investigating *n*-3 PUFA intake and diabetes show mixed findings[4–8]. An ecological study investigating data across forty-one countries found that, in countries with a high prevalence of obesity, those with a higher intake of fish and seafood products had a lower prevalence of T2D than those with lower fish intake, showing a potentially weight-dependent effect[9].

To date, most studies of the relationship between *n*-3 PUFA levels and risk of T2D have only investigated dietary intake of *n*-3 PUFA, with few incorporating biochemical markers of *n*-3 PUFA intake. The *n*-3 index (*n*-3I), defined as the sum of EPA and DHA present in the erythrocyte membranes expressed as a percentage of the total fatty acids, has been documented to be a reliable marker of long-term dietary *n*-3 PUFA intake and is a good indicator of tissue levels[10]. The *n*-3I has been shown to be negatively correlated with IR in obese children[11]; however, the relationship between *n*-3 PUFA status and T2D in older persons is unknown. The aim of this study was to determine whether *n*-3 PUFA status is associated with T2D in older Australians, and to test the hypothesis that the *n*-3I is negatively correlated with IR in obese older adults.

Methods

Subjects

The present study was a sub-study of the Retirement Health and Lifestyle Study (RHLS), a cross-sectional study of older Australians living in the Central Coast of NSW, Australia.

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; IR, insulin resistance; *n*-3I, *n*-3 index; RHLS, Retirement Health and Lifestyle Study; T2D, type 2 diabetes.

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Participants were eligible for the RHLS study if they were ≥65 years; if their primary residence was located within the Wyong or Gosford Local Government Areas; and if they had been living in their current address for ≥12 months. Exclusion criteria for the RHLS included not living independently or residing in a communal setting other than a retirement village; if another member of their household was taking part in the study; having language and/or other communicative difficulties that limited participation; being cognitively impaired; and/or unable to provide informed consent. Participants were included in the present study if they had anthropometric measures and blood samples collected; if their diabetic status could be determined; and if they did not report having type 1 diabetes or insulin dependence. This research was conducted according to the Declaration of Helsinki. Participants provided written informed consent, and ethics approval for the study was obtained from the University of Newcastle Human Research Ethics Committee (reference no. H-2008-0431) and the Northern Sydney Central Coast Health Human Research Ethics Committee (reference no. 1001-031M). An overview of participant recruitment is presented in Fig. 1.

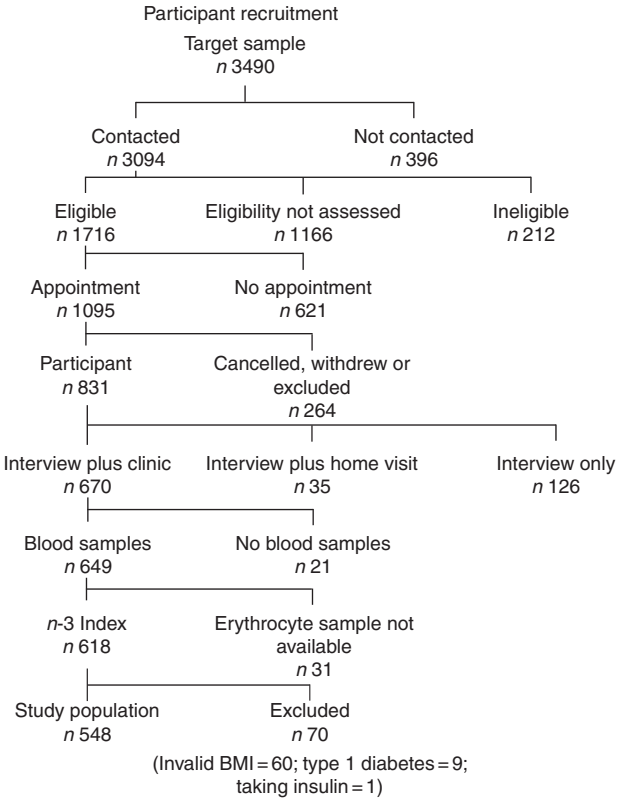

Fig. 1. Overview of participant recruitment.

Demographics and medical history

Demographic data were collected in an interviewer-administered questionnaire (IAQ). Self-administered questionnaires were used to collect information on medical history and medication/supplement use.

Dietary intake and physical activity

Nutrient intake was determined using a self-administered semi-quantitative FFQ, which was adapted from a validated FFQ[12]. Dietary intake was analysed using FoodWorks Professional Edition 2009 version 6.0.2562 (Xyris software), and mean daily energy (kJ/kcal) and macronutrient intakes were calculated using the following databases: Abbott products, AusFoods (brand) 2006, AusNut (all foods) 2007, Australia (fatty acids) and The New Zealand Vitamin and Mineral Supplement 1999.

Physical activity was assessed as part of the IAQ using questions that measured the frequency, duration and intensity of physical activity undertaken in the previous week. Questionnaire items captured information about time spent doing household chores and gardening; gentle exercise such as walking; moderate and vigorous physical activity; and strength training. Responses were used to categorise participants according to their level of physical activity: sedentary, low, moderate or vigorous. The questions used were adapted from validated surveys measuring physical activity[13–15].

Anthropometrics

Anthropometric measures were taken by trained research officers. Height was measured with shoes off, to the nearest mm using a portable stadiometer (design no. 1013522 Surgical and Medical Products). Weight in light clothing and without shoes was measured to the nearest 100 g on digital scales (Tanita HD-316 (Tanita Health Equipment H.K. Ltd) and Wedderburn UWPM150 (W.W. Wedderburn Pty Ltd)). Waist circumference was measured with a non-elastic flexible measuring tape at the mid-point between the iliac crest and the costal margin in the mid-auxiliary line, with the participant in the standing position. All measures were taken twice, with a third measure taken if there were discrepancies outside of specified tolerance limits. Height and weight were used to calculate BMI (kg/m^2). BMI was used to categorise participants into a healthy/underweight group (BMI < 27 kg/m^2) and an overweight/obese group (BMI ≥ 27 kg/m^2). This BMI cut-off was selected as evidence suggests that in adults aged >70 years the risk of all-cause mortality is only increased for individuals with BMI over 27 kg/m^2[16].

Blood samples and biochemical analysis

Blood samples were collected by a trained phlebotomist following a minimum of a 10-h overnight fast. Blood samples were analysed by Hunter Area Pathology Service for fasting insulin, fasting blood glucose and HbA1c levels using standardised laboratory protocols. Fasting insulin (mU/l) and blood glucose levels (BGL) (mmol/l) were used to calculate homeostatic model assessment of insulin resistance (HOMA-IR) scores (fasting insulin × fasting glucose/22·5).

Fatty acid composition was analysed by GC based on methods established by Lepage and Roy[17]. Erythrocyte pellets were washed three times by adding 5 ml of distilled water, centrifuging at 3000 ***g*** at 4°C for 10 min and discarding the supernatant to obtain erythrocyte membranes. 2 ml of methanol–toluene mix (4:1 by volume) with C19 : 0 as an internal standard was added to the membrane pellet. While vortexing, 200 µl of acetyl chloride was added drop-wise and the sample was heated at

100°C for 60 min. Samples were cooled to room temperature by running under distilled water, and the reaction was stopped by adding 5 ml of 6 % K_2CO_3; the sample was vortexed to mix and then centrifuged at 3000 ***g*** for 10 min to separate the layers. The upper layer containing toluene and fatty acid methyl esters was transferred to a 2-ml glass vial, and the vial crimp was sealed with a teflon-lined cap for GC analysis.

Methylated fatty acid samples were analysed by GC using a fixed carbon-silica column 30 m × 0·25 mm (DB-225) (J and W Scientific). The GC was equipped with a flame ionisation detector, autosampler and autodetector. Injector and detector ports were set at 250°C. Oven temperature was programmed for 170°C for 2 min, and then increased 10°C/min up to 190°C where it remained stationary for 1 min. Temperature was then increased 3°C/min up to 220°C, which was maintained for a total run time of 30 min per sample. A split ratio of 10:1 and an injection volume of 3 µl was used. A known fatty acid mixture was used to identify peaks according to retention time. Fatty acid concentration was determined using a Hewlett Packard 6890 Series GC with Chemstations version A. 04.02.

The *n*-3I was calculated by summing the erythrocyte membrane EPA and DHA, and the result was expressed as a percentage of the total fatty acids present[18]. Diabetic status was defined by participants having a fasting blood glucose >7·0 mmol/l or taking diabetic medication[19]. IR was categorised according to HOMA-IR score >3·8[20].

Statistical analysis

Data were assessed for normality, with the majority of data normally distributed, and thus parametric analyses were used. Descriptive statistics were calculated for all participants (total group), by sex, diabetic status (diabetic *v.* non-diabetic) and weight status (overweight/obese *v.* healthy/underweight). Differences between groups were assessed using independent sample *t* tests. Participants were categorised into quartiles according to their *n*-3I. Differences in the proportion of people with T2D and IR across the quartiles of *n*-3I were reported as OR (95 % CI), using the lowest quartile as a reference. Bivariate and multivariate relationships between fasting plasma insulin, fasting plasma glucose, HbA1c, HOMA-IR scores and *n*-3I were analysed using Pearson's correlations and hierarchical regression analyses. Statistical analyses were conducted using SPSS software version 17.0 (SPSS Inc.). Statistical significance was set as $P < 0{\cdot}05$. All values were reported as the mean values and standard deviation.

Results

A total of 618 RHLS participants were recruited to the current study. In all, seventy participants were excluded: sixty had no valid BMI recorded; nine reported a diagnosis of type 1 diabetes; and one reported using insulin. Following application of exclusion criteria, 548 participants were included in the analyses. A total of 56·1 % (*n* 308) of participants were female, and 60·9 % (*n* 334) of participants were classified as being overweight/obese (BMI ≥ 27 kg/m^2). Men had a higher fasting plasma glucose level (5·7 (SD 1·1) mmol/l *v.* 5·4 (SD 1·0) mmol/l; $P < 0{\cdot}001$) and larger waist circumference (104·1 (SD 12·1) cm *v.* 94·1 (SD 12·6) cm; $P < 0{\cdot}001$) than women; therefore, participants were stratified by sex for all analyses. In all, eighty-two participants (15·0 %) had T2D (plasma glucose level >7·0 mmol/l and/or the participant self-reported taking diabetic medications). Participants with diabetes had significantly higher BMI and waist circumferences than participants without diabetes, as well as significantly higher fasting BGL, HbA1c, fasting plasma insulin levels and HOMA-IR scores (Table 1). There were no significant associations found between reported levels of physical activity and either diabetic status or *n*-3I (data not presented).

Table 1. Characteristics of participants by sex and diabetic status*
(Mean values and standard deviations)

	Total (*n* 548)					Men (*n* 240)					Women (*n* 308)				
	Diabetic* (*n* 82)		Non-diabetic (*n* 466)			Diabetic* (*n* 44)		Non-diabetic (*n* 196)			Diabetic* (*n* 38)		Non-diabetic (*n* 270)		
	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*
Age (years)	76·7	5·9	77·8	7·1	–	75·6	6·4	77·5	6·7	–	78·0	5·1	78·1	7·4	–
Sex (M/F)	44/38		196/270		–	44/0		196/0		–	0/38		0/270		–
Ethnicity															
Caucasian (*n*)	79		452		–	4		192		–	36		260		–
Aboriginal or Pacific Islander (*n*)	1		4		–	0		0		–	1		4		–
Asian (*n*)	1		1		–	0		0		–	1		1		–
Don't know/no response (*n*)	1		9		–	1		4		–	0		5		–
Height (m)	1·6	0·1	1·6	0·1	<0·05	1·7	0·1	1·7	0·1	–	1·6	0·1	1·6	0·1	–
Weight (kg)	84·5	16·6	74·5	14·8	<0·001	90·7	16·8	81·4	14·2	<0·001	77·2	13·3	69·5	13·1	<0·01
BMI (kg/m^2)	30·9	4·7	28·1	4·8	<0·001	30·6	4·5	28·1	4·4	<0·01	31·3	13·3	28·2	5·1	<0·001
WC (cm)	107·2	12·7	96·9	12·8	<0·001	110·6	12·0	102·7	11·6	<0·001	103·7	12·7	92·7	11·9	<0·001
Fasting plasma glucose (mmol/l)	7·3	1·4	5·3	0·6	<0·001	7·4	1·3	5·4	0·6	<0·001	7·2	1·4	5·2	0·5	<0·001
HbA1c (%)	6·8	0·9	5·7	0·4	<0·001	6·8	0·9	5·7	0·4	<0·001	6·9	1·0	5·7	0·3	<0·001
HbA1c (mmol/mol)	51·0	4·0	39·0	10·0	<0·001	51·0	4·0	39·0	10·0	<0·001	52·0	4·0	39·0	11·0	<0·001
Fasting insulin (mU/l)	11·3	7·4	7·3	4·7	<0·001	10·6	7·8	7·1	4·5	<0·001	12·1	6·8	7·4	4·9	<0·001
HOMA-IR	3·7	2·4	1·8	1·3	<0·001	3·5	2·7	1·8	1·2	<0·001	3·8	2·0	1·8	1·3	<0·001

M/F, male/female; WC, waist circumference; HOMA-IR, homeostatic model assessment of insulin resistance.
* Diabetic status determined according to fasting blood glucose level >7·0 mmol/l or on diabetic medications.

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Macronutrient intake

Participants' macronutrient intake is summarised in online Supplementary Table S1. There were minimal differences between the reported dietary intake of diabetic and non-diabetic participants. Protein contributed a greater proportion of energy in diabetic men than in non-diabetic men (19·6 (SD 3·3) % *v.* 18·5 (SD 3·1) %; $P<0·05$), and diabetic women had a significantly lower intake of carbohydrate (180·0 (SD 57·3) g *v.* 212·7 (SD 96·4) g; $P<0·05$) and sugars (94·8 (SD 41·8) g *v.* 115·0 (SD 57·9) g; $P<0·05$) compared with those without diabetes. There were no significant differences in the PUFA intake between diabetics and non-diabetics in either sex. Dietary fat, protein and carbohydrate intake had no significant correlations with fasting glucose, HbA1c, insulin or HOMA-IR (data not presented).

Erythrocyte membrane fatty acid composition

Fish oil supplement use was reported by 28·1 % (*n* 154) of participants. There was no significant difference between the fatty acid composition of erythrocyte membranes or markers of glycaemic control (i.e. fasting BGL, HbA1c, fasting plasma insulin and HOMA score) between those who reported consuming fish oil supplements and those who did not (data not shown), so participants were pooled for analysis. Women had a significantly higher *n*-3I than men (8·8 (SD 2·5) % *v.* 8·1 (SD 2·1) %; $P<0·001$; data not shown). In women, diabetics had a significantly lower *n*-3I than non-diabetics (8·0 (SD 2·0) % *v.* 8·9 (SD 2·5) %; $P<0·05$), with both EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) significantly lower in diabetics than in non-diabetics (Table 2). Oleic acid (OA; 18 : 1*n*-9) was significantly higher and linoleic acid (LA; 18 : 2*n*-6) was significantly lower in diabetics when compared with non-diabetics for both sexes (Table 2).

n-3 Index and markers of glycaemic control

There was a weak but significant inverse correlation between *n*-3I and fasting plasma glucose (r −0·092; $P<0·05$), as well as HbA1c (r −0·092; $P<0·05$) (data not shown); however, these did not remain significant once adjusted for age and BMI (Table 3). There was a significant and proportionate decrease in the prevalence of IR as *n*-3I increased in women only (online Supplementary Table S2). When further stratified by sex and weight status, the relationship was evident only in the overweight/obese female sub-group, with no relationship between *n*-3I and IR in overweight/obese men or in the healthy/underweight sub-group for either sex (data not presented).

n-3 Index and diabetes

Fig. 2 shows the prevalence of T2D in each quartile of *n*-3I. Among the whole group, participants with a low *n*-3I were significantly more likely to be diabetic than those with a higher *n*-3I, with OR (95 % CI) decreasing across ascending quartiles (using the lowest quartile as a reference point): 1·0 (reference), 0·95 (95 % CI 0·51, 1·77), 0·72 (95 % CI 0·38, 1·39), 0·51

Table 2. Erythrocyte membrane fatty acid (% w/w) composition of Australian adults aged 65–95 years by sex and diabetic status*
(Mean values and standard deviations)

	Total (*n* 548)					Male (*n* 240)					Female (*n* 308)				
	Diabetic† (*n* 82)		Non-diabetic (*n* 466)			Diabetic† (*n* 44)		Non-diabetic (*n* 196)			Diabetic† (*n* 38)		Non-diabetic (*n* 270)		
	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*
ΣSFA	42·2	1·5	42·0	2·1	–	42·4	1·0	42·0	2·3	–	41·9	1·9	42·1	2·0	–
C16 : 0	23·0	1·1	22·8	1·2	–	23·0	0·9	22·9	1·3	–	23·0	1·2	22·8	1·2	–
C18 : 0	18·5	1·1	18·6	1·4	–	18·7	1·1	18·5	1·4	–	18·4	1·2	18·6	1·3	–
C20 : 0	0·7	0·7	0·6	0·1	–	0·7	0·7	0·6	0·2	–	0·6	0·1	0·6	0·1	–
ΣMUFA	18·0	1·5	17·1	1·6	<0·001	17·9	1·4	17·1	1·6	<0·01	18·0	1·6	17·1	1·6	<0·01
C16 : 1*n*-7	0·6	0·3	0·6	0·3	–	0·6	0·3	0·5	0·3	–	0·7	0·3	0·6	0·3	–
C18 : 1*n*-9	15·2	1·2	14·4	1·3	<0·01	15·2	1·2	14·5	1·3	<0·01	15·2	1·3	14·4	1·3	<0·01
C18 : 1*n*-7	1·8	0·4	1·7	0·4	–	1·7	0·3	1·7	0·4	–	1·8	0·4	1·7	0·4	–
C20 : 1*n*-9	0·4	0·4	0·3	0·1	<0·05	0·4	0·6	0·3	0·1	–	0·3	0·6	0·3	0·1	–
Σ*n*-6 PUFA	27·6	2·1	27·4	2·7	–	27·8	1·9	27·7	2·7	–	27·3	2·3	27·2	2·8	–
C18 : 2*n*-6	8·2	1·4	8·8	1·5	<0·001	8·4	1·3	9·0	1·5	<0·05	8·0	1·4	8·8	1·4	<0·01
C18 : 3*n*-6	0·2	0·1	0·2	0·2	–	0·2	0·1	0·2	0·2	–	0·2	0·2	0·2	0·2	–
C20 : 2*n*-6	0·3	0·3	0·3	0·2	–	0·3	0·4	0·3	0·3	–	0·3	0·1	0·3	0·1	–
C20 : 3*n*-6	1·4	1·1	1·5	1·1	–	1·7	1·1	1·5	1·2	–	1·2	1·1	1·4	1·1	–
C20 : 4*n*-6	17·4	2·4	16·6	2·5	<0·01	17·2	2·4	16·7	1·5	–	17·7	2·3	16·6	2·5	<0·05
Σ*n*-3 PUFA	11·9	2·3	12·4	2·8	–	11·9	2·2	12·0	2·6	–	11·9	2·5	12·8	2·9	–
C18 : 3*n*-3	0·2	0·1	0·3	0·1	–	0·2	0·1	0·3	0·1	<0·05	0·3	0·1	0·3	0·1	–
C20 : 5*n*-3	1·5	0·7	1·8	1·2	–	1·4	0·7	1·6	0·9	–	1·5	0·8	1·9	1·3	<0·05
C22 : 5*n*-3	3·6	0·6	3·6	0·7	<0·05	3·6	0·5	3·6	0·7	–	3·6	0·7	3·6	0·7	–
C22 : 6*n*-3	6·6	1·4	6·8	1·5	–	6·7	1·4	6·5	1·4	–	6·5	1·4	7·0	1·4	<0·05
n-3I‡	8·1	1·9	8·6	2·4	–	8·1	1·9	8·0	2·1	–	8·0	2·0	8·9	2·5	<0·05

HOMA-IR, homeostatic model assessment of insulin resistance; –, NS; *n*-3I, *n*-3 index.
* Group differences between diabetics and non-diabetics were assessed using independent-samples *t* tests (two-tailed).
† Diabetic status determined according to fasting blood glucose level > 7·0 mmol or on diabetic medications[19].
‡ *n*-3I = erythrocyte EPA% + DPA% of total fatty acids[18].

Table 3. Correlations between erythrocyte fatty acids (% w/w) and markers of glycaemic control in Australian adults aged 65–95 years, adjusted for age and BMI*

	Fasting plasma glucose (mmol/l)		Fasting plasma insulin (mU/l)		HbA1c (%)		HOMA-IR†	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
ΣSFA	−0·007	–	0·024	–	−0·042	–	0·027	–
C16 : 0	−0·033	–	−0·037	–	−0·031	–	−0·018	–
C18 : 0	−0·042	–	0·075	–	−0·054	–	0·062	–
C20 : 0	0·013	–	−0·021	–	0·077	–	−0·021	–
ΣMUFA	0·140	<0·01	0·061	–	0·117	<0·01	0·094	<0·05
C16 : 1*n*-7	0·006	–	0·031	–	−0·088	<0·05	0·023	–
C18 : 1*n*-9	0·181	<0·001	0·082	–	0·174	<0·001	0·125	<0·01
C18 : 1*n*-7	−0·062	–	−0·034	–	−0·074	–	−0·041	–
C20 : 1*n*-9	0·044	–	−0·032	–	0·053	–	−0·024	–
Σ*n*-6 PUFA	−0·001	–	0·000	–	0·039	–	0·006	–
C18 : 2*n*-6	−0·147	<0·01	−0·093	<0·05	−0·093	<0·05	−0·119	<0·01
C18 : 3*n*-6	0·020	–	−0·033	–	−0·034	–	0·011	–
C20 : 2*n*-6	0·004	–	−0·075	–	0·004	–	−0·052	–
C20 : 3*n*-6	0·009	–	−0·011	–	0·058	–	−0·013	–
C20 : 4*n*-6	0·080	–	0·069	–	0·071	–	0·084	0·05
Σ*n*-3 PUFA	−0·063	–	0·000	–	−0·092	<0·05	−0·014	–
C18 : 3*n*-3	−0·063	–	−0·020	–	−0·075	–	−0·029	–
C20 : 5*n*-3	−0·075	–	−0·024	–	−0·092	<0·05	−0·039	–
C22 : 5*n*-3	0·020	–	−0·056	–	−0·072	–	−0·032	–
C22 : 6*n*-3	−0·066	–	0·048	–	−0·063	–	0·022	–
n-3I‡	−0·077	–	0·018	–	−0·083	–	−0·005	–

HOMA-IR, homeostatic model assessment of insulin resistance; –, NS; *n*-3I, *n*-3 index.
* All correlations were assessed using Pearson's coefficient and significance determined using two-tailed *t* tests, adjusted for age and BMI.
† HOMA-IR = fasting blood glucose × fasting plasma insulin/22·5.
‡ *n*-3I = erythrocyte EPA% + DPA% of total fatty acids[18].

(95 % CI 0·25, 1·03) ($P_{trend} < 0·05$). Adjusting for age and BMI did not markedly change these values. When stratified by sex, there was a significant inverse relationship between *n*-3I and T2D in women, with OR again decreasing across ascending quartiles: 1·0 (reference), 0·81 (95 % CI 0·33, 2·01), 0·56 (95 % CI 0·23, 1·37), 0·26 (95 % CI 0·09, 0·82) ($P_{trend} < 0·01$). However, no significant relationship was detected between *n*-3I and T2D in men, with an OR across ascending quartiles of 1·0 (reference), 0·93 (95 %CI 0·36, 2·39), 0·95 (95 %CI 0·37, 2·43) and 0·93 (0·33, 2·58) ($P_{trend} = 0·903$). When further stratified by weight status, there was no significant relationship evident between *n*-3I and diabetes in the healthy/underweight sub-group for either sex, although in healthy/underweight men there was a non-significant positive association between T2D and *n*-3I with an OR across quartiles of 1·0 (reference), 1·24 (95 % CI 0·2, 9·4), 1·72 (95 % CI 0·2, 13·3), 5·2 (95 % CI 0·8, 32·0) ($P_{trend} = 0·067$). There was a significant inverse association in overweight/obese women (BMI ≥ 27 kg/m^2), with an OR of 1·0 (reference), 0·82 (95 % CI 0·31, 2·18), 0·56 (95 % CI 0·21, 1·52) and 0·22 (95 % CI 0·06, 0·82) in the first to fourth quartiles, respectively ($P_{trend} < 0·05$). Overweight/obese men showed a similar trend, with an OR across quartiles of 1·0 (reference), 0·86 (95 % CI 0·32, 2·29), 0·76 (95 % CI 0·25, 2·33) and 0·46 (95 % CI 0·14, 1·54); however, this finding did not reach significance ($P_{trend} = 0·211$).

When considered independently, neither EPA nor DHA was significantly associated with T2D in women in either the healthy weight or overweight/obese subgroups. Among men, a higher erythrocyte EPA was associated with a decrease in the odds of having T2D, with an OR of 0·533 (95 %CI 0·283, 0·936; $P < 0·05$) for every 1 unit increase in EPA. Conversely, a higher DHA was associated with an increased odds of T2D, with an OR of 1·427 (95 %CI 0·1012, 2·013; $P = 0·042$) for every 1 unit increase in erythrocyte DHA. When split into healthy and overweight/obese subgroups, the only significant association that remained was between DHA and T2D in healthy-weight men, with an OR of 2·789 (95 %CI 1·255, 6·197; $P = 0·012$) for every 1 unit increase in DHA.

Discussion

This study set out to determine whether erythrocyte *n*-3 PUFA status, as measured by the *n*-3I, is associated with T2D in a population of older adults, and to test the hypothesis that the *n*-3I is inversely correlated with IR in an overweight subgroup. Our results show a significant inverse relationship between *n*-3I and T2D in overweight/obese women, but not in men. To date, the literature articulating a role of dietary marine-based *n*-3 PUFA in the development of T2D has been unclear. Multiple systematic reviews have provided inconsistent findings, with one concluding that dietary *n*-3 PUFA intake increases the risk of T2D[4], whereas two have reported an inverse association[5,7], and a further two showed no association[6,8]. The majority of studies included in these reviews used questionnaires to determine dietary intake of *n*-3 PUFA, which may be prone to both reporting and measurement errors. One study that did compare objective biomarkers of dietary *n*-3 PUFA intake with the prevalence of T2D and markers of glycaemic control in older adults reported that a higher concentration of

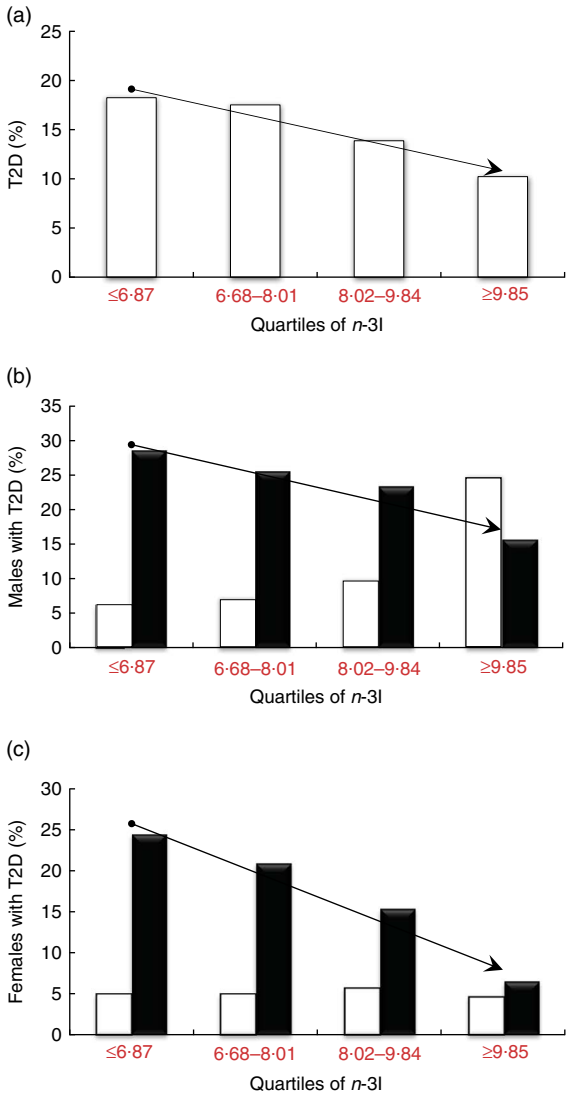

Fig. 2. (a) Prevalence of type 2 diabetes (T2D) in each quartile of *n*-3 index (*n*-3I). (b) Percentage of males with T2D in each quartile of *n*-3I according to weight status. (c) Percentage of females with T2D in each quartile of *n*-3I according to weight status. Diabetic status determined according to fasting blood glucose level > 7·0 mmol or on diabetic medications[19]. *n*-3I = erythrocyte EPA% + DPA% of total fatty acids[18]. Trends in proportions assessed using linear-by-linear association. a: □, Total population; b,c: □, healthy/underweight (BMI < 27 kg/m^2); ■, overweight/obese(BMI ≥ 27 kg/m^2).

plasma EPA and DHA was associated with a lower risk of incident diabetes[21]. Furthermore, a recent study in pre-diabetic men and women found baseline serum EPA and DHA to be associated with a reduced incidence of T2D over an 11-year follow-up[22]. Conversely, Mahendran *et al.*[23] did not find a relationship between erythrocyte *n*-3 PUFA and incident diabetes in a large prospective cohort study of middle-aged men over a 5-year follow-up. Similar to our findings, Zhang *et al.*[24] found that plasma levels of DHA were inversely correlated with the presence of IR in women ($P = 0{\cdot}03$) but not in men. Results of the present study highlight differences in response based on both weight status and sex, a novel finding that provides direction for future research.

In the current study, no direct correlation between *n*-3I and HOMA-IR score in the overweight sub-group was found; however, those in the highest quartile of *n*-3I were the least likely to be categorised as insulin resistant. The *n*-3I has been shown to be negatively correlated with HOMA-IR scores in children with obesity[11], whereas no correlation was found in predominately healthy-weight adolescents[25], consistent with a weight-dependent effect. Supporting this, randomised control trials in obese children, adolescents and women have found *n*-3 PUFA supplementation to reduce IR[26,27]. Conversely, some studies using high-dose *n*-3 PUFA supplementation (>2·5–3 g/d) have found no affect on insulin sensitivity[28,29]. These findings collectively suggest that potential benefits of *n*-3 PUFA in glycaemic control may be limited to overweight populations, and regular low dose EPA and DHA provided through habitual dietary intake, rather than short-term high dose supplementation, may be of optimal benefit.

It should be noted that the mean *n*-3I values obtained in this study are considerably higher than previous studies have reported[10]. This could possibly be because of the older age of our participants, as the *n*-3I has been shown to increase with age[30]. As the dietary data collected for this study did not include a specific breakdown of *n*-3 PUFA intake, it cannot be ruled out that a higher mean *n*-3I was because of a high intake of fish or seafood, or a higher intake of *n*-3 PUFA supplements. It is also possible that the high *n*-3I values are because of minor differences in the technique used to obtain and calculate the *n*-3I by different laboratories. Although the *n*-3I values from this study might not be directly comparable to previous studies, the relationships and trends found are valid and findings are robust.

A number of significant differences in the erythrocyte membrane fatty acid composition between diabetics and non-diabetics were noted. LA (C18 : 2*n*-6) was significantly lower in diabetic individuals, whereas arachidonic acid (C20 : 4*n*-6) and oleic acid (OA; C18: 1 *n*-9) were significantly higher, possibly reflecting an up-regulation of stearoyl-CoA desaturase and Δ-6 desaturase enzyme activities[31]. The *n*-3I was significantly lower in diabetic compared with non-diabetic women, with both EPA and DHA content showing significant differences. Women in our study had a significantly higher *n*-3I than men, consistent with literature that women have a greater ability to synthesise EPA and DHA from their precursor α-linolenic acid than men[32]. Sex differences in the physiological response to EPA and DHA supplementation have been reported in previous metabolic studies[33,34], although not directly in studies that included measures of glycaemia or diabetes.

Although a relationship between the *n*-3I and T2D was evident in women only, when results were further split into either a healthy weight (BMI < 27 kg/m^2) or overweight/obese (BMI ≥ 27 kg/m^2) subgroup there was no significant relationship in the healthy-weight group for either sex. Although the inverse association between *n*-3I and T2D was only significant in women who were overweight or obese, men displayed a similar trend, with the prevalence of T2D in the highest quartile for *n*-3I being half that of the lowest quartile. Interestingly, although non-significant, *n*-3I was positively associated with T2D in healthy/underweight men. Potential mechanisms for the differing relationships for the *n*-3I and T2D between healthy

and overweight/obese men are currently unknown. Findings from this study suggest that DHA is likely to play a significant role, with a more than 2-fold increase seen in the odds of having T2D for every 1 unit increase of erythrocyte DHA seen in healthy-weight, but not overweight/obese, men. The lack of significance found for the association between *n*-3I and T2D in healthy-weight men may be because of the small number of healthy-weight men in our study, and further studies with a larger sample of healthy-weight men should be conducted to confirm this finding, as well as mechanistic studies to elucidate potential mechanisms for why DHA is positively associated with T2D in healthy, but not overweight/obese, men. Overall, these results suggest that a higher *n*-3 PUFA status may be a contributing factor to metabolically healthy obesity.

There were few significant differences in macronutrient intake between diabetics and non-diabetics for either sex. Given that individuals with a high *n*-3I have previously been shown to be generally health conscious and consume diets high in fruits, vegetables and wholegrain cereals[35], which is known to be protective for the development of T2D[36], it is crucial to determine whether there is a causal relationship between *n*-3I and T2D. Given that there were few differences between the macronutrient intake of the diabetic and non-diabetic groups, it is likely that there is an independent mechanism implicated.

Although adiponectin was not assessed as part of this study, this adipokine is involved in the insulin signalling pathway and may play a role in the development of obesity-related IR[37]. As body fat increases, adiponectin levels decrease, which correlates with increasing levels of IR. Moreover, increasing adiponectin levels have been demonstrated to increase insulin sensitivity[37]. Fish oils rich in *n*-3 PUFA have demonstrated an ability to increase adiponectin levels[38], with EPA and DHA acting as ligands for PPARγ, stimulating adiponectin transcription[39]. As adiponectin decreases with increasing levels of obesity, this may be why the *n*-3I is negatively associated with T2D in overweight/obese, but not healthy-weight, men and women. The modulation of adiponectin by *n*-3 PUFA via PPARγ offers a biologically plausible mechanism by which a higher *n*-3 PUFA status results in a reduced prevalence of T2D in overweight individuals, and adiponectin should be included in future studies.

This analysis has some limitations. HOMA-IR is considered to be the least sensitive method of evaluating IR, and results may be different if more sensitive methods, such as the hyperinsulinaemic euglycaemic clamp had been utilised in the study. Other limitations include a lack of specific *n*-3 PUFA dietary data collected. However, this was overcome through the use of objective biomarkers; cross-sectional study design, which means that a causal relationship between *n*-3 PUFA status and T2D cannot be drawn; and primarily Caucasian study population, which limits transferability. However, this study also had some strengths, including a large sample size and biochemical data collection in a population of independently living older adults. The proportions of women and diabetics included in the study are comparable to those reported for people over 65 years of age within the general Australian community, making this a somewhat representative sample.

Conclusion

There is a significant inverse relationship between *n*-3 PUFA status and T2D in overweight/obese women, with a similar trend noted in overweight/obese men. However, higher *n*-3 PUFA status has a positive relationship with T2D in healthy-weight men. Although a causal relationship cannot be drawn from this cross-sectional study, results support a potential role for dietary *n*-3 PUFA intake in improving the metabolic health of individuals with obesity. Further investigations are warranted to elucidate sex-based differences and to determine whether dietary interventions to improve *n*-3 PUFA status can prevent the onset of T2D in at-risk individuals.

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Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114516000258

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