

Efficacy of the Omega-3 Index in predicting non-alcoholic fatty liver disease in overweight and obese adults: a pilot study

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is an independent predictor of CVD in otherwise healthy individuals. Low *n*-3 PUFA intake has been associated with the presence of NAFLD; however, the relationship between a biomarker of *n*-3 status – the Omega-3 Index – and liver fat is yet to be elucidated. A total of eighty overweight adults (fifty-six men) completed the anthropometric and biochemical measurements, including the Omega-3 Index, and underwent proton magnetic resonance spectroscopy assessment of liver fat. Bivariate correlations and multiple regression analyses were performed with reference to prediction of liver fat percentage. The mean Omega-3 Index was high in both NAFLD (intrahepatic lipid concentration $\geq 5.5\%$) and non-NAFLD groups. The Omega-3 Index, BMI, waist circumference, glucose, insulin, TAG, high-sensitive C-reactive protein (hsCRP) and alanine aminotransferase (ALT) were positively correlated, and HDL and erythrocyte *n*-6:*n*-3 ratio negatively correlated with liver fat concentration. Regression analysis found that simple anthropometric and demographic variables (waist, age) accounted for 31% of the variance in liver fat and the addition of traditional cardiometabolic blood markers (TAG, HDL, hsCRP and ALT) increased the predictive power to 43%. The addition of the novel erythrocyte fatty acid variable (Omega-3 Index) to the model only accounted for a further 3% of the variance ($P=0.049$). In conclusion, the Omega-3 Index was associated with liver fat concentration but did not improve the overall capacity of demographic, anthropometric and blood markers to predict NAFLD.

Key words: Non-alcoholic fatty liver: *n*-3 PUFA: Omega-3 Index: Biomarkers

Non-alcoholic fatty liver disease (NAFLD) affects up to 40% of the adult population^(1–3), but this can exceed 85% in obese individuals⁽³⁾. NAFLD is defined by hepatic fat $\geq 5.5\%$ in the absence of excessive alcohol consumption or other genetic, viral or drug-induced causes of liver fat accumulation⁽⁴⁾. Although for some patients, the clinical course of 'simple steatosis' is benign, approximately 10–61% progress to non-alcoholic steatohepatitis^(5,6) – which can then lead to cirrhosis⁽⁷⁾ – and to hepatocellular carcinoma⁽⁸⁾. At present, NAFLD can only be conclusively diagnosed by biopsy, and proton magnetic resonance spectroscopy (¹H-MRS) is used for quantification in research settings⁽⁹⁾. In clinical practice, increased levels of serum aminotransferases are commonly used to infer risk of NAFLD, although blood liver enzyme levels are an unreliable measure of liver damage⁽¹⁰⁾, and thus alternative useful markers are needed.

NAFLD is an independent predictor of CVD⁽¹¹⁾ and the metabolic syndrome⁽¹²⁾, and thus strategies to reduce liver fat are required. Weight loss by surgery or lifestyle therapy reduces liver fat^(5,13); however, the required weight loss is difficult to achieve and maintain⁽¹⁴⁾. Furthermore, there are safety concerns about the long-term use of current pharmacological agents to reduce liver fat⁽¹⁵⁾.

Published studies have suggested a potential role for *n*-3 PUFA in the treatment of NAFLD⁽¹⁶⁾; however, the effect of current *n*-3 PUFA status on NAFLD risk is unknown. Given that erythrocytes have a lifespan of approximately 120 d, the Omega-3 Index, or the erythrocyte membrane content of EPA plus DHA, expressed as a percentage of total membrane fatty acids, is likely to be a robust marker of medium- to long-term *n*-3 intake that is unaffected by recent diet^(17,18). The index is a promising biomarker for CVD⁽¹⁹⁾, and therefore may be a useful indicator of NAFLD risk.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; hsCRP, high-sensitive C-reactive protein; ¹H-MRS, proton magnetic resonance spectroscopy; IHL, intrahepatic lipid.

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NAFLD may exist undiagnosed in overweight and obese, but otherwise healthy, men and women. This group does not fulfil the traditional NAFLD target group criteria of morbid obesity and multiple comorbidities, and the Omega-3 Index may therefore assist in identifying those at increased risk for NAFLD and CVD. This study aimed to assess whether the index as a marker of long-term PUFA intake is a robust marker of hepatic steatosis in apparently healthy, overweight and obese men and women.

Methods

Participants

From June 2011 to September 2013, a community sample of men and women were recruited via notice boards and electronic bulletins. To be eligible, participants had to be non-smokers, aged between 18 and 60 years with a BMI > 25.0 kg/m² and with a waist circumference⁽²⁰⁾ that exceeded the cut-off point for 'increased waist circumference' (>94 and 80 cm for males and females, respectively)⁽²⁰⁾. Volunteers were excluded if they had biochemistry indicative of overt disease, had been previously diagnosed with diabetes mellitus or liver disease (other than NAFLD), were taking medication for hyperlipidaemia or if they reported taking supplements containing *n*-3 PUFA within the last 6 months. Other exclusion criteria included a change in antihypertensive medication within the last 12 months (if taking such medication), a regular alcohol intake exceeding 20 g alcohol/d and reported recent (within 3 months) significant changes to diet and exercise habits or body weight (>5% weight change). This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving humans were approved by the Sydney Local Health District Ethics Review Committee (RPAH Zone), protocol number X10-0115, clinical trial registration number ANZCTR12610000351011. Written informed consent was obtained from all the subjects.

Experimental design

Participants were screened for eligibility via telephone, and those deemed likely to be eligible attended the initial visit. At the first visit, measurements included anthropometry (height, weight, waist and total body fat) and a fasting blood test (8 h fast). The second visit included an MRI and MRS scan, which were completed within 7 d of visit one.

Measurements

Anthropometry assessment. Standing height was recorded to the nearest 0.5 cm. Weight was measured in light clothing using a digital platform scale accurate to 0.1 kg (Tanita BC-418 Body Composition Analyzer; Tanita Corporation); total body fat was measured by bioelectrical impedance analysis at the same time. Waist circumference was measured to the nearest millimetre at the midpoint between the twelfth rib and the iliac crest, according to International Diabetes Federation guidelines⁽²⁰⁾.

Blood pressure. Resting systolic (SBP) and diastolic (DBP) blood pressure and heart rate were measured in duplicate after 5 min of rest (seated) using an automated blood pressure monitor (Omron M5; Omron Healthcare Co. Ltd).

Biochemical parameters

Following an overnight (>8 h) fast, a 20 ml EDTA venous blood sample was collected and the plasma and erythrocyte portions were separated. The erythrocytes were stored at -80°C in an antioxidant (1 mg butylated hydroxytoluene) until fatty acid analysis.

Erythrocyte fatty acid derivatisation. The fatty acid profile was analysed via direct transesterification of the washed erythrocyte fraction of blood followed by GC⁽²¹⁾. In brief, methanol-toluene 2 ml (4:1, v/v) (containing C19 : 0 (20 µg/ml) as internal standard) was added to the sample. Acetyl chloride (200 µl) was added while vortexing and then heated (1 h, 100°C). The tubes were cooled in water and K₂CO₃ 6% (5 ml) was subsequently added and centrifuged (3000 g, 5 min, 4°C). The upper toluene phase was collected and stored in a GC vial at -20°C for GC analysis.

Gas chromatography 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 injector and detector ports were set at 250°C and the oven temperature was programmed as follows: 170°C for 2 min, increased by 10°C/min up to 190°C where it remained stationary for 1 min. The temperature was then increased by 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 ml were used. A known fatty acid mixture was used to compare with the analysed samples in order to identify peaks according to retention time, and their concentrations were determined using a Hewlett Packard 6890 Series GC (Hewlett Packard) with ChemStation version A.04.02.

The Omega-3 Index was calculated as the %EPA+%DHA content of erythrocyte cell membranes. The total *n*-3 content was calculated as the summed total of *n*-3 PUFA in the erythrocyte membranes: C18 : 3*n*-3, C20 : 5*n*-3, C22 : 5*n*-3 and C22 : 6*n*-3. Total *n*-6 content was calculated as the total of C18 : 2*n*-6, C18 : 3*n*-6, C20 : 2*n*-6, C20 : 3*n*-6 and C20 : 4*n*-6 contents.

Other biochemical parameters. Biochemical assessments were carried out by a commercial laboratory (Royal Prince Alfred Hospital Pathology). Glucose, albumin, serum aminotransferases (alanine aminotransferase (ALT) and aspartate aminotransferase) and lipids (total cholesterol, TAG, LDL-cholesterol and HDL-cholesterol) were analysed by photometric reactions using a C702 Cobas8000 Autoanalyser (Roche Diagnostics). Insulin was analysed by immunoassay using electrochemiluminescence on an E602 Cobas8000 Autoanalyser (Roche Diagnostics). HbA_{1c} was analysed by HPLC (Biorad), homocysteine was analysed from plasma by HPLC (Schimazu) and high-sensitive C-reactive protein (hsCRP) was analysed by rate nephelometry on a BN II (Siemens).

Magnetic resonance imaging and proton magnetic resonance spectroscopy

Magnetic resonance imaging and proton magnetic resonance spectroscopy data acquisition. All MRI and ^1H -MRS measurements were acquired using a 1.5-Tesla Achieva whole-body system (Philips Medical Systems). Visceral and subcutaneous adipose volumes were measured by MRI with the patient in the supine position. Axial T1-weighted fast field echo images were acquired from the diaphragm to the pelvis (TR=11 ms, TE=4.5 ms, flip angle=40°), with slice thickness of 10 mm and inter-slice gap of 10 mm. Images were acquired during suspended end-expiration with breath-hold duration of approximately 10 s per acquisition.

Liver fat (intrahepatic lipid (IHL)) concentration and composition were measured *in vivo* by ^1H -MRS according to the methods detailed previously⁽²²⁾. Image-guided, localised (3.0 × 2.0 × 2.0 cm voxel) ^1H -MRS data were acquired using the whole-body (Q body) (transmit) coil and a circular polarised surface (flex M multi-channel surface) (receive) coil, with volumes of interest centred within the right lobe of the liver. The subjects lay in the supine position and spectra were acquired with respiratory gating (end-expiration). Spectra were acquired using the PRESS (point resolved spectroscopy) technique (TR=5000 ms, TE=45 ms, 64 measurements, 1024 sample points). Fully automated high-order shimming was performed on the volume of interest to ensure maximum field homogeneity. Excitation water suppression was used to suppress the water signal during data acquisition. Unsuppressed water spectra were acquired *in vivo* for use as the internal standard.

Cross-sectional areas of both the visceral⁽²³⁾ and subcutaneous adipose tissue (VAT and SAT) depots were computed by automated software (Hippo Fat™ version 2.11)⁽²⁴⁾ with manual editing of contour lines and Gaussian curves as necessary. Volumes of VAT and SAT from the diaphragm to the pelvis were calculated by the summation of VAT and SAT area from the abdominal slices, adjusted for slice thickness and inter-slice gap.

Magnetic resonance imaging and proton magnetic resonance spectroscopy data processing. Spectral data were post-processed by the magnetic resonance user interface software (jMRUI version 3.0, EU Project). After Fourier transformation and manual phasing of the spectra, the water peak was identified and nominated 4.69 ppm and the signal amplitude was measured using HLSVD (Hankel Lanczos Squares Singular Values Decomposition). For the water-suppressed signal *in vivo*, the remaining water resonance (if evident) was first removed by the SVD (singular values decomposition) filter after which a five-resonance model was used to fit the lipid peaks: hepatic lipid olefinic methene protons ((CH)n; approximately 5.3 ppm), bulk methylene protons (-CH₂n; approximately 1.3 ppm), methylenic protons in the α (-CH₂; approximately 2.3 ppm) position relative to the carboxyl group, allylic protons (-CH₂n; approximately 2.0 ppm) and methyl protons (-CH₃; approximately 0.9 ppm) with metabolite signal amplitude quantified using the QUEST algorithm (QUantitation based on QUantum ESTimation), as we have described previously^(22,25).

To ensure consistency across subjects, all MRI and MRS analyses were performed by a single investigator who was blinded to treatment allocation.

Calculations. *In vivo* IHL% and hepatic fat composition were calculated as we have described recently⁽²⁵⁾. In brief, IHL% was measured as the ratio of hepatic methylene fatty acid amplitude to hepatic water amplitude corrected for T_2 effects, and hepatic lipid saturation was measured as the inverse of the hepatic allylic fatty acid resonance as a fraction of total hepatic fatty acids⁽²²⁾. Participants were subsequently grouped as those with NAFLD (IHL ≥ 5.5%) and those without NAFLD (IHL < 5.5%)⁽⁴⁾.

Statistical analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS; Release 17.0; SPSS Inc.). Data are reported as the mean values with their standard errors from the mean or frequencies as appropriate. Groups were compared using the χ^2 and the unpaired two-tailed *t* tests. Pearson coefficients (*R*) were used for all correlations. Hierarchical regression analyses were used to examine the contribution of variables to IHL%. Simple anthropometric and demographic variables (waist, age) were entered into block 1. Clinically relevant biochemical variables (TAG, HDL, hsCRP and ALT) were entered into block 2 of the regression analysis, and, finally, the novel marker, erythrocyte content of %EPA + %DHA (Omega-3 Index), was entered into the regression in block 3. Pairwise exclusion for missing data was employed in all analyses. Significance was set at $P < 0.05$.

Results

Participants

A total of eighty eligible volunteers (70% male) participated in the study (Fig. 1). Participant characteristics are summarised in Table 1. Study participants were aged 38.8 ± 1.3 years (range: 18–59 years), with BMI of 29.6 ± 0.4 kg/m² (range: 25.3–45.7 kg/m²) and waist circumference of 99.8 ± 0.9 cm (males: 101.9 ± 0.9 cm; females: 94.9 ± 2.3 cm). Liver fat was 5.1 ± 0.5% (range: 0.2–18.9%). In all, thirty participants (37.5% of the cohort) were classified as having NAFLD (liver fat ≥ 5.5%)⁽²⁶⁾, while the remaining fifty were not; fifteen participants satisfied the criteria for diagnosis of the metabolic syndrome⁽²⁷⁾, eleven of these participants had NAFLD.

Compared with those without NAFLD, participants with NAFLD had higher mean BMI, DBP and visceral adipose tissue ($P < 0.05$ for all; Table 1). Waist circumference was also higher in the group with NAFLD ($P = 0.004$); however, when males and females were studied separately, this observation was statistically significant for females only ($P = 0.003$ and 0.082 for females and males, respectively). As expected, participants with NAFLD had higher fasting glucose, insulin, hsCRP and TAG and lower HDL ($P < 0.05$ for all; Table 1). The IHL saturation index (the level of SFA in liver TAG) tended to be higher in the NAFLD group compared with the non-NAFLD group ($P = 0.058$; Table 1). There were no differences in serum aminotransferases

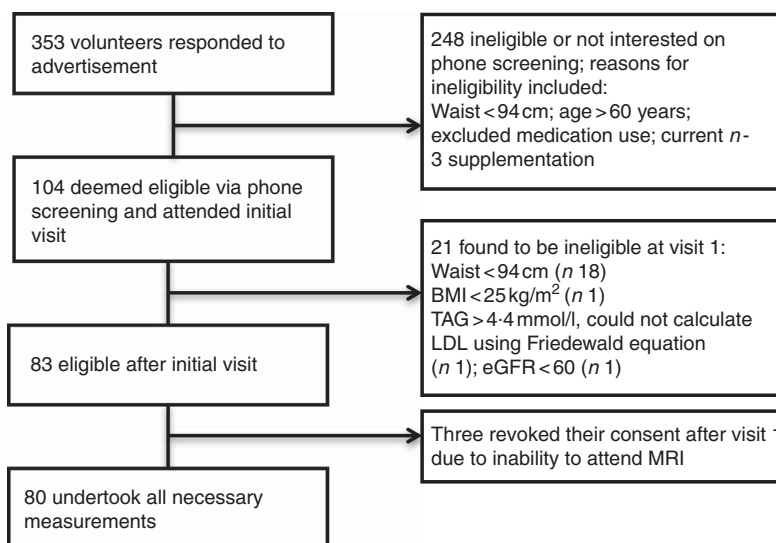


Fig. 1. Flow diagram of participant screening and recruitment. eGFR, estimated glomerular filtration rate.

or erythrocyte fatty acid composition between those with and without NAFLD (Table 1).

Bivariate correlations

Bivariate correlations between IHL and biochemical and anthropometric variables are summarised in Table 2. Higher IHL% was associated with higher BMI, waist circumference, glucose and TAG levels, as well as with lower HDL levels (Table 2). IHL% was positively correlated with the Omega-3 Index (Fig. 2) and negatively correlated with the erythrocyte *n*-6:*n*-3 ratio. The Omega-3 Index was positively correlated with BMI and glucose level, but not other components of the metabolic syndrome. Waist circumference, BMI, glucose and insulin were all moderately, positively correlated with one another, and these correlations were highly significant (Table 2).

Hierarchical multiple regression analysis

Demographic and anthropometric variables (waist circumference, age) accounted for 31% of the variance in liver fat ($P < 0.001$; Table 3), although only waist circumference was a statistically significant predictor of IHL%. The addition of biochemical measures (TAG, HDL, hsCRP and ALT) accounted for a further 11% ($P = 0.009$) of the variance in liver fat ($\Delta R^2 = 0.11$). Finally, adding Omega-3 Index into the model (block 3; Table 3) raised the total variance explained by the model to 46% ($P = 0.049$).

Discussion

This study sought to examine the associations between traditional anthropometric, biochemical markers and a novel marker – the Omega-3 Index – and liver fat level in a group of apparently healthy, but overweight, adults. We found that all the components of the metabolic syndrome⁽²⁰⁾ except blood pressure were associated with IHL%, and the relationships were as expected: glucose, TAG and waist circumference were positively correlated

and HDL was negatively correlated with IHL%. The Omega-3 Index was significantly associated with IHL% in bivariate correlations, but did not meaningfully improve the statistical power of the regression model for predicting NAFLD. These data from a small pilot sample suggest that measurement of the Omega-3 Index does not improve prediction of NAFLD in overweight individuals compared with previously established simple anthropometric and biochemical risk factors.

Observational studies have linked lower *n*-3 PUFA status in liver tissue⁽²⁸⁾ and low intake of *n*-3 PUFA^(29,30) with the presence of NAFLD, and experimental research has shown that *n*-3 PUFAs regulate mRNA expression of genes involved in hepatic lipid storage, such as *PPAR- α* , *SREBP-1c* and *ChREBP*^(31–33). Furthermore, human studies of *n*-3 supplementation have demonstrated that *n*-3 PUFA can ameliorate fatty liver⁽¹⁶⁾. The Omega-3 Index, or the percentage of erythrocyte cell membrane content of EPA + DHA⁽¹⁹⁾, is a robust long-term marker (≥ 3 months) of *n*-3 intake⁽¹⁹⁾, and therefore is a potentially useful marker of NAFLD risk. As NAFLD commonly exists with few or no symptoms, readily available single biomarkers are needed, particularly given that current methods used to identify those at risk and to ascertain disease progression are unsuitable for the growing prevalence of NAFLD. Thus, liver function tests are poor predictors of liver disease⁽¹⁰⁾, which is supported by the findings of this study, and quantification of liver fat, fibrosis and inflammation by invasive liver biopsy carries a risk for complications. The only non-invasive method validated to quantify liver fat – MRS – is expensive and generally restricted to research⁽⁹⁾. In this study, anthropometric and biochemical variables such as BMI, waist circumference and glucose and insulin levels were all correlated with one another and also with IHL% in both men and women (Tables 2 and 3). Taken together with the correlations also observed between TAG, HDL and IHL%, it would appear that increased NAFLD risk may be best identified via a cluster of anthropometric and biochemical markers suggestive of a ‘sub-clinical’ or pre-metabolic syndrome.

Although regression analyses did not find the Omega-3 Index to be predictive of fatty liver in this group of individuals, the

Table 1. Baseline participant characteristics (Mean values with their standard errors)

	Total group		NAFLD (<i>n</i> 30)		Non-NAFLD (<i>n</i> 50)		<i>P</i> *
	Mean	SE	Mean	SE	Mean	SE	
Demographics and anthropometry							
Age (years)	38.8	1.3	41.2	2.3	37.3	1.6	0.149
Sex (male/female)	56/24		20/10		36/14		0.614
BMI	29.6	0.4	31.3	1.0	28.6	0.3	0.003
Body fat (%; BIA)	26.9	0.9	28.0	1.7	26.2	1.0	0.341
Waist (cm)	99.8	1.0	103.5	1.8	97.6	1.1	0.004
Males	101.9	0.9	104.1	2.1	100.7	0.8	0.082
Females	94.9	2.3	102.4	3.7	89.6	1.9	0.003
SBP (mmHg)	129.6	1.4	131.9	2.5	128.2	1.7	0.206
DBP (mmHg)	82.6	0.9	84.7	1.3	81.3	1.1	0.049
MetSyn (Y/N)†	15/65		11/19		4/46		0.001
SAT (cm ²)‡	327.7	11.5	344.6	23.3	317.9	12.1	0.264
VAT (cm ²)‡	112.6	7.4	140.6	14.8	96.5	7.2	0.004
Biochemistry§							
Glucose (mmol/l)	4.4	0.1	4.7	0.1	4.2	0.1	<0.001
Insulin (pmol/l)	59.8	4.1	75.8	8.4	50.3	3.6	0.002
ALT (U/l)	30.0	1.5	32.6	2.3	28.5	1.9	0.167
AST (U/l)	25.9	1.0	24.9	1.0	26.5	1.5	0.420
hsCRP (mg/l)	2.6	0.3	3.5	0.6	2.1	0.3	0.026
Lipids§							
Total cholesterol (mmol/l)	5.5	0.1	5.7	0.2	5.4	0.1	0.256
TAG (mmol/l)	1.4	0.1	1.8	0.2	1.2	0.1	0.001
HDL (mmol/l)	1.4	0.0	1.3	0.1	1.5	0.1	0.016
LDL (mmol/l)	3.4	0.1	3.5	0.2	3.4	0.1	0.642
Erythrocyte membrane fatty acids (%)							
SFA	37.6	0.1	37.3	0.3	37.7	0.2	0.117
MUFA	15.5	0.1	15.6	0.2	15.5	0.2	0.628
<i>n</i> -6 PUFA	30.8	0.2	30.6	0.3	30.9	0.2	0.347
<i>n</i> -3 PUFA (total)	16.1	0.2	16.5	0.4	15.9	0.3	0.176
Omega-3 Index	8.6	0.2	9.0	0.3	8.4	0.3	0.151
EPA	2.3	0.1	2.5	0.2	2.2	0.2	0.142
DHA	6.3	0.1	6.5	0.2	6.2	0.2	0.329
<i>n</i> -6: <i>n</i> -3 ratio	1.9	0.0	1.9	0.1	2.0	0.0	0.260
¹H-MRS liver measurements							
IHL%	5.1	0.5	9.5	0.7	2.4	0.2	<0.001
Hepatic SI	0.946	0.006	0.959	0.006	0.937	0.008	0.058

NAFLD, non-alcoholic fatty liver disease; BIA, bioelectrical impedance analysis; SBP, systolic blood pressure; DBP, diastolic blood pressure; MetSyn, metabolic syndrome; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; ALT, alanine aminotransferase; AST, aspartate aminotransferase; hsCRP, high-sensitive C-reactive protein; ¹H-MRS, proton magnetic resonance spectroscopy; IHL%, intrahepatic lipid per cent; SI, saturation index.

* *P* value for NAFLD v. non-NAFLD using Student's *t* test for continuous variables and the χ^2 test for categorical variables.

† Metabolic syndrome criteria⁽²⁷⁾ – individuals must satisfy three or more of the following criteria: fasting glucose ≥ 5.6 mmol/l; fasting TAG > 1.7 mmol/l; fasting HDL < 1.29 or < 1.03 mmol/l for females and males, respectively; blood pressure $\geq 130/85$; waist circumference (midpoint between iliac crest and twelfth rib) > 88 or > 94 cm for females and males, respectively.

‡ Visceral and subcutaneous adipose tissue measurements at the level of the umbilicus.

§ Normal reference ranges: glucose, 3.0–5.4 mmol/l; insulin, 10–96 pmol/l; ALT, 5–55 U/l; AST, 5–55 U/l; hsCRP 25/50/75/100th percentiles: 0.72/1.47/2.96/9.00 mg/l; total cholesterol, ≤ 5.2 mmol/l; TAG, ≤ 2.5 mmol/l; HDL, 1.0–2.5 mmol/l; LDL, ≤ 3.5 mmol/l.

evidence linking NAFLD with low *n*-3 PUFA (intake and status) is strong, and more research in large cohorts using quantitative MRS is, therefore, warranted.

Strengths and limitations

Potentially limiting the strength of associations found in the present study between Omega-3 Index and liver fat was that the mean Omega-3 Index was relatively high in this group of participants compared with other free-living populations^(19,34). The healthy but overweight population studied was recruited from an affluent area within a capital city and had 'high' mean Omega-3 Index levels⁽¹⁹⁾, with 64% of participants having Omega-3 Index above 8.0%, which is considered to be the level for optimum cardiovascular

protection⁽¹⁹⁾. In addition, the observed variance in IHL% was relatively low, which may also have reduced the capacity to examine the strength of this association. Although a moderately large sample size was achieved, given the unexpectedly low variance in Omega-3 Index and IHL% between participants, a larger sample with a wider range of individuals from various demographic and socio-economic spectra may yield further insights into the relationship between Omega-3 Index and liver fat. Furthermore, the gender split was not even, with only twenty-four females included in the analyses, preventing sub-analysis of the major outcomes by sex, which may have affected the relationship between liver fat, Omega-3 Index and other variables.

As NAFLD is likely to occur in apparently healthy individuals as well as in obese and/or those with comorbidities, it would be

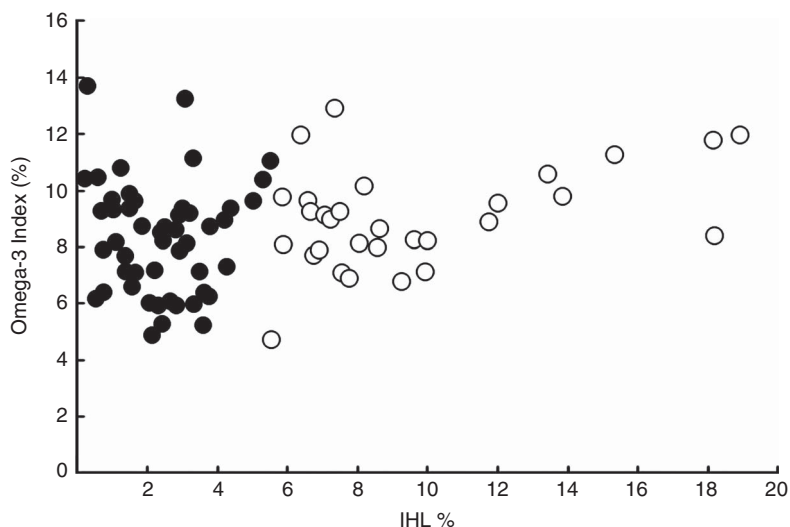


Fig. 2. Intrahepatic lipid concentration (IHL%) v. Omega-3 Index for those with (○) and without (●) non-alcoholic fatty liver disease.

Table 2. Descriptive statistics and correlations of metabolic, anthropometric and erythrocyte outcomes (Mean values with their standard errors)

	Mean	SE	1	2	3	4	5	6	7	8	9	10	11
1. IHL%	5.1	0.49											
2. BMI	29.6	0.45	0.444***										
3. Waist	99.8	0.10	0.545***	0.595***									
4. Glucose	4.4	0.07	0.456***	0.420***	0.539***								
5. Insulin	59.9	4.08	0.423***	0.503***	0.404***	0.380**							
6. ALT	30.0	1.45	0.296**	-0.011	0.352**	0.122	0.098						
7. AST	25.9	0.99	0.018	-0.133	0.127	-0.124	-0.133	0.576***					
8. TAG	1.4	0.09	0.379**	0.057	0.193	0.347**	0.277*	0.077	-0.099				
9. HDL	1.4	0.04	-0.318**	0.005	-0.222*	-0.119	-0.317**	-0.105	0.058	-0.364**			
10. hsCRP	2.6	0.31	0.302**	0.593***	0.355**	0.321**	0.365**	0.028	-0.179	0.084	0.011		
11. Erythrocyte Omega-3 Index	8.6	0.21	0.244*	0.421***	0.101	0.221*	0.132	-0.079	-0.115	0.175	0.198	0.200	
12. Erythrocyte n-6:n-3 ratio	1.9	0.03	-0.237*	-0.187	-0.332**	-0.112	-0.131	-0.164	-0.133	-0.036	0.129	0.012	-0.510***

IHL%, intrahepatic lipid per cent; ALT, alanine aminotransferase; AST, aspartate aminotransferase; hsCRP, high-sensitive C-reactive protein.
 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3. Hierarchical regression model for prediction of liver fat

	R	R ²	R ² change	F change	df	P	β
Block 1	0.558	0.311	0.311	17.415	2,77	<0.001	
Waist							0.525*
Age							0.120
Block 2	0.653	0.426	0.114	3.640	4,73	0.009	
Waist							0.353*
Age							0.106
TAG							0.187
HDL							-0.179
hsCRP							0.132
ALT							0.147
Block 3	0.675	0.456	0.030	4.016	1,72	0.049	
Waist							0.340*
Age							0.062
TAG							0.156
HDL							-0.221*
hsCRP							0.111
ALT							0.160
Erythrocyte Omega-3 Index							0.193*

hsCRP, high-sensitive C-reactive protein; ALT, alanine aminotransferase.
 * $P < 0.05$.

beneficial to examine the utility of the Omega-3 Index and other biomarkers across the spectrum of both Omega-3 Index and liver health/disease.

Although further research on the Omega-3 Index is warranted, clinicians should continue to investigate the potential for NAFLD in all overweight and obese patients, particularly those who exhibit anthropometry and biochemistry suggestive of, or approaching, metabolic syndrome. The data from this study reinforce the usefulness of simple markers, particularly waist circumference, age, TAG, HDL and hsCRP in predicting those at risk of NAFLD.

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The authors' contributions are as follows: H. M. P. was involved in the study design, subject recruitment, data collection, statistical analysis, data interpretation and drafting of the manuscript. H. T. O. was involved in the study design, data interpretation and drafting of the manuscript. S. E. K. was involved in the study design, subject recruitment and data collection. J. S. C. was involved in obtaining the required funding, study design and manuscript preparation. M. L. G. was involved in data analysis and manuscript preparation. I. D. C. and J. G. were involved in obtaining the required funding and manuscript preparation. N. A. J. was involved in obtaining the required funding, study design, statistical analysis, data interpretation and drafting of the manuscript. N. A. J. had overall responsibility for the study; H. M. P. and N. A. J. had full access to all the data regarding the study and took responsibility for the integrity of the data and the accuracy of data analysis.

H. M. P., H. T. O., S. E. K., J. S. C. and M. L. G. declare no conflicts of interest. N. A. J. has received honoraria for speaking engagements for Merck Sharp & Dohme. I. D. C. has performed and still performs clinical trials for obesity treatment and prevention, some of which have been funded by the government, but others by the pharmaceutical industry. Current trials are funded by the NHMRC (3), NovoNordisk, Amylin Corporation, the Egg Board. He serves on the steering committees of international trials (SCOUT and EXSCLE) and has received honoraria for this. He has given talks for NovoNordisk, Servier Laboratories, Pfizer and iNova pharmaceuticals in the last 3 years. He serves on the scientific advisory board of the Sansom Institute for Health Research, University of SA, the board of the Children's Medical Research Institute, and chairs the Executive Management Committee of the bariatric surgical register for the Obesity

Surgery Society of Australia and New Zealand. J. G. has no conflicts to declare in relation to this submission.

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