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Association between erythrocyte omega-3 polyunsaturated fatty acid levels and fatty liver index in older people is sex dependent

Meagan Rose [a], Martin Veysey [b], Mark Lucock [c], Suzanne Niblett [b], Katrina King [b], Surinder Baines [d], Manohar L. Garg [a, *]

[a] Nutraceuticals Research Group, School of Biomedical Sciences & Pharmacy, University of Newcastle, NSW, Australia
[b] School of Medicine & Public Health, University of Newcastle, NSW, Australia
[c] School of Environmental & Life Sciences, University of Newcastle, NSW, Australia
[d] School of Health Sciences, University of Newcastle, NSW, Australia

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ABSTRACT

Background/Objectives: Non-alcoholic fatty liver disease (NAFLD) is highly prevalent in older people but currently no specific drugs are available for its treatment. Omega-3 polyunsaturated fatty acids (n-3PUFA), known for their lipid-lowering, anti-inflammatory and anti-hypertensive properties, may have therapeutic potential for the management of NAFLD. The aim of this study was to determine whether n-3PUFA levels are associated with the prevalence of NAFLD in older adults.

Methods: A cross-sectional sample of older adults aged 65–95 years (n = 620) from the Retirement Health and Lifestyle Study (RHLS) was analysed. Fatty Liver Index (FLI) scores, used as an indicator of NAFLD risk, were calculated using a validated algorithm that incorporates body mass index, waist circumference, plasma triglycerides and γ-glutamyl transferase. Omega-3 index scores (O3I, %eicosapentaenoic acid plus %docosahexaenoic acid) were determined by analysing the fatty acid composition of erythrocyte membranes by gas chromatography.

Results: Following application of exclusion criteria, 475 participants were included in the analysis (age 77.9 ± 7.0 years; 60.4% females). Of these, 216 participants had FLI scores (≥60) suggestive of NAFLD (age 77.0 ± 6.6 years; 49.1% females). O3I was significantly lower in participants with NAFLD compared to those without NAFLD ($p < 0.01$). A significant inverse relationship was found between O3I and FLI ($r = -0.165$; $p < 0.001$). This relationship was gender specific with women, but not men, showing a significant association ($r = -0.206$; $p < 0.001$).

Conclusions: The current study demonstrated a sex-dependent inverse relationship between erythrocyte n-3PUFA concentrations and NAFLD in older adults. The finding supports the proposal for sex-stratified n-3PUFA intervention trials in this high-risk age group.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as the accumulation of fat (>5%) in the hepatocytes of the liver [1–3], represents a broad spectrum of conditions ranging from steatosis to the more severe non-alcoholic steatohepatitis (NASH) [3]. If left undetected or untreated, NAFLD can progress to fibrosis, cirrhosis and potentially liver failure [4]. In 2012, NAFLD was reported to be the most prevalent of all the liver diseases in Australia and was estimated to affect 5.5 million Australians including approximately 40% of adults aged 50 years or older [5]. By the year 2030, the number of Australians diagnosed with NAFLD is expected to exceed 7 million [5]. Furthermore, it has also been reported that NAFLD is becoming more prevalent in the older population (>60 years) [6] with post-menopausal women more likely to develop NAFLD than men of the same age [6].

Recent studies suggest that NAFLD is the hepatic expression of the metabolic syndrome with risk factors including insulin resistance, obesity, hyperlipidaemia, and hypertension [7–10]. Two stages have been proposed in the pathogenesis of the disease [1,6,11,12]. The first stage is the accumulation of free fatty acids (FFA) and triglycerides (TG) in the liver due to excessive storage of

* Corresponding author. 305C Medical Science Building, University of Newcastle, Callaghan, NSW 2308, Australia.
E-mail address: manohar.garg@newcastle.edu.au (M.L. Garg).

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fat in the adipose tissue [13] and is associated with high dietary fat/sugar intake or *de-novo* lipogenesis [13]. The second is mitochondrial dysfunction, oxidative stress and inflammation in addition to steatosis and results from the increase in serum FFA and serum TG [11]. However, not all patients with steatosis progress to NASH, indicating that additional stages may be involved in disease progression [14,15].

Currently, there is no specific treatment for NAFLD. Management options include lifestyle modifications (dietary energy restrictions, increased physical activity and weight loss) [16,17] and pharmacological agents (anti-hypertensive, lipid lowering, insulin sensitizing and weight loss medications) [14,15]. However, these options are complicated by the need for long-term compliance and concerns regarding the safety of medications such as insulin-sensitizing agents [14]. Development of safe and efficacious treatments and prevention strategies is therefore highly desirable [15].

A promising therapy for the management and treatment of NAFLD is increasing omega-3 polyunsaturated fatty acids intake (n-3PUFA) [3]. N-3PUFA are recognised to have lipid-lowering, anti-inflammatory [18] and anti-hypertensive properties, although the mechanisms underlying these effects have not been fully elucidated. Evidence suggests that eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids may reduce serum and hepatic TG, and decrease the production of the pro-inflammatory cytokines tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6) [19]. Other studies have also shown that n-3PUFA compete with omega-6 fatty acids for metabolism, thereby promoting the production of less-inflammatory eicosanoids (thromboxane and prostaglandins of the 3-series), and reducing the formation of pro-inflammatory 2-series eicosanoids [20]. In addition, EPA and DHA may serve as precursors for the synthesis of resolvins and protectins, promoting resolution of the inflammatory component of NAFLD [17]. Observational and interventional studies have reported an inverse association between n-3PUFA and NAFLD. Previous studies have been conducted in younger populations using objective measures of NAFLD [3]. Further work is needed to establish the relationship between long-term n-3PUFA status and NAFLD.

Typically, imaging procedures, such as ultrasound, computerized tomography scans and magnetic resonance imaging, have been used to diagnose fatty liver disease. However, an alternative validated non-invasive method for diagnosing NAFLD is the Fatty Liver Index (FLI) [21–23]. This index assesses risk of fatty liver disease using an algorithm based on four criteria; body mass index (BMI), TG, waist circumference and γ-glutamyl transferase (GGT) [18]. Our study used the FLI to assess NAFLD risk in a group of adults aged 65 years or older. While previous studies have been conducted with younger populations using non-FLI diagnostic tools [19,24,25], the current study was the first to examine the potential relationship between n-3PUFA status and NAFLD in older Australians. The omega-3 index (O3I), the sum of %EPA and %DHA in erythrocyte membranes [26], was used as a validated measure of long-term n-3PUFA status.

2. Subjects and methods

2.1. Subjects and study design

A cross-sectional analysis was conducted using data collected for the Retirement Health and Lifestyle Study (RHLS) between 2010 and 2012. The RHLS eligibility criteria included: $\geq$65 years of age, living independently in a retirement or community dwelling for 12 months or more and living within the Gosford/Wyong Local Government Areas. People were ineligible for the RHLS if: they were not independently living or were residing in a communal setting other than a retirement village, their listed address was not their primary residence, or another member of their household was taking part in the study. People with language and/or other communicative difficulties, or who were cognitively impaired and/or unable to provide informed consent, were also excluded. Participants were included in the current analyses if they had erythrocyte samples available for fatty acid analysis, valid height, weight, waist circumference, GGT and TG measures, and their liver disease status and daily alcohol intake could be determined. Participants were excluded if they reported liver disease other than fatty liver disease, or an alcohol intake exceeding 20.5 g per day.

All RHLS participants (n = 831) took part in an interviewer-administered questionnaire (IAQ) that collected information relating to demographics and health status including lifestyle factors and physical activity [27]. A subset of participants also took part in a clinical assessment that included measurement of anthropomorphic characteristics and sitting blood pressures. The majority of clinic participants provided a fasted blood sample and completed a self-administered food frequency questionnaire (FFQ) [28] as part of a physical activity questionnaire [29–32]. Erythrocyte samples were available for determination of O3I for 620 participants.

The study protocols were approved by 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. 1001-031 M) and all participants provided written informed consent.

2.2. Anthropometric measures

Anthropometric measurements were taken by trained research officers using standardised protocols outlined by the World Health Organisation [33]. Participants were weighed in light clothing and without shoes to the nearest 100 g using standardised digital scales (Tanita HD-316 scales, Tanita Corporation, Tokyo, Japan; or Wedderburn UWPM150 Digital Platform Scales, Wedderburn Scales, Australia). Height was measured without shoes to the nearest 0.1 cm using a portable stadiometer (Design No. 1013522, Surgical and Medical Products, Seven Hills, NSW, Australia). Waist circumference was measured between the lower costal margin and the iliac crest using a non-elastic flexible measuring tape (150 cm $\times$ 12 mm, Sullivans International), to the nearest 0.1 cm. Subjects were asked to remove any heavy clothing and belts, and measurements were conducted either on bare skin or over loose fitting clothing. Hip measurements, to the nearest 0.1 cm, were taken from the greatest posterior protuberance of the buttocks using a non-elastic flexible measuring tape. All anthropometric measurements were taken twice. If the measurements disagreed by more than set tolerance limits (weight, 800 g; height, 1 cm; waist circumference, 2 cm; and hip circumference, 2 cm) a third measure was taken. Each measure was presented as the mean of the two observations or the mean of the two closest measurements if a third was taken. Height and weight measurements were used to calculate BMI (kg/m^2).

2.3. Sitting blood pressure and pulse measures

Sitting blood pressure measurements were conducted by trained research officers following protocols outlined by the National Heart Foundation of Australia [34]. Participants were asked to avoid strenuous exercise for 24 h prior to measurement, fast overnight and abstain from smoking on the morning of measurement. Two measurements were taken at 1 min intervals using an Omron 1A2 digital automatic blood pressure monitor (Omron, Australia) with participants seated in a chair, feet flat on the ground and legs uncrossed. If the two readings differed by more than