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# Dietary fat intake and red blood cell fatty acid composition of children and women from three different geographical areas in South Africa

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ABSTRACT

Dietary fat intake, particularly the type of fat, is reflected in the red blood cell (RBC) fatty acid (FA) profile and is vital in growth, development and health maintenance. The FA profile (%wt/wt) of RBC membrane phospholipids (as determined by gas chromatography) and dietary intake (as determined by 24 h recall) was assessed in 2–6 y old South African children and their caregivers randomly selected from three communities, i.e. an urban Northern Cape community (urban-NC; $n=104$), an urban coastal Western Cape community (urban-WC; $n=93$) and a rural Limpopo Province community (rural-LP; $n=102$). Mean RBC FA values across groups were compared using ANOVA and Bonferroni post-hoc test while controlling for age and gender (children); median dietary intake values were compared using a Kruskal–Wallis test. Dietary intakes for total fat, saturated FAs and polyunsaturated FAs were higher in the two urban areas compared to the rural area. Total fat intake in rural-LP, and omega-3 FA dietary intake in all three areas were lower than the South African adopted guidelines. Dietary SFA intake in both urban areas was higher than recommended by South African guidelines; this was reflected in the RBC membrane FA profile. Rural-LP children had the lowest intake of omega-3 and omega-6 FAs yet presented with the highest RBC docosahexaenoic acid (DHA) profile and highest arachidonic acid percentage. Although differences observed in dietary fat intake between the two urban and the rural area were reflected in the RBC membrane total phospholipid FA profile, the lowest total fat and α-linolenic acid (ALA) intake by rural children that presented with the highest RBC DHA profile warrants further investigation.



## 1. Introduction

The fatty acid (FA) composition of human red blood cells (RBC) and plasma is greatly influenced by different dietary patterns and especially the type of fats consumed [1]. The type of dietary fat ultimately affects the individual's health, contributing towards either disease prevention or promotion, and also child development [1,2]. Dietary fat intake recommendations throughout the human life cycle are based on the requirements to meet essential fatty acid (EFA) needs, to support neurodevelopment and cardiovascular health, and to prevent degenerative diseases [2]. Certain RBC FAs have been shown to be influenced by FA intake, and this measure is used as a good indicator of a longer term FA intake [3].

The quality of fat consumed in the diet is largely determined by the quantities of polyunsaturated fatty acids (PUFA), thus omega-6 and omega-3 fatty FAs consumed [4]. The importance and supply of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) by the diet throughout the life cycle has increasingly gained more interest and evidence relates health benefits of these FAs to fish intake. Research suggests that this is due to the consumption of pre-formed EPA and DHA in the fish [5,6]. These FAs play vital roles in cell membrane functioning, brain and nervous system development and functioning, and in the manufacture of eicosanoids [7]. DHA is also considered as a conditional EFA, especially during the early developmental years [8].

Even though α-linolenic acid (ALA), found in food sources other than fish, is a precursor of EPA and DHA, it is questionable if the conversion in the human body is sufficient to meet the need [7,9]. Also, a diet high in linoleic acid (LA), thus more than 10% of total

*Abbreviations:* AI, adequate intake; ALA, alpha-linolenic acid; ANOVA, analysis of variance; AA, arachidonic acid; CHO, carbohydrate; DHA, docosahexaenoic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAMEs, fatty acid methyl esters; IQR, Inter-quartile range; kJ, kilojoule; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; RBC, red blood cell; Rural-LP, rural Limpopo Province; SD, standard deviation; SFA, saturated fatty acids; Urban-NC, urban Northern Cape; Urban-WC, urban Western Cape; %E, percentage of total energy; GLA, γ-linolenic acid

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energy intake, reduces the conversion of ALA to EPA and DHA [10,11]. Therefore, the habitual diet and balance between LA and ALA consumption are important factors in determining the long chain polyunsaturated fatty acid profile of RBC membranes, particularly in non-fish eaters [8,10].

There is a concern that for low income communities that do not consume fish regularly, particularly those situated inland [10], omega-3 FA intake is possibly low, with possible increased intakes of omega-6 FAs [8,12]. However, limited literature is available from developing countries on the FA status, dietary intake and composition, particularly regarding omega-3 and omega-6 FAs [10].

South Africa is a widely diverse country with many varied income, living conditions and resources. In addition, South Africa has diverse ethnic groups with different traditional eating patterns [13]. These eating patterns could result in different FA intakes. The more industrialised lifestyle and current 'Western diet' that many South Africans particularly in urban areas, already follow or are transitioning towards, has resulted in an increased consumption of saturated, omega-6 PUFA and trans FAs [13,14]. In contrast to this, the dietary habits of rural living South African communities are described as more prudent, lower in fat, with a higher intake of starch-rich staple foods [13]. The South African National Food Consumption Survey found milk (56.3%), meat and offal (47.9%) and vegetable fats (36.8%) to be the three main food groups contributing fat to the diet of children 1–5 years of age. Fish was consumed by 7.6% of children [15]. The pattern in adults were found to be similar, as meat and offal (57.4%), vegetable fats and oil (47.9%) and milk (30.6%) were the food groups which supplied fat to the diet [16]. However, specific data on EFA intake and prevalence of EFA deficiency for the South African population is lacking.

The aim of this study was to assess the FA profile (%) of RBC membrane phospholipids and the dietary fat intake of South African preschool children and women from three different geographical areas, each with known distinct dietary patterns. The FA profile and possible relations between the RBC FA profile and dietary intake were examined.

## 2. Study population and methods

### 2.1. Study population

This cross-sectional study forms part of a sub-study nested within a parent study that aimed to determine the vitamin A intake and status of South African children and that of their primary caregivers, located in four geographical sites, and over a wide range of dietary patterns [17]. Three of the four geographical areas were selected for the current nested study as these were assumed to have the most distinct differences in eating patterns between groups. The study was done in an urban community in the Northern Cape (urban-NC), an urban coastal community in the Western Cape (urban-WC) and a rural community in Limpopo Province (rural-LP). The urban-NC area is situated in the Karoo where sheep farming is common and mutton consumption is frequent. The urban-WC area is a coastal community and fish consumption particularly that of fresh fish, was thought to be higher. The rural-LP area was predicted to have a more prudent diet pattern with a lower fat intake and a more frequent consumption of green leafy vegetables [18].

For the parent study in which this study was nested, 200 randomly selected preschool children as well as their respective caregivers were recruited from each geographical area, as described in Faber et al. [17]. If more than one child in the household fitted the age range, one of them was randomly selected. If the mother was unable to attend, a female caregiver preferably from the household, was recruited. The measurements of all geographical areas were taken in 2011.

The parent study showed that $>90\%$ of households in all three areas had access to electricity; nearly all households had a flush toilet in the two urban areas and a pit toilet in the rural area, respectively. Most households in the two urban areas had access to tap water, versus only 13.7% in rural-LP, where most of the households had to fetch water from the river or natural spring or had to buy from a water truck. While electricity was mostly used for cooking in the two urban areas, wood (mostly as an open fire inside the dwelling) and gas/paraffin were used in the rural area [17].

For the current study, approximately 100 children aged 2–6 years and their caregivers per area, for whom all the measurements were completed for both the caregiver and the child, were included.

Anthropometric measurements were taken as described in Faber et al. [17].

### 2.2. Biochemical analysis, blood sampling

A nursing sister collected a 10 ml non-fasting blood sample by antecubital venipuncture from each child and woman and transferred it into EDTA anti-coagulant tubes. The blood was then centrifuged at 3500 rpm for 10 min where after the plasma was harvested. The red blood cells were washed three times with normal saline and RBC samples were stored at –80 °C until analysed within 3 months after collection.

Total phospholipid FA extraction and analysis were done following an adapted method of Folch et al. [19] as described by Baumgartner et al. [20]. The total lipid extraction with chloroform/methanol (2:1; v/v) containing butylated hydroxytoluene was done using 300 μl of the thawed RBC sample.

The total phospholipid fraction was separated from the neutral lipids by using pre-coated thin layer chromatography silica gel 60 plates (10 cm × 20 cm; Merck) that were developed in a filter paper lined separation tank containing diethyl ether, petroleum ether and acetic acid (30:90:1; v-v:v) as developing agent. The total phospholipid fraction was removed by scraping it off the plate through a funnel into a clean Teflon-lined screw-capped test tube.

Once 2 ml of trans-methylating solution (methanol:sulphuric acid; 95:5; v-v) had been added to the phospholipid sample, it was incubated at 70 °C for 120 min, yielding the FA methyl esters (FAMEs). FAMEs were extracted with 2 ml hexane and 1 ml distilled water.

The hexane phase containing the FAMEs was evaporated under nitrogen at 45 °C. After this, 45 μl of hexane was added and mixed well to re-dissolve the FAMEs. The resulting FAMEs were analysed by using quadrupole (QP) Gas Chromatography–electron impact–mass spectrometry (GC–EI–MS) on an Agilent Technologies 7890A gas chromatography (GC) System equipped with an Agilent Technologies 5975C VL mass selective detector. A BPX 70 capillary column (60 m; 0.25 mm 0.25 μm; SGE Analytic Sciences) was used in the GC separation of the FAMEs. The GC inlet and MS line were maintained at a temperature of 280 °C and 230 °C, respectively. Helium at 197.86 kPa was the carrier gas with a flow rate of 1.3 ml/min. The injection volume of the sample solution was 1 μl using a split ratio of 1:1. The oven temperature was programmed at 130 °C to 240 °C, rose from 130 °C to 200 °C at 2 °C/min, was held isothermally at 220 °C for 5 min, it was increased by 10 °C/min to 240 °C, where it was retained for 5 min. The complete analysis took 53 min. QP–GC–MS with 70 eV electron impact functioned in full scan acquisition mode. All mass spectra were acquired over the $m/z$ range of 50–500. FAMEs were quantified with the use of the selected ion extraction method based on the response of two selected diagnostic ions.