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Lipid-soluble nutrient status of healthy Omani school children before and after intervention with oily fish meal or re-esterified triacylglycerol fish oil

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ABSTRACT

Objectives: Over the past two decades, the Omani diet has changed considerably to resemble a high calorie and a low nutrient density Western diet. We investigated the fat soluble nutrient status of children before and after intervention with fish diet or fish oil.
Methods: Children ages 9 and 10 y (n = 314) were recruited from three randomly selected schools. The schools were assigned to a fish, fish oil, or control group and the children were given a lightly grilled oily fish, a re-esterified triacylglycerol fish oil capsule, or no fish for 12 wk.
Results: Plasma vitamin A, beta carotene, vitamin E concentrations, and vitamin E/total lipid ratio at baseline were 2.7 ± 0.85 μmol/L, 0.68 ± 0.48 μmol/L, 21.1 ± 4.8 μmol/L, and 5.0 ± 0.81 μmol/mmol, respectively, and none of the children were deficient. They were severely deficient (<27.5 nmol/L; 10.5% boys and 28.5% girls), deficient (27.5–44.9 nmol/L; 47.6% boys and 49.4% girls) or insufficient (50–74.9 nmol/L; 34.6% boys and 21.5% girls) in vitamin D; only 7.3% boys and 0.6% girls had optimal status (≥75 nmol/L). Parathyroid hormone (5.0 ± 1.7 versus 5.8 ± 2.1 pmol/L; $P < 0.0001$) and alkaline phosphatase (225.2 ± 66.6 versus 247.8 ± 73.7 U/L; $P < 0.01$) levels were lower in boys. Postintervention, the fish oil (54.1 ± 17.5 nmol/L; $P < 0.001$) and fish (49.2 ± 17.4 nmol/L; $P < 0.05$) groups had elevated levels of vitamin D compared with the controls (42.3 ± 17.5 nmol/L).
Conclusions: Vitamin D deficiency is prevalent in Omani school children, but it can be mitigated with omega-3 fatty acid supplementation. Vitamin D plays a crucial role in skeletal and extra-skeletal systems. Hence, there is a need for a child-focused program of food fortification and outdoor activities to alleviate the problem.

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A. Al-M coordinated the implementation of the study and liaised with ministries, schools and parents; E.S. processed samples, conducted laboratory analysis and collated data; H.S. Al-O contributed significantly with the conception of the study, acquisition of funds and the refining of the proposal and study protocol; I.S.H. contributed substantially to the conception and design of the study, writing of the proposal, and enrolment and follow up of the children; K.G (PI) contributed to the conception, design and implementation of the study, analysed and interpreted data and drafted the manuscript; S.M. Al-S assisted significantly with the implementation of the study, data collation and statistical analysis; S.S. Al-G recruited, screened, enrolled and followed the children, liaised with teachers and parents, collected and processed samples and acquired data; Y.M. participated substantially in writing of the proposal, data collation, analyses and interpretation, and manuscript drafting.

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Introduction

Oman has made remarkable progress in socioeconomic development in the last four decades [1,2]. This advancement is reflected in dramatic reductions in maternal, child, and infant mortality, common infectious diseases, and extreme poverty rates, and a significant increase in life expectancy [3,4]. Conversely, the prevalence of obesity and non-communicable diseases, such as vascular and respiratory diseases, diabetes, and cancer, has reached epidemic proportions [5,6] in adults and children. The Oman World Health Survey, a country-wide community-based household survey involving about 5000 subjects conducted in 2008, revealed that 40.3% of those surveyed had hypertension, 12.3% diabetes, 21.4% obesity, and 33.6% elevated blood cholesterol [7].

It is plausible that genetic predisposition may be a factor for the rise in non-communicable diseases in Oman and the other Gulf countries. However, the fact that the rise occurred in a short time and coincided with a period of economic prosperity suggests that modifiable lifestyle factors, such as physical inactivity, smoking, and unhealthy diets could be the main culprits. It has been reported that the traditional diet of the gulf countries, primarily composed of dates, milk, rice, brown bread, fish, and vegetables, [1,8] has changed to resemble a more Western diet, which is high in vegetable oils and animal fat (~30% daily calorie), refined sugar (~51% daily calorie), and low fiber cereals (mainly highly extracted wheat flour and polished rice, 35–42% of daily energy) [1]. The Western diet is high in calories, glycemic index carbohydrates, total and saturated fats, and trans and omega-6 fatty acids while being low in omega-3 fatty acids, essential vitamins, and trace elements.

Childhood dietary habits tend to track to adult life [9,10], and some non-communicable chronic diseases are thought to begin in early life and progress to become clinical diseases in adulthood [11–14]. Moreover, perhaps because of the impact of marketing strategies [15,16], fast food meals have become very popular among children and adolescents in Arab countries [17,18].

The aims of this study were to investigate 1) plasma fat-soluble micronutrient status of preadolescent school children who are being exposed to fast foods at home and outside, and 2) the effect of fish-based menu or docosahexaenoic acid (DHA) enriched fish oil supplement on plasma fat soluble micronutrient status of the children. There is tentative evidence that oily meals enhance the availability of these nutrients.

Subjects and methods

Subjects and recruitment

Three hundred fourteen children (boys, n = 139, girls, n = 175), accounting for a 4.6% of school pupils aged between 9 and 10 y from the Muscat Governorate, were recruited using a two-stage sampling procedure. In the first stage, 3 of 39 schools in the Governorate, and in the second stage, three Grade 4 classes from five, were randomly selected and assigned to fish menu, fish oil (re-esterified triacylglycerol fish oil capsule), or control group. The children were given a 100-g lightly grilled fish sandwich with some vegetables (fish group), a fish oil capsule containing 403 mg DHA and 53 mg eicosapentaenoic acids (fish oil group), or their habitual food (control group) at lunch on school days for 12 wk. One hundred grams of the fish used in the study (grouper, sea bream, kingfish, emperor, and snapper) provided 150 to 200 mg of omega-3 fatty acids, and the dishes were prepared tastefully by professional chefs to enhance compliance at the Intercontinental City Hotel, Muscat. Body weight, height, and body mass index were assessed at baseline, as well as a non-fasting blood sample, about 8 mL, obtained in EDTA at baseline, and another sample taken after 12 wk of intervention.

The study was approved by the Research Ethics Committee of the Ministry of Health, Sultanate of Oman (Ref. MH/DGP/R&S/Proposal_Approved/8/2012), and the National Research Ethics Committee North West, Haydock, UK (REC reference no. 12/NW/0760) and registered with ISRCTN Register (Reg. No. ISRCTN93233285). Informed and signed consent was obtained from the parents or guardians of the children, and the study was conducted in accordance with the provisions of the ethical approval of the two ethics committees and the principles of Helsinki Declaration.

Methods

Sample processing

Plasma and red blood cells were separated from whole blood specimen by centrifugation at 1200 g, 4°C, for 10 min. The plasma was carefully siphoned and transferred to another tube. The buffy coat was discarded and the red cell pellet washed three times by suspension in physiological saline (0.85% NaCl) and cold centrifugation. The plasma and red blood cells were stored at −70°C until analysis.

Anthropometry

Weight in kilograms and height in centimeters were assessed with a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham, UK) and a measuring board (Schorr, Weight and Measure, LLC, Olney, Maryland, USA) respectively.

Plasma vitamin A and vitamin E and beta carotene analyses

An aliquot of 200 μL of plasma in duplicate was used for analysis. The plasma was deproteinised with 4 mL of absolute ethanol and vortexing for 3 min. Subsequently, 10 mL of hexane was added to the plasma-ethanol mixture, vortexed for 3 min, and centrifuged at 1200 g, 4°C, for 10 min. The top organic layer containing the required analytes was carefully transferred to another tube, dried at 30°C under a gentle stream of nitrogen, suspended in 1000 μL of methanol containing 0.01% butylated hydroxytoluene. An aliquot of 50 μL was taken for analysis. The target analytes (retinol, alpha-tocopherol, and beta carotene) were separated by Agilent 1100 high-performance liquid chromatography system (Agilent Technologies, Waldbronn, Germany) with the use of a 5 micron C18 reverse-phase column, 150 × 4.6 mm, (HiChrom Limited). The analytes were eluted with 100% high-performance liquid chromatography–grade methanol at a flow rate of 2 mL/min and detected with a diode array UV/Vis detector (Agilent Technologies). Vitamin A, vitamin E, and beta carotene were detected at 325 nm (1.5 min), 292 nm (4.8 min), and 453 nm (30 min), respectively. Concentrations were determined from a standard curve computed with the use of ChemStation (Agilent Technologies).

Plasma triacylglycerols

Concentration of plasma triacylglycerols was determined enzymatically (Glycerol phosphate oxidase assay) using the method described by Fossati and Prencipe [19] and McGowan et al. [20] with the use of a reagent kit supplied by Abbot Laboratories (Ref: 7 D74-21, 304350/R1, Abbott, Wiesbaden, Germany).

Plasma cholesterol

An enzymatic method (cholesterol esterase cholesterol oxidase-peroxidase) described by Roeschlau and Allain [21] with a reagent kit obtained from Abbott Laboratories (Ref: 7 D62-21, 304342/R1, Abbott) was used to analyze total cholesterol.

Vitamin D (25-hydroxy vitamin D)

Plasma total vitamin D, 25-hydroxylated cholecalciferol (vitamin D3), and ergocalciferol (vitamin D2) were determined with a competitive electrochemiluminescence protein binding assay [22] using the Cobas e 601 immunoassay auto-analyzer and reagents obtained from Roche Diagnostics (Sandhoferstrasse, Mannheim, Germany).

Parathyroid hormone

Intact plasma parathyroid hormone was quantified by Architect Intact PTH assay, a two-step chemiluminescent microparticle immunoassay, using the automatic immunoassay analyzer ARCHITECT i2000 SR (Abbott Diagnostics, Abbott Park, IL, USA) and reagents from Abbott Diagnostics (Ref: 8 K25, 84-6434/R5, Abbott, Wiesbaden, Germany).

Alkaline phosphatase

Alkaline phosphatase was measured on an Architect c8000 analyzer (Abbot Diagnostics) with a reagent kit supplied by Abbott Diagnostics (Ref: 7 D61-20, 30-3979/RS, Abbott).

Calcium

Plasma total calcium was determined with the use of an Architect c8000 analyzer (Abbot Diagnostics) with the Arsenazo III dye binding method and a reagent kit from Abbott Diagnostics (Ref: 7 D61-20, 30-3979/RS, Abbott).