

Vitamin D deficiency is prevalent among healthy Omani school children: Omega-3 fatty acids have a mitigating effect.

Al-Ghannami SS^{1, 3}, Sedlak E¹, Hussein IS¹, Min Y¹, Al-Shmmkhi SM³, Al-Oufi HS², Al-Mazroui A², Ghebremeskel K¹

¹ Lipidomics and Nutrition Research Centre, Faculty of Life Sciences and Computing, London Metropolitan University, London, UK; ² Ministry of Agriculture and Fisheries Wealth, Muscat, Sultanate of Oman; ³ Ministry of Health, Muscat, Sultanate of Oman

Short title: Vitamin D Deficiency in Omani School Children

Corresponding author: Professor Kebreab Ghebremeskel
Lipidomics and Nutrition Research Centre
Faculty of Life Sciences and Computing
London Metropolitan University
166-220 Holloway Road, London N7 8DB, UK
Email: k.ghebremeskel@londonmet.ac.uk
Phone: +44 20 7133 2440
Fax: +44 20 7133 2453

ABSTRACT

Background - The traditional diet of Oman which comprises primarily of dates, milk, rice, brown bread, fish and vegetables has changed considerably to resemble a Western diet which is high in calorie, high glycaemic index carbohydrates, total fat and saturated, trans and omega 6 fatty acids, and low in omega-3 fatty acids and essential micronutrients.

Aim - To investigate fat-soluble nutrient (vitamin A, D, E and beta carotene) status of Omani school children before and after intervention with fish diet and docosahexaenoic acid (DHA)-enriched fish oil supplement.

Method - Three hundred fourteen children aged between 9 and 10 years were recruited from three schools in Muscat. The schools were randomly assigned to fish, fish oil or control group and the children given 100 g lightly grilled fish (fish group), omega-3 capsule containing 403 mg DHA and 53 mg eicosapentaenoic acid (EPA) or their habitual diet. Baseline body weight, height and body mass index were assessed and a non-fasting blood sample collected at baseline and after 12 weeks of intervention.

Results – Mean plasma vitamin A, beta carotene, vitamin E concentrations and vitamin E/total lipid ratio at baseline were 2.7 ± 0.85 $\mu\text{mol/L}$, 0.68 ± 0.48 $\mu\text{mol/L}$, 21.1 ± 4.8 $\mu\text{mol/L}$ and 5.0 ± 0.81 $\mu\text{mol/mmol}$ respectively. None of the children were deficient in either of the nutrients and there was no gender difference. Most of the children 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 vs. 5.8 ± 2.1 pmol/L; $p < 0.0001$) and alkaline phosphatase (225.2 ± 66.6 vs. 247.8 ± 73.7 U/L; $p < 0.01$) levels were lower in the boys than in the girls. After 12 weeks intervention, the fish oil (1.2 ± 0.70 $\mu\text{mol/L}$) and fish (1.20 ± 0.7 $\mu\text{mol/L}$) groups compared with the placebo

(0.85 ± 0.43 $\mu\text{mol/L}$) had higher concentrations of beta carotene ($p < 0.0001$). Similarly, the children who received fish oil (54.1 ± 17.5 nmol/L ; $p < 0.001$) and fish (49.2 ± 17.4 nmol/L ; $p < 0.05$) had elevated levels of vitamin D than those who did not (42.3 ± 17.5 nmol/L).

Conclusion – This study demonstrate that vitamin D insufficiency is highly prevalent in Omani young school children and it can be mitigated with omega-3 fatty acid supplementation. Vitamin D plays a crucial role in skeletal and extraskkeletal systems. Therefore, there is an urgent need for a well-thought-out programme which incorporates a fortification of foods and drinks favoured by children and outdoor activities to help tackle this major public health problem.

INTRODUCTION

Oman has made a remarkable progress in socio-economic development in the last four decades (Musaiger 1998, Ibpus 2013). This advancement is reflected in dramatic reductions in maternal, child and infant mortality rates, common infectious diseases, extreme poverty, and a significant increase in life expectancy (UNICEF 2009, Al-Lamki 2010, and UNDP 2010). Conversely, the prevalence of obesity in adults and children, and non-communicable diseases, such as vascular and respiratory diseases, diabetes and cancer has reached epidemic proportions (Musaiger 2002, Badran and Laher 2011, Abdul Rahim et al 2014). 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 (Al Riyami et al 2012).

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. Indeed, it has been reported that the traditional diet of the gulf countries which comprises primarily of dates, milk, rice, brown bread, fish and vegetables (Musaiger 1998, Galal 2002) 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 fibre cereals (mainly highly extracted wheat flour and polished rice, 35-42% of daily energy) (Musaiger 1998, Badran and Laher 2011). The Western diet is high in calorie, high glycaemic index carbohydrates, total fat and saturated, trans and omega-6 fatty acids, and low in omega-3 fatty acids and essential micronutrients.

Childhood dietary habits tend to track to adult life (Mikkila et al 2004, Ventura and Worobey 2013) and some of the non-communicable chronic diseases are thought to begin in early life

and progress to become clinical diseases in adulthood (Holman et al 1958, Holman 1961, McNamara et al 1971, Newman et al 1986, Barker 1995, McGill et al 2000, 2008, 2009). Moreover, perhaps because of the impact of marketing strategy (Robinson et al 2007, Andreyeva et al 2011,) fast food meals have become very popular among children and adolescents in the Arab countries (Musaiger 1987, Chakar and Salameh 2006) and elsewhere. Therefore, children and young adults must be an integral part of programmes for prevention and control of non-communicable diseases.

Recently, we conducted a comprehensive study designed to define health, physical fitness and cognitive ability of school children within the context of the rapid nutrition transition in Oman. This communication reports plasma fat-soluble micronutrient status at baseline and after intervention with fish-based menu or docosahexaenoic acid (DHA) enriched fish oil supplement.

SUBJECTS AND METHODS

Subjects and Recruitment

Three hundred fourteen children (boys, n=139, Girls, n=175) accounting for a 4.6% of the school pupils aged between 9 and 10 years from the Muscat Governorate were recruited using a two stage sampling procedure. In the first stage, three (n=3) of thirty nine (n=39) schools in the Governorate and in the second stage, three Grade 4 classes from five were randomly selected. The three schools were assigned to fish, fish oil or control group and the children were given for lunch a 100 gram lightly grilled fish sandwich with some vegetables (fish group), their habitual food or omega-3 fatty capsule containing 403 mg docosahexaenoic (DHA) and 53 mg eicosapentaenoic (EPA) acids. One hundred gram (100g) of the fish used in the study (Grouper, Sea bream, Kingfish, Emperor and Snapper) provided 150 to 200 mg

omega-3 fatty acids and the dishes were prepared tastefully to enhance compliance by professional chefs at the Intercontinental City Hotel, Muscat. Body weight, height and body mass index were assessed baseline and a non-fasting blood sample, about 8 ml, obtained in EDTA at baseline and after 12 weeks 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_Aproved/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/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 minutes. The plasma was siphoned out carefully 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 kilogram and height in centimetre were assessed with a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham B5 5QB, 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 aliquate of 200 µL of plasma in duplicate was used for analysis. The plasma was deproteinised by with 4 ml of absolute ethanol and vortexing thoroughly for 3 minutes. Subsequently, 10 ml of hexane was

added to the plasma-ethanol mixture, vortexed for 3 minutes and centrifuged at 1200 g, 4°C, for 10 minutes. 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 aliquate, 50 µL, was taken for analysis. The target analytes (retinol, alpha-tocopherol and beta carotene) were separated by Agilent 1100 high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) with the use of a 5 micron C18 reverse-phase column, 150 X 4.6 mm, (HiChrom Limited UK). The analytes were eluted with 100% HPLC grade methanol at a flow rate of 2 ml/min and detected with a diode array UV/Vis detector (Agilent Technologies, Waldbronn, Germany). 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). Concentrations were determined from a standard curve computed with the use of ChemStation (Agilent Technologies, Waldbronn, Germany).

Plasma triglycerides – Concentration of plasma triglycerides was determined enzymatically (Glycerol phosphate oxidase assay) based on the method described by Fossati and Prencipe (1982) and McGowan et al (1983) with the use of a reagent kit supplied by Abbot Laboratories (Ref: 7D74-21, 304350/R1, Abbott, Max-Planck-Ring 2, 65205 Wiesbaden, Germany).

Plasma cholesterol – An enzymatic method (cholesterol esterase- cholesterol oxidase-peroxidase) described by Roeschlau and Allain (1974) with a reagent kit obtained from Abbott Laboratories (Ref: 7D62-21, 304342/R1, Abbott, Max-Planck-Ring 2, 65205 Wiesbaden, Germany) was used to analyse total cholesterol.

Vitamin D (25-hydroxy vitamin D) - Plasma total vitamin D, 25-hydroxylated cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), was determined with a competitive electrochemiluminescence protein binding assay (Abdel-Wareth et al 2013)

using the Cobas e 601 immunoassay auto-analyser and reagents obtained from Roche Diagnostics (Sandhoferstrasse, Mannheim, Germany).

Parathyroid Hormone (PTH) – Intact plasma PTH was quantified by Architect Intact PTH assay, a two-step chemiluminescent microparticle immunoassay, using the automatic immunoassay analyser ARCHITECT i2000SR (Abbott, Abbott Park, IL, USA) and reagents from Abbott Diagnostics (Ref: 8K25, 84-6434/R5, Abbott, Max-Planck-Ring 2, 65205 Wiesbaden, Germany)

Alkaline Phosphatase (ALP) – ALP was measured on an Architect c8000 analyser (Abbot Diagnostics, Abbot Park, IL, USA) with a reagent kit supplied by Abbott Diagnostics (Ref: 7D61-20, 30-3979/RS, Abbott, Max-Planck-Ring 2, 65205 Wiesbaden, Germany).

Calcium - Plasma total calcium was determined with the use of an Architect c8000 analyser (Abbot Diagnostics, Abbot Park, IL, USA) with the Arsenazo III dye binding method and a reagent kit from Abbott Diagnostics (Ref: 7D61-20, 30-3979/RS, Abbott, Max-Planck-Ring 2, 65205 Wiesbaden, Germany).

Phosphate – Plasma inorganic phosphate was analysed based on the Molybdenum blue colorimetric method with a reagent kit obtained from Abbott Laboratories (Ref: 7D71-20 and 7D71-30, 30-3926//R5, Abbott, Max-Planck-Ring 2, 65205 Wiesbaden, Germany) using the ARCHITECT c8000 auto-analyser (Abbott, Abbot Park, IL. USA).

Data Analyses

The data are expressed as mean and standard deviation (S.D.). Independent (unpaired) and paired t-tests, respectively, were used to determine statistical significance between the genders at baseline and between pre- and post-intervention within gender. Group comparison was performed with a one-way ANOVA, and Boneferroni post hoc test when a significant

difference is indicated. Differences were considered significant if the p value was less than 0.05. All analyses were carried out with SPSS version 21 (IBM SPSS, IBM Corporation, Armonk, NY, USA).

RESULTS

Pre-intervention (baseline) gender comparison

Body weight, height, body mass index and plasma vitamin A (all-trans retinol), beta carotene, vitamin E (alpha-tocopherol), total lipid (triglycerides and cholesterol), alpha-tocopherol and total lipid ratio, total vitamin D, parathyroid hormone, alkaline phosphatase, calcium and inorganic phosphate levels of the children are shown in Table 1. The male compared with the female students had higher vitamin D ($p < 0.0001$) and lower parathyroid hormone ($p < 0.0001$) and alkaline phosphatase ($p < 0.01$) levels.

Post-intervention group comparison

Compared with the control group, the children (both male and female students) who were given fish oil supplement for twelve weeks had increased concentrations of beta carotene and vitamin D ($p < 0.001$). Similarly, both carotene ($p < 0.001$) and vitamin D ($p < 0.05$) levels were higher in those who received fish meals (Table 2).

Gender-stratified post-intervention group comparison

In the male students, beta carotene and vitamin D concentrations in the fish oil ($p < 0.001$) and in the fish ($p < 0.05$) groups were higher than in their counterparts who did not receive fish oil or fish meals (Table 3). Likewise, in the female students who were given fish oil and fish meals had elevated level of vitamin D ($p < 0.001$ and $p < 0.05$) compared with the control group students (Table 3).

Pre- and post-intervention comparison

Post-intervention plasma vitamin D (Fig. 1) and parathyroid hormone (Fig. 2) concentrations compared with pre-intervention (baseline) were higher in the children who received fish oil and fish meals ($p < 0.0001$). There was no difference between the pre- and post-intervention concentrations of the two analytes in the control group.

DISCUSSION

Urbanisation, heavy reliance on imported foods (primarily beef, dairy and poultry products and refined cereals, vegetable oils and sugar) and sweetened beverages and the proliferation of food supermarkets and convenience fast-food restaurants have led to a drastic change in dietary habits in Oman. The impact of the change, which is characterised mainly by intakes of calorie-rich and essential micronutrient-poor foods, on lipid-soluble nutrient (vitamin A, D and E, and beta carotene) status of Omani children has not been fully investigated. Accumulating evidence demonstrates, these micronutrients, in addition to their well-characterised classical functions (Goodman et al 1966, Bonet et al 2003, DeLuca 2004, Black et al 2008, Sathe and Patel 2010, Nike and Traber 2012) play a role in gene expression (Zhang et al 1992, Azzi et al 2004, Bastien and Rochette-Egly 2004, Hossein-nezhad et al 2013), cognitive function (Grodstein et al 2007, Olson and Mello 2010, Soni et al 2012, Ulatowski et al 2014) and antioxidant defense (Burton and Ingold 1984, Lin et al 2005). Moreover, deficiency/insufficiency of these nutrients is thought to be associated with increased risks of non-communicable diseases.

A biannual supplementation programme with a high dose vitamin A in infants and children aged six to fifty-nine months (UNICEF 2007, WHO 2011a) is effective in reducing morbidity, mortality and vision impairment (Beaton et al 1993, Fawzi et al 1993, Imdad et al

2010, Mayo-Wilson et al 2011). Regardless, vitamin A deficiency is still the foremost cause of these preventable problems, particularly in South East Asia and Africa. Based on the prevalence of night blindness and biochemical vitamin A deficiency based estimates, 45 and 122 countries respectively have vitamin A deficiency of public health significance (WHO 2009). A $0.70 \mu\text{mol/l}$ serum/plasma vitamin A concentration is the cut-off point of deficiency (WHO 1996, De Pee and Dary 2002) which is used as a marker for assessing severity and public health significance in most age groups (WHO 2011b). A cross-sectional survey (MHO 2006) on food fortification and micronutrient deficiencies conducted in Oman in 2004 found vitamin A deficiency in children aged 6-59 (5.5%), 6-23 (18%) and 24-59 (3%) months, and in non-pregnant women of reproductive age (0.5%). In the current study, deficiency ($\leq 0.70 \mu\text{mol/l}$) was not detected in any of the children and only three of them had a marginal status ($<1.05 \mu\text{mol/l}$). Some of the milk and dairy products in the Omani market are fortified with vitamin A (Alasfoor et al 2007) and it is mandatory for edible vegetable oils sold in the country to incorporate 60 IU/g of the vitamin. It appears that the children were able to maintain adequate status by consuming foods fortified with preformed vitamin A as well as vegetables containing provitamin A.

There is a wide variability in blood beta carotene concentration among healthy individuals (Winklhofer-Roob et al 1997). The variations are primarily a reflection of intake, vitamin A status and genetic make-up (Lacher et al 2005, Tourniaire et al 2009, Borel 2012). Gender-related differences in concentration have also been reported (Hercberg et al 1994, Olmedilla et al 1994). Nevertheless, depending on the amount of fruits and vegetables consumed, blood beta carotene levels generally fall between 0.2 and $1.5 \mu\text{mol/l}$ (Burri 1997). The present study did not detect gender-related effect on plasma beta carotene, and the values were within the aforementioned range in 87% ($0.63 \pm 0.31 \mu\text{mol/l}$), below in 7% ($0.16 \pm 0.04 \mu\text{mol/l}$) and above in 6% ($1.87 \pm 0.28 \mu\text{mol/l}$) of the children. Fresh fruits, which are an important component of

the Omani foods, are eaten three or more times a week (Opara et al 2007). This Omani dietary habit may provide and an explanation for the 'adequate' beta carotene status in most of the children in the current study.

In contrast to vitamin A, the concentration of plasma beta carotene increased significantly in the fish diet and fish oil groups after intervention for 12 weeks (Table 2). This finding, which is consistent with the report that fish oil supplementation enhances the absorption of beta carotene in human (Nair et al 1993) and rats (Blakely 1992), is rather intriguing since dietary fat is thought to facilitate fat-soluble vitamin uptake and transport at the intestinal level.

As is the case with the other fat-soluble vitamins, the blood level of vitamin E (alpha-tocopherol) varies considerably between individuals and population groups (Winklhofer-Roob et al 1997, Valtueña et al 2011, Péter et al 2013, Traber 2014). Nevertheless, a concentration of less than 12 $\mu\text{mol/l}$ in plasma is defined as a cut-off point of vitamin E inadequacy (IOM 2000) and the minimum concentration required to help prevent cardiovascular diseases and cancer is thought to be 30 $\mu\text{mol/l}$ (Gey 1995). 96.6% of the children in this study had concentrations higher and 3.4% lower than the cut-off point (12 $\mu\text{mol/l}$). Two children had levels (7.38 and 7.89 $\mu\text{mol/l}$) less than 8 $\mu\text{mol/l}$, a value which is associated with neurological disease. Since vitamin E is intimately associated with circulating lipids, the ratio of the two nutrients was used to evaluate vitamin E status. All of the children had vitamin E/total lipid ratio higher than the cut-off point 1.11 $\mu\text{mol/mmol}$ suggesting that the 'deficiency' in the 3.4% of the children indicated by plasma vitamin E concentration may not be related to a low intake.

There was no difference in the post-intervention plasma vitamin E concentration between the three groups (Table 2, 3 and 4). In contrast, the post-intervention vitamin E/total lipid ratio was significantly higher in the children who received the fish oil supplement compared with the fish diet and control groups. This difference was most likely due to the combined effect of

omega-3 fatty acids which reduces blood lipid content and the vitamin E incorporated in the supplement to prevent lipid peroxidation.

Consistent with the previous reports of Omani non-pregnant (Al-Kindi 2011) and pregnant (Al Kalbani et al 2011) women, school children aged 9 to 12 years (Kilani et al 2013) and adults aged 18 to 55 (Abiaka et al 2013), most of the children in the current study 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 a sufficient level (≥ 75 nmol/L) level. As it is borne out by the elevated levels of parathyroid hormone and alkaline phosphatase (Table 1) the insufficiency was more pronounced in the female children. The high prevalence of vitamin D deficiency in the children should be a major public health concern because childhood is a period of skeletal mineral acquisition and bone modelling and there is evidence that vitamin D plays a critical role in muscle growth and development, cognitive function and modulation of innate and adaptive immunity.

Omega-3 fatty acids have been shown to enhance bone mineralisation in human and experimental animals but their effect on blood vitamin D has not been investigated. The children who were given oily fishes, which are thought to contain variable amounts of vitamin D, and DHA-enriched fish oil supplement, which was stripped out of vitamin D, had elevated plasma 25-hydroxy vitamin D concentration compared with the control group (Table 2, 3, 4). It is not obvious how fish oil increases plasma vitamin D. However, since it did not have any effect on PTH or alkaline phosphatase the action may be mediated by the facilitation of vitamin D transport across the intestinal mucosa. Indeed, there is evidence that dietary fat promotes vitamin D absorption (Dawson-Hughes et al 2014).

Bone mineral density of the children and vitamin D status of rural children with limited access to school transport were not assessed. These limitations will be addressed in future

investigations. This study demonstrates that vitamin D insufficiency is highly prevalent in Omani young school children and it could be mitigated with omega-3 fatty acid supplementation. Various studies have reported vitamin D deficiency/insufficiency in non-pregnant and pregnant Omani women. It is conceivable that Omani children may be at risk of developmental and health problems caused by prenatal and postnatal vitamin D deficiency. Vitamin D plays a crucial role in skeletal and extraskeletal systems. Therefore, there is an urgent need for a well-thought-out programme which incorporates a fortification of foods and drinks favoured by children and outdoor activities to help tackle this major public health problem.

ACKNOWLEDGEMENTS

Very sincere thanks are due to the children, parents, teachers and headmistresses of the schools for their participation and support, staffs and management of the Ministries of Agriculture and Fisheries Wealth, Health and Education who helped with the design, implementation and monitoring of the study, Dr Manal Al Kindi and colleagues in the pathology laboratory, Royal Hospital, for blood sample processing and biochemical assay, members of Seeb Polyclinic for blood specimen collection, catering service team of Intercontinental Hotel for preparing fish meals and Sheikh Saif Sultan Mohammed Al Habsi for providing transport. In addition, we are very grateful to Mr Peter Clough and Efamol Ltd. UK for providing omega-3 capsules for the study free of charge. The study was supported by the 8th Five Year Development Plan, Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman.

Table 1. Baseline weight, height, body mass index and plasma A, D and E, beta carotene, total lipid, parathyroid hormone, calcium and phosphate levels of Omani school children

Variables	Male and Female students (n=314)	Male students (n=139)	Female students (n=175)
Weight (kg)		30.1 ± 7.8	29.9 ± 8.2
Height (cm)		133.1 ± 6.4	133.1 ± 6.6
Body mass index (kg/m ²)		16.8 ± 3.4	16.7 ± 3.5
Retinol (µmol/L)	2.7 ± 0.9	2.7 ± 0.8	2.6 ± 0.9
Beta carotene (µmol/L)	0.7 ± 0.5	0.7 ± 0.5	0.7 ± 0.5
α-tocopherol (µmol/L)	21.1 ± 4.8	20.9 ± 4.9	21.3 ± 4.7
Total lipid (mmol/L)	5.0 ± 0.8	4.9 ± 0.8	5.1 ± 0.8
α-tocopherol (µmol) / total lipid (mmol) ratio	4.3 ± 0.9	4.3 ± 0.9	4.3 ± 0.9
Vitamin D (25-hydroxy vitamin D) (nmol/L)	43.1 ± 17.1	49.3 ± 17.5	38.5 ± 15.3**
Parathyroid hormone (pmol/L)	5.5 ± 1.9	5.0 ± 1.7	5.8 ± 2.1**
Alkaline phosphatase (U/L)	237.2 ± 73.5	225.2 ± 66.6	247.8 ± 73.7*
Calcium (mmol/L)	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Phosphate (mmol/L)	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2

** $p < 0.0001$, * $p < 0.01$ - Male students vs. Female students

Table 2. Plasma vitamin A, D and E, beta carotene, total lipid, parathyroid hormone, calcium and phosphate levels of male and female school children after intervention with DHA-rich fish oil formulation or fish meal.

Variables	Control group (n=116)	Fish oil group (n=86)	Fish meal group (n=108)
Retinol ($\mu\text{mol/L}$)	2.2 ± 0.7	2.3 ± 0.8	2.3 ± 0.8
Beta carotene ($\mu\text{mol/L}$)	0.9 ± 0.4	$1.2 \pm 0.7^{**}$	$1.2 \pm 0.7^{**}$
α -tocopherol ($\mu\text{mol/L}$)	20.0 ± 4.8	20.5 ± 4.5	20.5 ± 4.7
Total lipid (mmol/L)	5.1 ± 0.8	5.1 ± 0.8	5.2 ± 0.8
α -tocopherol (μmol) / total lipid (mmol) ratio	4.2 ± 0.9	$4.6 \pm 1.0^{\$}$	4.2 ± 0.8
Vitamin D (nmol/L)	42.3 ± 17.5	$54.1 \pm 17.5^{**}$	$49.2 \pm 17.4^*$
Parathyroid hormone (pmol/L)	5.8 ± 2.1	5.8 ± 2.1	6.4 ± 2.4
Alkaline phosphatase (U/L)	250.4 ± 72.8	245.9 ± 65.7	253.6 ± 85.0
Calcium (mmol/L)	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Phosphate (mmol/L)	1.9 ± 0.4	1.6 ± 0.1	1.6 ± 0.1

** $p < 0.001$ Control group vs. Fish oil group; * $p < 0.05$ Control group vs. Fish meal group

$\$ p < 0.001$ Fish oil group vs. Fish meal and Control Groups

Table 3. Plasma vitamin A, D and E, beta carotene, total lipid, parathyroid hormone, calcium and phosphate levels of the male school children after intervention with DHA-rich fish oil formulation or fish meal for 12 weeks.

Variables	Control group (n=51)	Fish oil group (n=37)	Fish meal group (n=48)
Retinol ($\mu\text{mol/L}$)	2.2 ± 0.7	2.1 ± 0.7	2.4 ± 0.6
Beta carotene ($\mu\text{mol/L}$)	0.9 ± 0.4	$1.4 \pm 0.8^{**}$	$1.2 \pm 0.8^*$
α -tocopherol ($\mu\text{mol/L}$)	20.2 ± 5.6	21.1 ± 4.0	20.5 ± 3.6
Total lipid (mmol/L)	5.1 ± 0.8	5.0 ± 0.7	5.3 ± 0.8
α -tocopherol (μmol) / total lipid (mmol) ratio	4.2 ± 0.9	$4.6 \pm 0.8^{\$}$	4.1 ± 0.8
Vitamin D (nmol/L)	50.6 ± 18.9	$63.1 \pm 17.3^{**}$	$58.0 \pm 16.5^*$
Parathyroid hormone (pmol/L)	5.5 ± 1.9	4.9 ± 1.4	5.9 ± 2.1
Alkaline phosphatase (U/L)	237.2 ± 61.8	226.6 ± 39.2	256.7 ± 82.6
Calcium (mmol/L)	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Phosphate (mmol/L)	2.4 ± 0.4	1.6 ± 0.1	1.6 ± 0.1

** $p < 0.001$ Control group vs. Fish oil group; * $p < 0.05$ Control group vs. Fish meal group

⁺ $p < 0.05$ Fish oil group vs. Fish meal and Control groups; ^{\\$} $p < 0.05$ Fish oil group vs. Control and Fish meal groups

Table 4. Plasma vitamin A, D and E, beta carotene, total lipid, parathyroid hormone, calcium and phosphate levels of the female school children after intervention with DHA-rich fish oil formulation or fish meal for 12 weeks.

Variables	Control group (n=65)	Fish oil group (n=49)	Fish meal group (n=60)
Retinol ($\mu\text{mol/L}$)	2.2 ± 0.7	2.2 ± 0.9	2.3 ± 0.8
Beta carotene ($\mu\text{mol/L}$)	0.8 ± 0.5	1.1 ± 0.7	0.9 ± 0.8
α -tocopherol ($\mu\text{mol/L}$)	19.9 ± 4.2	20.1 ± 4.9	20.6 ± 5.5
Total lipid (mmol/L)	5.1 ± 0.8	5.2 ± 0.8	5.2 ± 0.8
α -tocopherol (μmol) / total lipid (mmol) ratio	4.2 ± 0.8	$4.6 \pm 1.0^{\$}$	4.2 ± 0.8
Vitamin D (nmol/L)	36.1 ± 13.5	$47.3 \pm 14.6^{**}$	$41.8 \pm 14.6^*$
Parathyroid hormone (pmol/L)	6.0 ± 2.2	6.5 ± 2.3	6.8 ± 2.6
Alkaline phosphatase (U/L)	261.2 ± 79.6	260.8 ± 77.6	251.2 ± 87.5
Calcium (mmol/L)	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Phosphate (mmol/L)	1.6 ± 0.2	1.7 ± 0.2	1.6 ± 0.1

** $p < 0.001$ Control group vs. Fish oil group; * $p < 0.05$ Control group vs. Fish meal group

$^{\$}p < 0.05$ Fish oil group vs. Control and Fish meal groups

Figure 1. Plasma vitamin D concentrations before (baseline) and after intervention with fish oil and fish meal for 12 weeks

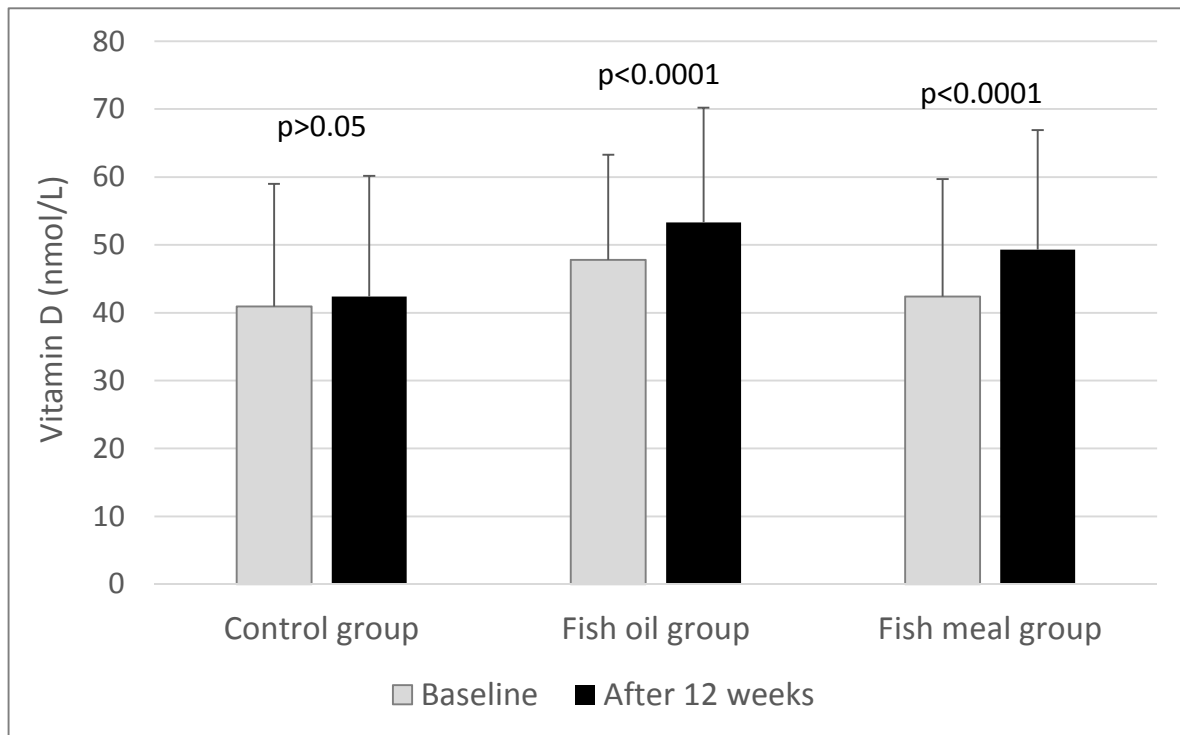
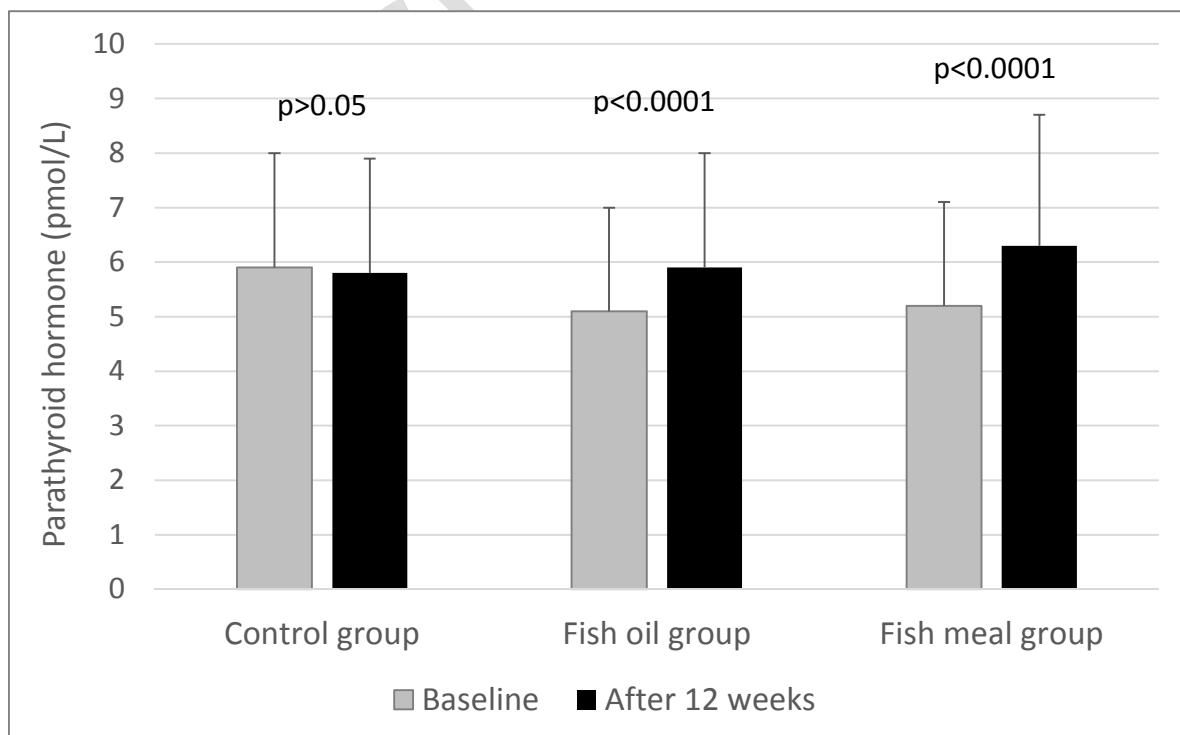


Figure 2. Plasma parathyroid hormone levels before (baseline) and after intervention with fish oil and fish meal for 12 weeks



References

1. Abdel-Wareth L, Haq A, Turner A, Khan S, Salem A, Mustafa F, Hussein N, Pallinalakam F, Grundy L, Patras G and Rajah J (2013) Total Vitamin D Assay Comparison of the Roche Diagnostics “Vitamin D Total” Electrochemiluminescence Protein Binding Assay with the Chromsystems HPLC Method in a Population with both D2 and D3 forms of Vitamin D. *Nutrients* 5:971-980.
2. Abdul Rahim HF, Sibai A, Hwalla N, Fadhil I, Alsiyabi H, Mataria A, Mendis S, Mokdad AH and Hussein A (2014) Health in the Arab World: a view within 2. Non-communicable diseases in the Arab world. *The Lancet* 338:356-367.
3. Abiaka C, Delghandi M, Kaur M and Al-Saleh M (2013) Vitamin D Status and Anthropometric Indices of an Omani Study Population. *SQU Med J* 13 (2): 224-233.
4. Alasfoor D, Rajab H and Al-Rassasi B (2007) Food based dietary guidelines technical background and description. Task force for the development and implementation of the Omani Food Based Dietary Guidelines
5. Al Kalbani M, Elshafie O, Rawahi M, Al-Mamari A, Al-Zakwani A and Woodhouse N (2011) Vitamin D Status in Pregnant Omanis A disturbingly high proportion of patients with low vitamin D stores. *SQU Med J* 11 (1): 52-55.
6. Al-Kindi MK (2011) Vitamin D Status in Healthy Omani Women of Childbearing Age Study of female staff at the Royal Hospital, Muscat, Oman. *SQU Med J* 11 (1): 56-61.
7. Al Lamki (2010) UN Millennium Development Goals and Oman Kudos to Oman on its 40th National Day. *SQU Med J* 10 (3):301-305.
8. Al Riyami A, Attia M, Abd Elaty A, Morsi M, Al Kharusi H, Al Shukaily W, Jaju S (2012) Oman World Health Survey: Part 1 – Methodology, sociodemographic profile and epidemiology of non-communicable diseases in Oman. *Oman Med J* 27(5):425-443.

9. Azzi A, Gysin R, Kempná P, Munteanu A, Negis Y, Villacorta L, Visarius T and Zingg JM (2004) Vitamin E mediates cell signaling and regulation of gene expression. *Ann N Y Acad Sci* 1031:86-95.
10. Badran M and Laher I (2011) Obesity in Arab-speaking countries. *Journal of Obesity* doi:10.1155/2011/686430.
11. Barker DJP (1995) Fetal origins of coronary heart disease. *BMJ* 311:171-174.
12. Bastien J and Rochette-Egly C (2004) Nuclear retinoid receptors and the transcription of retinoid-target genes. *Gene* 328:1–16.
13. Beaton GH, Martorell R and Aronson KL (1993) Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. ACC/SCN State-of-the-Art Series policy discussion paper no. 13. World Health Organization, Geneva, Switzerland.
14. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. (2008) Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 371:243–260.
15. Blakely SR, Dodge WR, Grundel E, Mitchell GV and Jenkins MY (1992) Enhanced accumulation of beta carotene and canthaxanthin in rat liver and plasma with moderately and plasma with moderately high corn oil diets. *Proc Soc Exp Biol Med* 200:277
16. Boonyasit Y, Chinvongamorn C, Chailapakul O and Laiwattanapaisal W (2012) Simple spectrophotometric sequential injection analysis system for determination of serum calcium. *Am J Anal Chem* 3:131-137.
17. Bonet ML, Ribot J and Felipe FA (2003) Vitamin A and the regulation of fat reserves. *Cell Mol Life Sci* 60:1311–21.

18. Borel P (2012) Genetic variations involved in interindividual variability in carotenoid status. *Mol Nutr Food Res* 56: 228–2410
19. Burri BJ (1997) Beta-carotene and human health: a review of current research. *Nutr Res* 17 (3): 547-580.
20. Chakar H and Salameh (2006) Adolescents obesity and Lebanese private schools. *Eu J Public Health* 16 (6):648-651.
21. DeLuca HF (2004) Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 80:1689S–1696S.
22. De Pee S and Dary O (2002) Biochemical indicators of vitamin A deficiency: Serum retinol and serum retinol binding protein. *J Nutr* 132 (9 Suppl):2895S-2901S.
23. Fawzi W., Chalmers T, Herrera M and Mosteller F (1993) Vitamin A supplementation and child mortality: a meta-analysis. *JAMA* 269:898–903.
24. Fossati P and Prencipe L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 28:2077–2080.
25. Galal OM (2002) The nutrition transition in Egypt: obesity, and the food consumption context. *Public Health Nutr* 5 (1A):141-148.
26. Gey KF (1995) Cardiovascular disease and vitamins. Concurrent correction of 'suboptimal' plasma antioxidant levels may, as important part of 'optimal' nutrition, help to prevent early stages of cardiovascular disease and cancer, respectively. *Bibl Nutr Dieta* 52:75 – 91.
27. Goodman DS, Blomstrand R, Werner B, Huang HS, Shiratori T (1966) The intestinal absorption and metabolism of vitamin A and P-carotene in man. *J Clin Invest* 45:16 15-23.

28. Grodstein F, Kang JH, Glynn RJ, Cook NR and Gaziano JM (2007) A Randomized Trial of Beta Carotene Supplementation and Cognitive Function in Men. The Physicians' Health Study II. *JAMA Internal Medicine* 167(20):2184-2190.
29. Hercberg S, Preziosi P, Galan P, Devanlay M, Keller H, Bourgeois C, Potier de Courcy G and Cherouvrier F (1994) Vitamin status of a healthy French population: dietary intakes and biochemical markers. *Int J Vitam Nutr Res* 64(3):220-232.
30. Holman RL, McGill HC Jr., Strong JP, Geer JC (1958) The natural history of atherosclerosis: the early aortic lesions as seen in New Orleans in the middle of the 20th century. *Am J Pathol* 34:209-235.
31. Holman RL (1961) Atherosclerosis - A pediatric nutrition problem. *Am J Clin Nutr* 9:565-569.
32. Hossein-nezhad A, Spira A and Holick MF (2013) Influence of Vitamin D Status and Vitamin D3 Supplementation on Genome Wide Expression of White Blood Cells: A Randomized Double-Blind Clinical Trial. *PLOS ONE* 8 (3): e58725
33. Ibpus -International business publications, USA (2013) Oman – Economic and development strategy hadbook, volume 1, strategic information and program 2020.
34. Imdad A, Herzer K, Mayo-Wilson E, Yakoob MY and Bhutta ZA (2010) Vitamin A supplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. *Cochrane Database Syst Rev* 12:CD008524. doi:10.1002/14651858.CD008524.pub2.
35. IOM - Institute of Medicine, food and nutrition board (2000) Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids, pp. 186 – 283, National Academy Press, Washington.

36. Kilan H, Alyaarubi S, Zayed K, Alzakwani I, Bererhi H, Shukri R and Alrasadi K (2013) Physical fitness attributes, vitamin D, depression, and BMD in omani's children. *Eur Sci J* 9 (30):156-173.
37. Lacher DA, Hughes JP and Carroll MD (2005) Estimate of biological variation of laboratory analytes based on the Third National Health and Nutrition Examination Survey. *Clin Chem* 51:450–452.
38. Lin AM, Chen KB and Chao PL (2005) Antioxidative effect of vitamin D3 on zinc-induced oxidative stress in CNS. *Ann N Y Acad Sci* 053:319-29.
39. Mikkila V, Rosanen L, Pietinen P and Vikari J (2004) Longitudinal changes in diet from childhood into adulthood with respect to risk of cardiovascular diseases. The cardiovascular risk in young Finns study. *Eu J Clin Nutr* 58:1038-1045.
40. McGill HC Jr, McMahan CA, Herderick EE, Malcom GT, Tracy RE and Strong JP (2000) Origin of atherosclerosis in childhood and adolescence. *Am J Clin Nutr* 72 (suppl):1307S-1315S.
41. McGill HC Jr., McMahan CA and Gidding SS (2008) Preventing heart disease in the 21st century: Implications for the pathological determinants of atherosclerosis in youth (PDAY study). *Circulation* 117:1216-1227.
42. McGill HC Jr., McMahan CA and Gidding SS (2009) Are pediatricians responsible for prevention of adult cardiovascular disease? *Nat Clin Pract Cardiovasc Med* 6(1):10-21.
43. McGowan MW, Artiss JD, Strandbergh DR and Zak B (1983) A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29:538–542.
44. McNamara JJ, Molot MA, Stremple JF, Cutting RT (1971) Coronary artery disease in combat casualties in Vietnam. *JAMA* 216(7):1185-1187.

45. MHO – Ministry of Health of Sultanate of Oman (2006) National micronutrient status and fortified food coverage survey, Oman, 2004
46. Musaiger AO (1987) The state of food and nutrition in the Arabian Gulf countries. *Wld Rev Nutr Diet* 54:105-173.
47. Musaiger AO (1998) Change in dietary habits, lifestyle and trend in diseases in GCC countries. *Bahrain Medical Bulletin* 20(3):87-90.
48. Musaiger AO (2002) Diet and prevention of coronary heart disease in the Arab Middle East Countries. *Med Principles Pract* 11 (suppl 2):9-16.
49. Nair PP, Judd JT, Berlin E, Tailor PR, Sliatni S, Sam E and Bhagavan HN (1993) Dietary fish oil-induced changes in the distribution of a-tocopherol, retinol, and beta carotene in plasma, red blood cells, and platelets: modulation by vitamin E. *Am J Clin Nut* 58:98-102
50. Newman WP 3rd, Freeman DS, Voors AW, Gard PD, Srinivasan SR, Cresanta JL et al. (1986) relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis. The Bogalusa Heart Study. *N Eng J Med* 14(3):138-144.
51. Niki E and Traber MG (2012) A history of vitamin E. *Ann Nutr Metab* 61:207 – 212.
52. Olmedilla B, Granado F, Blanco I and Rojas-Hidalgo E.(1994) Seasonal and sex-related variations in six serum carotenoids, retinol, and alpha-tocopherol. *Am J Clin Nutr* 60(1):106 -110.
53. Olson CR and Mello CV (2010) Significance of vitamin A to brain function, behavior and learning. *Mol. Nutr. Food Res* 54, 489–495.
54. Opara LU, Al-Said FA and Al-Abri A (2007) Assessment of what the consumer values in fresh fruit quality: Case study of Oman. *NZ J Crop & Hort Sci* 35:235-242.

55. Péter S, Moser U, Pilz S, Eggersdorfer M and Weber P (2013) The challenge of setting appropriate intake recommendations for vitamin E: considerations on status and functionality to define nutrient requirements. *Int J Vitam Nutr. Res* 83 (2): 129-136.
56. Robinson TM, Borzekowski DLG, Matheson DM and Kraemer HC (2007) Effects of Fast Food Branding on Young Children's Taste Preferences. *Arch Pediatr Adolesc Med* 161(8):792-797
57. Roeschlau P, Bernt E and Gruber WA (1974) Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem* 12:226.
58. Sathe MN and Patel AS (2010) Update in pediatrics: focus on fat-soluble vitamins. *Nutr Clin Pract* 25:340–346.
59. Soni M, Kos K, Lang IA, Jones K, Melzer D and Llewellyn DJ (2012) Vitamin D and cognitive function. *Scand J Clin Lab Invest Suppl.* 243:79-82.
60. Tourniaire F, Gouranton E, von Lintig J, Keijer J, Bonnet ML, Amengua J, Lietz G and Landrier J-F (2009) Beta-Carotene conversion products and their effects on adipose tissue. *Genes Nutr* (2009) 4:179–187.
61. Traber MG (2014) Vitamin E inadequacy in humans: Causes and consequences *Adv Nutr* 5: 503–514.
62. UNDP (2010) Human Development Report 2010. The Real Wealth of Nations: Pathway to Human Development.
63. UNICEF (2007) Vitamin A supplementation: A decade of progress, New York. 8-ISBN: 978-92-806-4150-9
64. UNICEF (2009). Situation analysis of children and women in Oman update 2009: Fact Sheet, pp. 5-6.

65. Valtueña J, Breidenassel C, J. Folle J and González-Gross M (2011) Retinol, β -carotene, α -tocopherol and vitamin D status in European adolescents; regional differences and variability: A review. *Nutr Hosp* 6(2): 280-288.
66. Ventura AK and Worobey J (2013) Early influences on the development of food preferences. *Curr Biol* 23(9):R401-408.
67. WHO (1996) Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes, Geneva (WHO/ NUT/96.10).
68. WHO (2009) Global prevalence of vitamin A deficiency in populations at risk 1995–2005: WHO Global Database on Vitamin A Deficiency.
69. WHO (2011a) Guideline: Vitamin A supplementation in infants and children 6–59 months of age, Geneva.
70. WHO (2011b) Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations (WHO/NMH/NHD/MNM/11.3).
71. Winklhofer-Roob BM, van't Hof MA and Shmerling DH (1997) Reference values for plasma concentrations of vitamin E and A and carotenoids in a Swiss population from infancy to adulthood, adjusted for seasonal influences *Clin Chem* 43(1): 146–153.
72. Zhang L, Cooney RV and Bertram JS (1992) Carotenoids up-regulate connexin43 gene expression independent of their pro-vitamin A or antioxidant properties. *Cancer Res* 52:5707-12.