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Improving Nutritional Status of Egyptian School Children by A Food-Based Natural Enhancer (Orange and Fish) to Iron Absorption and Omega 3 Supplement

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Abstract

Background: Iron Deficiency is a major health problem in Egypt, especially among children. Several approaches have been used to alleviate such a problem, one of which is diet-based programs. Ascorbic acid has been shown to be an enhancer of iron bioavailability and is readily available in Egypt. Omega-3 is polyunsaturated essential fatty acid which can't be constructed by the body and must be obtained through diet and is important for human health. Omega-3 fatty acids sources include salmon, sardine, tuna, mullet and olive and canola oils. Omega-3 fatty acids have anti-inflammatory properties and are helpful in treatment of many diseases.

Aim of Study: The present study was designed to determine if nutritional status could be improved by using a simple school food-based approach of an enhancer (orange and fish) to iron absorption and omega 3 fatty acid supplements.

Subjects and Methods: The first phase of the study started in January 2016, and the second phase in November 2016, and the oranges and fish (tuna sandwich) were served within the school lunch program to a group of school children. The period of intervention was 2 months in the 1 st phase, and 4 months in the 2nd phase, to assess the effect of longer periods of intervention. The oranges and fish (tuna sandwich) were provided 6 days a week for both phases duration. A school meal (biscuits fortified with iron) was also provided to those receiving the oranges and tuna sandwich and to the control group who did not receive either orange or fish (tuna sandwich). The children were tested for their hemoglobin, serum ferritin and omega 3 index levels at the beginning and at end of the designated intervention period. Stool analysis for presence of parasites was also performed.

Results: During the first phase, there was slight improvement of the mean levels of hemoglobin, ferritin and omega 3 index due to the short duration of orange and tuna sandwich intake. The mean hemoglobin increased from 12.1g/dL to 12.33g/dL, the mean ferritin increased from 26.2ng/ml to 27.6ng/ml while the omega 3 index increased from 1.8% to 2.2%. One hundred and fifty of the intervention children were below 12g/dL (25%). At the post-test, mean hemoglobin for intervention children was 12.43±1.04g/dL. Control group had a mean hemoglobin level of 11.67g/dL at the end. The same rising trend was also seen in the levels of the serum ferritin and the omega 3 index. About forty percent of the intervention group was found to be infected with at least one parasite. Hemoglobin levels, ferritin and omega 3 index were higher in children with no parasites.

Conclusion: The results of this study showed significant improvement in the nutritional status among the intervention group, but not in the control group after providing the oranges and fish (tuna sandwich) for 4 months.

Key Words: *Nutritional status – Iron deficiency – Orange – Tuna sandwich – Omega 3 fatty acid – Dietary supplement and school children.*

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Introduction

IRON deficiency and iron deficiency anemia were and still major health problems in Egypt especially for children and pregnant women. The composition of the Egyptian diet is comprised mainly of non heme iron, a poor source of dietary iron. To further intensify the problem, these diets also contain several inhibitors of iron absorption (phytates and tannates) and lack the enhancers of iron absorption.

Ascorbic acid is a strong enhancer of non heme iron absorption. It may exert its "enhancing" effect by promoting acid conditions within the stomach so that the dietary iron is efficiently solubilized; by reducing ferric iron to its better absorbed ferrous form; by forming chelates with iron in the stomach; and by maintaining the solubility of non heme iron when the food enters the alkaline environment of the small intestine which counteracts the inhibitory effect of dietary ligands such as hydrates and tannins. The latter effect can be explained by the fact that ascorbic acid forms complexes with soluble

food iron at a lower pH than do inhibitory ligands. The overall result is that iron from the common non heme pool complexes with ascorbic acid in the stomach and passes into the intestine as an iron-ascorbate complex, thereby reducing the influence of the inhibitory ligands that bind iron in the more alkaline pH of the duodenum [1].

Iron absorption from a semi synthetic meal increased three-fold after adding 75mg ascorbic acid and four-fold after adding 100mg ascorbic acid [2-7]. The most profound effects of ascorbic acid occur when meals have a high content of "inhibitors" such as phytates and tannins, which are found in a traditional maize-based Latin American meal [11,12]. A similar level of iron absorption enhancement was obtained using synthetic ascorbic acid versus the same amount of natural ascorbic acid consumed in foods such as cauliflower and papaya [8-10]. This enhancing effect of ascorbic acid on iron absorption is dose-related, both from single meals such as a maize meal containing 100g maize [11], and a semi synthetic meal containing dextrimaltose, corn oil, ovalbumin, and 4.1mg iron. The increase in iron absorption (0.77 to 7.1 percent) was directly proportional to the amount of ascorbic acid added over the range of 25 to 1,000mg [2].

Polyunsaturated fatty acids are further subdivided into omega-3 fatty acids which are derived from a-Linolenic Acid (ALA) and omega-6 fatty acids which are derived from Linoleic Acid (LA). They share the same pool of enzymes and go through the same oxidation pathway while being metabolized. Fats from each of these families are essential “Essential Fatty Acids (EFAs)” that can't be constructed by the human body. The body can convert one omega-3 to another omega-3, but can't create an omega-3 from omega-6 or saturated fats [11].

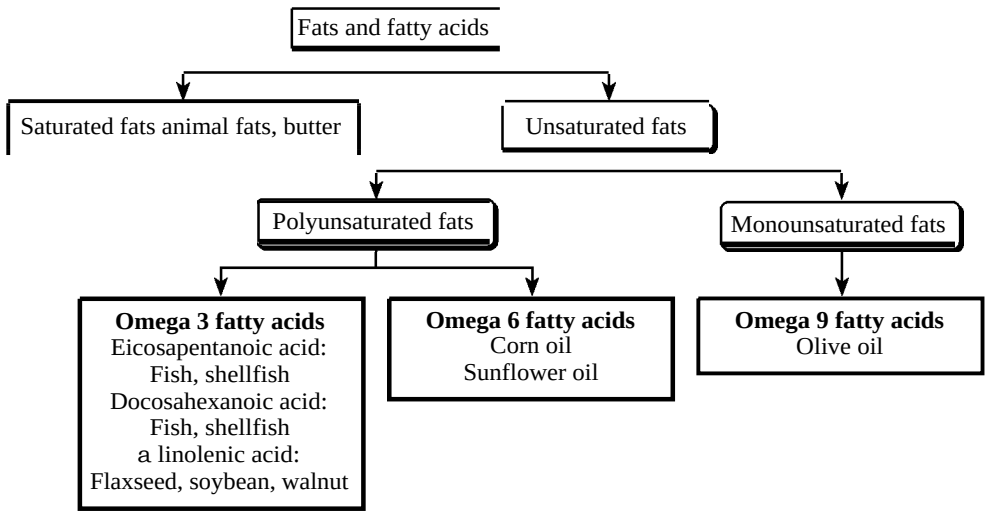

Fig. (1): Classification of fatty acids [12].

Omega-6 eicosanoids are generally pro-inflammatory and omega-3eicosanoids are much less so. The amounts and balance of these fats in a person's diet will affect the body's eicosanoid-controlled functions. Omega-3 fatty acids are considered an important anti-inflammatory factor able to reduce pro-inflammatory cytokines [11].

Fish are the major source of omega-3 fatty acids in diet specially Eicosapentaenoic Acid and Docosahexaenoic Acid (EPA and DHA) such as salmon, herring, mackerel and sardines [13].

Botanical sources are rich source of omega-3. Flaxseed oil consists of approximately 55% ALA. Flax contains approximately 3 times as much omega-3 as omega-6. Chicken eggs are one of the sources of omega-3 and if fish oil is added to the diet of chicken, it will increase its omega-3 concentrations [14].

Cod liver oil contains high levels of omega-3 FAs (EPA and DHA), and very high levels of vitamin A and D but fish oil is extracted from the tissues fatty fish like salmon and is a good source of EPA and DHA and contains very little levels of vitamin A and D [15].

Four major food oils (palm, soybean, rapeseed, and sunflower) provide more than 100 million metric tons oil annually, providing more than 32 million metric tons of omega-6 linoleic acid and 4 million metric tons of omega-3 alpha-linolenic acid [17]. Sources of omega-6 fatty acids include “Poultry, Avocado, Cereals, Whole-Grain Breads, Cottonseed Oil, Sunflower Seed Oil, Corn Oil,

Most Vegetable Oils, Safflower Oil, Evening Primrose Oil, and Borage Oil.

Table (1): Adequate intake for omega-3 fatty acids according to age **[18]**.

Adequate intake for Omega-3 Fatty Acids DHA		
Life stage	Age	Gram/day
Infants	At birth-12 months	0.5
Children	1-3 years	0.7
Children	4-8 years	0.9
Children	9-13 years	1.2

Omega-3 Fatty Acids (DHA).

Table (2): The Adequate Intake allowed (AI) a for infant formula/diet.

Fatty acid	Percent of fatty acids
LA b	10.00
ALA	1.50
AA c	0.50
DHA	0.35
EPA d (upper limit)	<0.10

Omega-3 Fatty Acids (LA b, AL a, AA c, DHA and EPA d).

The Adequate Intake (AI) for omega-3 is 1.6 g/day for men and 1.1g/day for women, while the Acceptable Macronutrient Distribution Range (AMDR) is 0.6% to 1.2% of total energy. Approximately 10 percent of the AMDR can be consumed as EPA and/or DHA **[19]**. Adequate intake for DHA for children is shown in (Tables 1,2) **[18]**.

Polyunsaturated fatty acids are vitally important structural elements of cell membranes and essential for formation of new tissues, as occurs during pregnancy and fetal development **[20]**.

Deficiencies of omega-3 fatty acids especially EPA and DHA have been linked to decreased cognitive abilities, increased aggression and memory loss. Sufficient dietary intake of omega-3 fatty acids has been shown to be effective in promoting cognitive and emotional health **[21,22]**.

Large doses of omega-3 reduce platelet aggregation but smaller amounts have platelet inhibitory effects. Dietary consumption of omega-3 fatty acids decreases platelet aggregation by the reduced formation of the pro-aggregatory TXA2 through an inhibitory effect of omega-3 fatty acids on cyclooxygenase (COX) enzyme which converts AA to TXA2 making thrombus formation less likely Figs. (2,3) **[23]**.

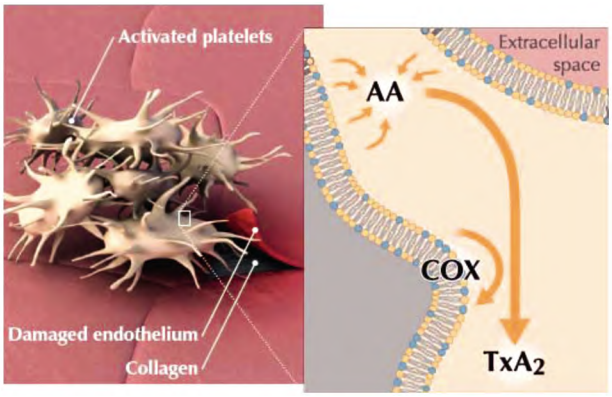

Fig. (2): The effect of omega-6 fatty acids on platelets **[24]**.

Omega-6 fatty acids (also referred as n-6 or ω-6) are a family of unsaturated fatty acids that have a common final carbon-carbon double bond in the n-6 position “sixth bond, counting from the methyl end of the fatty acid”. Linoleic Acid (LA) is a PUFA used in the biosynthesis of Arachidonic Acid (AA) and thus some Prostaglandins (PGs). It is found in the lipids of cell membranes. It is abundant in many vegetable oils (eg. sunflower, and corn oils) Fig. (2) **[16]**.

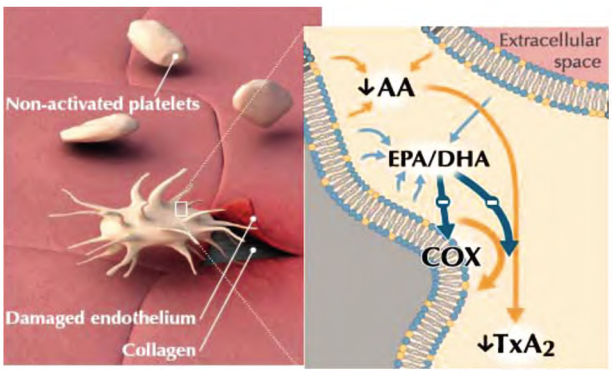

Fig. (3): The effect of omega-3 fatty acids on platelets **[24]**.

Omega-3 fatty acids support healthy levels of calcium, improve calcium absorption and promote calcium deposition in the bone tissue and therefore strengthen the bones **[25]**.

Several limitations are encountered in previous published studies addressing the effect of ascorbic acid on iron absorption and the effect of fish on Omega 3 supplementation. These limitations included: The effect is studied using single meals and not on whole meals, the effect is studied using synthetic ascorbic acid and omega 3 capsules and not of natural origin of both, the effect is studied in communities with low prevalence of iron and omega 3 deficiency, and the effect is studied for short periods.

Aim of the study:

The present study was designed to determine if nutritional status could be improved by a simple school food-based approach for an enhancer (tuna sandwich and orange) of both omega 3 fatty acid and iron absorption.

The main objective was: To decrease prevalence of iron deficiency among school children (10-12 years old) and to spotlight the importance of omega-3 fatty acid daily intake to the health of children.

This will be fulfilled through: (I) Introduction of natural ascorbic acid in the form of orange with the standard school lunch meal for the school children, (II) Introduction of natural omega 3 in the form of fish (tuna sandwich) in the standard school lunch. AND (III) Increase the bioavailability of absorbed iron for the children participating in the study to raise their hemoglobin levels to above 12gm%.

Patients and Methods

The study took place in Shebin El-Kom district, Menoufia Governorate. This study could be assessed as two phases. The first phase started on January 2016 and ended on November 2016, while the 2nd phase started on April 2016 and ended on January 2017. The intervention period for the 1st phase was for 2 months, while that for the 2nd phase was extended for 4 months.

A written consent was taken children parents' after explanation of the objectives of the study to them. The acquaintance with the community started and the explanation of the objectives of the study to the school personnel were undertaken. The local district authorities were the first to be met, and explanation took place. The school authority personals were the next in line for acquaintance, and the visiting team was accompanied by the district authority personnel. Three of the school personnel were chosen to be the key figures that were going to be used in the study later on. The three supervisors were advised to meet with the parents even in the evenings, in the district clubs or in the cafes and to explain the research and the benefits that their children will gain from it later on.

A sample of the school lunch and its comparative alternative (if school lunch is not available), were analyzed to estimate the iron, omega 3 and vitamin C content. The lunch program was irregularly served by the school and covered only 120-180 days of the whole school year.

In the 1st phase, 300 children from fourth and fifth primary grades (10-12 years) were randomly selected from nine classes in three schools to receive the orange and fish (tuna sandwich). There were 152 girls and 148 boys. The intervention included serving 150 children with a fresh orange and the school lunch (tuna sandwich) for six days a week for two months.

In the 2nd phase, 600 children from fourth and fifth primary grades (10-12 years) were randomly selected from 12 classes in four schools to receive the orange and fish (tuna sandwich). There were 302 girls and 298 boys. The intervention included giving all the children a fresh orange and a lunch (tuna sandwich) six days a week for four months. Controls (150 children in 1st phase, and 100 children in 2nd phase) also came from the same schools.

The biscuit and tuna sandwich were the food items given to all schools children. The biscuit weighed ~150 grams and contained a mean of 4mg of iron in the form of ferrous sulfate, the tuna sandwich weighed ~350 and contained 1.2gm of omega 3 fatty acid. The oranges weighed between 100-200 grams (mean 126 grams) and had between 50-75mg of Vitamin C (mean 65mg) and iron a mean of 5.1mg. The controls were served the lunch meals that lacked the orange.

Before providing the meal plus/minus the orange, all children were subjected to blood sampling using a vacutainer and a disposable needle for each vacutainer. The experienced team of doctors and personals collected all samples via the hand veins using strict aseptic conditions.

Each blood sample was collected in two vacutainers. One with EDTA for the hemoglobin, and the other empty vacutainer used for serum collection. Each vacutainer was labeled with a serial number and the children's name for double-checking.

Each child was given an empty labeled plastic container and was asked to provide a fresh stool sample the next morning. Only 94 children out of 300 children (1st phase), and 240 children out of 600 children (2nd phase) returned their stool samples.

The blood samples were taken to the hospital laboratory immediately. The hemoglobin levels were analyzed using the cell analyzer (Sysmic) on the same day. In addition, serum was separated and frozen at 70ºC on the same day. When all the samples were collected for the pre-and post-intervention, serum ferritin was analysed using the

ferritin autoanalyser kits and also omega 3 index was assessed.

We started giving the children daily school lunch (biscuits fortified with iron) with or without an orange and tuna sandwich immediately after blood sampling in the beginning of January 2016 for phase 1, and November 2016 for phase 2. They were provided these meals six days a week, for 2 months (1 st phase) and 4 months (2 nd phase).

The three supervisors ensured that the children ate the, biscuits, tuna sandwich and oranges in their lunch at school.

Statistical analysis:

Data were recorded and analyzed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). All tests of significance were two-tailed. *p*-values of <0.05 were considered statistically significant. Comparison of variables representing quantitative data before and after intervention was performed using the paired *t*-test for normally distributed variables and wilcoxon test for non-normally distributed variables.

Results

During the first phase, there was slight improvement of the mean levels of hemoglobin and ferritin due to the short duration of orange intake. The 138 children, of the intervention orange group, showed that the mean hemoglobin level was 12.09 ±0.61 g/dl (Table 3A). This mean was insignificantly different from the mean of the control group (134 children) of 12.22±0.48g/dl. Average improvement in the intervention group was 0.21 g/dl for hemoglobin and 1.42ng/ml for serum ferritin. Sixty of the intervention group (40%) had hemoglobin levels below 12g/dL before intervention, the numbers decreased to 50 children after the intervention (Table 4A). In addition, there were 40 intervention children with ferritin levels below 10ng/ml decreased to 18 after intervention. Specifically, those with hemoglobin levels below 12g/dl and serum ferritin levels below 10ng/ml improved significantly. Results indicated that among the intervention group, both hemoglobin and serum ferritin levels in the blood improved because of the food-based intervention. All children, despite the number of parasites had higher mean levels of hemoglobin and serum ferritin at the end of the intervention Fig. (4A) & (Table 5A). The mean hemoglobin levels were highest in children not infested with parasites being 12.25 ±0.51g/dl. The hemoglobin and ferritin means showed higher improvements in children infested with one parasite than two parasites.

Table (3): Phase I and II *t*-tests of hemoglobin and serum ferritin levels among cases and controls.

	Mean	Sample size
A = Phase I		
• *Cases:*		
HB before (g/dl)	12.09±0.61	150
HB after	12.30±0.52	
• *Controls:*		
HB before (g/dl)	12.22±0.48	150
HB after	12.12±0.60	
• *Cases:*		
S. Ferritin before (ng/ml)	26.18±3.31	150
S. Ferritin after	27.60±5.62	
• *Controls:*		
S. Ferritin before (ng/ml)	25.41 ±4.3	150
S. Ferritin after	24.09±3.1	
B = Phase II		
• *Cases:*		
HB before (g/dl)	11.81 ±0.41*	600
HB after	12.44±0.40	
• *Controls:*		
HB before (g/dl)	11.72±0.61	100
HB after	11.69±0.50	
• *Cases:*		
S. Ferritin before (ng/ml)	39.62±3.81*	600
S. Ferritin after	49.49±5.20	
• *Controls:*		
S. Ferritin before (ng/ml)	50.06±4.81	100
S. Ferritin after	49.49±3.30	

*: $p<0.01$.

Due to the positive but minimal increase in iron status in the 1 st phase, the period of intervention was extended to 4 continuous months in the second phase. The complete results of 600 children of the intervention group showed that the mean hemoglobin level was 11.81 ±0.41g/dl (Table 3B). This mean was insignificantly different from the mean of the control group of 11.72 ±0.61g/dl. Average improvement in the intervention group was 0.63g/dl for hemoglobin and 9.87ng/ml for serum ferritin which were both statistically significant ($p<0.01$). three hundred and fifty children of the intervention group (58.3%) had hemoglobin levels below 12g/dl (Table 4B). Also, there were 44 children with ferritin levels below 10ng/ml. Specifically, those with hemoglobin levels below 12g/dl and serum ferritin levels below 10 and above 10ng/ml improved significantly. Results indicated that among the intervention group, both hemoglobin and serum ferritin levels in the blood improved significantly as a result of the food-based intervention. The improvement included the iron deficient anemic children (low hemoglobin and ferritin) and iron deficient children (low hemoglobin and normal

ferritin). All children, despite the number of parasites had higher mean levels of hemoglobin and serum ferritin at the end of the intervention Fig. (4) & Table (5B). Forty percent of the intervention group was found to be infested with at least one parasite. The mean hemoglobin levels were highest in children not infested with parasites being 12.27 ± 0.51g/dl. The hemoglobin and ferritin means showed higher improvements in children infested with one parasite than two parasites.

Table (4): Phase I and II *t*-test of hemoglobin and serum ferritin levels among cases & controls above & below cut off points*.

	Mean	Sample size		Mean	Sample size
A = Phase I:			**B = Phase II:**		
• *Cases <12g/dl:*			• *Cases <12g/dl:*		
HB before	11.41±0.38*	60	HB before	10.96±0.60*	350
HB after	12.02±0.41		HB After	12.19±0.53	
• *Cases > 12g/dl:*			• *Cases > 12g/dl:*		
HB before	12.90±0.70	90	HB before	12.91±0.12	250
HB after	12.83±0.62		HB after	12.77±0.11	
• *Controls <12g/dl:*			• *Controls <12g/dl:*		
HB before	11.31±0.43	50	HB before	11.35±0.51	60
HB after	11.26±0.49		HB after	11.38±0.34	
• *Controls > 12g/dl:*			• *Controls > 12g/dl:*		
HB before	12.45±0.61	100	HB before	12.39±0.83	40
HB after	12.34±0.72		HB after	12.17±0.62	
• *Cases <10ng/ml:*			• *Cases <10ng/ml:*		
S. Ferritin before	7.35±0.60*	40	S. Ferritin before	7.51±0.80*	44
S. Ferritin after	21.21±5.42		S. Ferritin after	33.23±3.70	
• *Cases > 10ng/ml:*			• *Cases > 10ng/ml:*		
S. Ferritin before	33.01±7.42	110	S. Ferritin before	42.05±6.83*	556
S. Ferritin after	38.42±5.80		S. Ferritin after	50.43±7.31	
• *Controls <10ng/ml:*			• *Controls <10ng/ml:*		
S. Ferritin before	7.53±0.52	36	S. Ferritin before	7.66±0.42	20
S. Ferritin after	9.61±1.30		S. Ferritin after	10.59±1.61	
• *Controls > 10ng/ml:*			• *Controls > 10ng/ml:*		
S. Ferritin before	32.15±7.22	114	S. Ferritin before	51.93±8.22	80
S. Ferritin after	32.03±6.61		S. Ferritin after	51.23±6.81	
• *Cases <0.9%:*			• *Cases <0.9%:*		
S. Omega 3 index before	0.8±0.20*	50	S. Omega 3 index before	1.0±0.31 *	150
S. Omega 3 index after	1.6±0.42		S. Omega 3 index after	1.9±0.42	
• *Cases > 0.9%:*			• *Cases > 0.9%:*		
S. Omega 3 index before	1.4±0.33	100	S. Omega 3 index before	1.8±0.33	450
S. Omega 3 index after	1.8±0.53		S. Omega 3 index after	2.2±0.53	
• *Controls <0.9%:*			• *Controls <0.9%:*		
S. Omega 3 index before	1.2±0.38	30	S. Omega 3 index before	1.3±0.38	40
S. Omega 3 index after	1.5±0.41		S. Omega 3 index after	1.6±0.41	
• *Controls > 0.9%:*			• *Controls > 0. 9%:*		
S. Omega 3 index before	1.7±0.31	120	S. Omega 3 index before	1.8±0.31	60
S. Omega 3 index after	1.9±0.45		S. Omega 3 index after	2.0±0.45	

*: $p<0.01$.

Cutoff points selected according to:

WHO Technical Report Series 3. Nutritional Anemia 2011.
Worwood, M: erum Ferritin. Critical Reviews in Clinical Laboratory Sciences. Vol. 10 pages 171-204.
Dallman & Siimes: Percentile curves for hemoglobin and red cell volume in infancy and childhood. J. Pediatrics, 94: 26, 1979.
Reeves et al.: Iron deficiency in health and disease. In Zuckerman & Chase, Year Booh Pediatrics: 281, 1984.
Dallman M: Developmental changes in red blood cell production and function. In Rudolph, Pediatrics: 1091, 1991.

Table (5): Phase I (n=94) and II (n=240) mean hemoglobin & serum ferritin levels before & after interventions by number of parasites/child.

Number of parasites/child	0.0	1.00	2.00
A = Phase I:			
HB before (g/dl)	12.25±0.51	12.05±0.61	11.91±0.43
HB after (g/dl)	12.69±0.62	12.24±0.73	12.12±0.51
S. Ferritin before (ng/ml)	25.84±5.42	24.25±6.13	25.71±7.22
S. Ferritin after (ng/ml)	28.92±6.53	25.21±6.71	26.45±5.22
S. Omega 3 index before (%)	1.3±0.40	1.0±0.23	0.8±0.22
S. Omega 3 index after (%)	1.7±0.51	1.5±0.41	1.0±0.34
B = Phase II:			
HB before (g/dl)	12.27±0.51	11.55±0.41	11.73±0.43
HB after (g/dl)	12.73±0.62	12.33±0.72	12.20±0.61
S. Ferritin before (ng/ml)	45.91±5.54	39.26±4.84	49.67±5.22
S. Ferritin after (ng/ml)	56.72±7.53	54.21±6.32	47.43±4.91
S. Omega 3 index before (%)	1.4±0.31	1.1±0.21	0.9±0.20
S. Omega 3 index after (%)	2.0±0.37	1.6±0.40	1.1±0.33

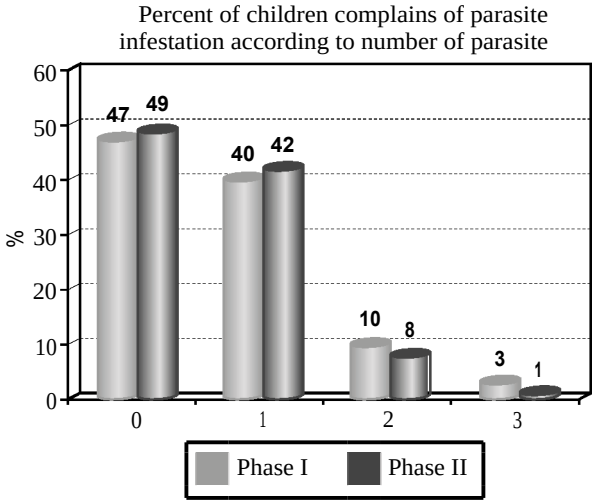

Fig. (4): Phase I (n=94) and Phase II (n=240) percent of intervention children with parasites.

Discussion

Ascorbic acid can improve iron absorption even in the presence of inhibitors such as phytates in cereals and soy, tannins in tea, and calcium **[10,26,28]**. The addition of ascorbic acid (50 or 100mg) significantly counteracted the inhibitory effect of phytate added to wheat rolls at various levels, i.e. 0, 25, and 250mg of phytate phosphorus **[26]**; the latter constitutes 28 percent of the phytic acid molecule. Similarly, 30mg of ascorbic acid overcame the inhibitory effects of 10 to 58mg of phytate phosphorus, and it was concluded that more than 50mg ascorbic acid would be required to overcome the inhibitory effects on iron absorption of any meal containing more than 100mg tannic acid **[29]**. The inhibitory effect of tannic acid in tea consumed with maize-meal porridge was counteracted by giving large amounts of ascorbic acid (250 or 500mg) to iron-deficient Indian women **[10]**. Likewise, the inhibitory effect of taking a calcium supplement (500mg calcium as calcium citrate malate) on iron absorption from a breakfast test-meal was overcome when postmenopausal women drank 450mL orange juice with the calcium supplement **[27]**.

These studies show that the greater the level and effect of inhibitors in a meal, the greater the amount of ascorbic acid required to overcome the inhibition. However, a given quantity of ascorbic acid causes a proportionately greater increase in the amount of iron absorbed from diets higher in inhibitors.

One limitation of the studies that have been conducted with ascorbic acid is that they were predominantly limited to measuring its impact on iron absorption from single meals. There is little information on the effectiveness of increasing ascorbic acid intake on iron status over the longer term, or at the population level. Long term ascorbic acid-induced increases in non heme iron bioavailability might be less than that observed from single meals, particularly among those who are not iron-deficient. For example, two g/day of ascorbic acid with meals for 16 weeks did not increase serum ferritin in non-iron-deficient volunteers in the U.S. eating self-selected diets **[28]**. This was not caused by adaptation to the high ascorbic acid intake because iron absorption from single meals was still stimulated by a dose of ascorbic acid at the end of the 16 weeks. Similarly, 100mg of ascorbic acid, fed with meals three times per day for eight weeks failed to increase serum ferritin **[28]**. Cook et al., (1991) **[29]**, observed that non heme iron absorption from an enhancing diet was 2.5-fold higher than that from an inhibitory diet, when each diet was fed over a two-week period. In contrast,

iron absorption from a single enhancing meal was 5.9-fold higher than from an inhibitory meal. Hunt et al., (1994) [30], reported no significant effect of ascorbic acid (500mg, three times per day) on serum ferritin although the observed increase (from 11.4 to 12.9g/L with predicted poorly-available iron, and from 10.7± to 11.9 with a typical diet in a developed country) may have become significant if the treatment had been given for more than five weeks. Iron balance was not improved but this measure is relatively insensitive to changes in absorption. The level of inhibitors tested in these longer-term studies was much lower (less than 500mg phytate per day) compared with many traditional diets, especially those that are maize-based. For example, in rural Mexico, adult women consume more than 4,000mg of phytate per day, and adult men consume 5,000mg [31]. As Hallberg et al., (1986) [37] pointed out, "a more marked long-term effect of ascorbic acid on iron balance can only be expected when the diet has a fairly high content of inhibitors. In addition, improvements in iron status would be more apparent in iron-deficient populations.

In a recent study by Cook and Reddy (2001) [32], the effect of Vitamin C on non heme-iron absorption from a complete diet rather than from single meals was examined. Iron absorption from a complete diet was measured during 3 separate dietary periods in 12 subjects by having the subjects ingest a labeled wheat roll with every meal for 5 days. The diet was freely chosen for the first dietary period and was then altered to maximally decrease or increase the dietary intake of Vitamin C during the second and third periods. There was no significant difference in mean iron absorption among the 3 dietary periods despite a range of mean daily intakes of vitamin C of 51-247mg/day. When absorption values were adjusted for differences in iron status and the 3 absorption periods were pooled, multiple regression analysis indicated that iron absorption correlated negatively with dietary phosphate (p=0.0005) and positively with ascorbic acid (p=0.0069) and animal tissue (p=0.0285). The reasons for the diminished influence of dietary factors when iron absorption is measured from a complete diet rather than from individual meals are unknown. One possibility is that residual gastric contents from meals eaten throughout the day dampen the influence of dietary factors compared with that in fasting subjects. Another is that the range of meals consumed over a 5-day period is much greater than with an isolated meal and consequently the biochemical composition of the total diet is more varied. The negative influence of phosphorus intake on dietary absorption shown in this study supports the idea that the facilitating effect of ascorbic acid on iron absorption from a complete diet is at least partly offset by dietary inhibitors. For whatever reason, the influence of dietary ascorbic acid on iron absorption is substantially less than indicated by absorption studies with single meals.

The superior impact of food based approaches versus supplemental approaches have been highly stressed in several studies and could be the explanation for the previous observations. In a recent epidemiologic investigation including 634 young individuals' aged 8-18 years, iron stores as measured by serum ferritin were compared with dietary intake during the previous year as assessed by a food-frequency questionnaire [33]. Individuals with a pathologic elevation in serum ferritin were excluded and multiple regression analysis was used to control for sex, age, body mass index, total energy intake, smoking, and the use of medications known to affect blood loss.

There are two reports of the impact of ascorbic acid supplementation at the community level. These studies although showing the same improvement of iron status as the current study, they have used synthetic ascorbic acid and not natural ascorbic acid (orange). In India, 54 anemic preschool children were supplemented with 100mg synthetic ascorbic acid versus a placebo, at each of the two main meals, for two months [34]. Usual iron and ascorbic acid intakes were low. Ascorbic acid treatment improved hemoglobin concentrations significantly, from 9.38 to 11.30ng/ml on average. There was no change in controls (9.08 versus 9.18 ng/ml). Initially 96 percent of all the children had a microcytic hypochromic blood profile, but only 26 percent showed this post-intervention. In China, 65 children with mild anemia received 0, 25, 50, 100, or 150mg ascorbic acid daily for eight weeks (Mao and Yao, personal communications). Usual intakes of iron and ascorbic acid were 7.5mg and 30mg respectively. Weekly iron status assessment showed the 50mg ascorbic acid supplement to be most effective, and an improvement in iron status could be detected in six weeks. No community trials have been attempted on adults, and equally importantly, no interventions have been attempted using local, potentially sustainable food sources of ascorbic acid. Because citrus and other fruits and vegetables are also high in citric acid, these may have a stronger effect on iron absorption than synthetic ascorbic acid.

The impact of ascorbic acid as an enhancer of iron absorption seems to be pronounced in the

anemic children as shown in this study and other studies. There was significant improvement in the children with iron deficiency and iron deficiency anemia in the current study with no impact on the iron replete children of serum ferritin above 12ug/L (Table 4A,B). The results of iron absorption studies performed by Cook and Reddy (2001) **[32]**, augment such findings although the overall results show that iron absorption was minimal. Their subjects were adults and of iron replete status, on the basis of a serum ferritin concentration of >12ug/L, all but one subject was iron deficient and none of the participants were anemic. This subject although only iron deficient but not anemic showed a highest level of iron absorption reaching 26% from high Vitamin C diets compared to 9% from the low Vitamin C diets.

Daily supplementation with fish oil capsules providing omega-3 (EPA and DHA) and Vitamin E for 6 months with increasing sea foods intake can improve many symptoms of CF, breathing easier, coughed up significantly less sputum and the most important result was the sharp drop in needing antibiotic medications **[35]**.

Fish oil consumption has a beneficial effect on lung functions, promotes respiratory health and lessens the effects of oxidative stress and prevalence of asthma **[36]**. Several studies have reported improvement in cough and wheezes with higher dietary intake of omega-3 fatty acids rich foods **[37,38]**.

Using of fish oil and cod liver oil as supplementation in therapeutic diet during medical treatment of asthmatic patient help in reducing the severity of asthmatic attacks. Also it resulted in significant decrement of IgE level and this favorable effect could be attributed to omega-3 fatty acids content of these supplementations **[39]**.

Supplementation of 60 children with moderate persistent asthma, with Omega-3 fatty acids, Vitamin C and zinc either singly or in combination resulted in a significant improvement in pulmonary function tests and sputum inflammatory markers. There was significant improvement with the combined use of the three supplementations than single use of any one of them **[40]**.

One study evaluated intake of Omega-3 and Omega-6 fatty acids in association with development of autoimmunity to pancreatic islet cells as seen in type 1 diabetes. The researchers concluded that dietary intake of Omega-3 is associated with reduced risk of islet autoimmunity in children who are at increased risk for developing IDDM **[41]**.

Epidemiological studies have shown a reduced incidence of cancer in populations consuming high levels of dietary fish. Omega-3 fatty acids have regulatory effects on cell proliferation and apoptosis. They may help sensitizing tumors to different chemotherapeutics **[42]**, improving quality of life and reducing post-surgical morbidity in cancer patients **[42]**.

Fish oil ingestion increases the concentration of plasminogen activator and decreases the concentration of Plasminogen Activator Inhibitor 1 (PAI-1). In patients with types II b and IV hyper lipoproteinemia and in another double blind clinical trial, ingestion of Omega-3 fatty acids decreased the fibrinogen concentration. A recent study noted that fish and fish oil increase fibrinolytic activity, indicating that 200g/d of lean fish or 2g of Omega-3 EPA and DHA improve certain hematologic parameters implicated in the etiology of cardiovascular disease **[11]**.

Helland et al., (2003) **[43]** concluded that children whose mothers had been supplemented with fish oil and who had been breast fed for at least 3 months after birth had a better Intelligence Quotient (IQ). Adequate maternal intake of sea-foods during pregnancy improves verbal communication skills at 6 and 18 months, reduces the risk of preterm birth and low birth weight, improves the infant's problem solving capacity and eye and hand coordination **[43]**.

The pronounced impact in nutritional status of the 2nd phase of intervention compared to the 1st phase in the current study can be attributed to several factors. These factors include the higher incidence of omega three deficiency and iron deficiency anemia among the studied children of the 2nd phase reaching more than 50% compared to 30% of the children in the 1st phase (Table 4A,B). Also the longer period of orange and fish (tuna sandwich) intervention reaching 4 months in the 2nd phase could have influenced the positive nutritional status of those children. Another factor was the use of natural enhancer (orange and fish) that could have such positivity on nutritional status compared to previous studies that used medicinal Vitamin C and omega 3 capsules.

Conclusion:

The results of this study show improvement in the nutritional status of children in the intervention group who were iron or omega three deficient + anemic after giving them an orange and fish daily for four months. This simple and cheap approach can be easily implemented in the schools settings.

The diet-based approach has important implications as it can be used along with other means to overcome the problem of iron or omega three deficiency in school children, both in Egypt and in other countries. Longer periods of introducing the oranges and fish (4 months) in the 2nd phase, have proven to be of highly significant improvement in the nutritional status of the deficient children. This study should stress the importance of using food approaches as means of nutritional intervention and their superiority to using other medicinal supplemental approaches. The improvement of the nutritional status of the school children would improve their scholastic and daily activities with lesser load on the teacher to explain the curriculum. This would allow better performance of both the students and the teachers and lesser extra-scholastic teaching and studying.

Recommendations:

Food based approaches such as providing oranges and tuna sandwich to the meals for long periods would improve the nutritional status significantly. Other approaches such as de-worming and others should be also implemented simultaneously.

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تحسين الحالة الغذائية لآطفال المدارس المصرية بإستخدام محفز غذائى (البرتقال والسمك) لزيادة إمتصاص الحديد والآوميجا ٣

إن نقص الحديد هو مشكلة صحية كبيرة فى جمهورية مصر العربية، وخاصة بين الآطفال. وقد إستخدمت عدة آساليب للتخفيف من هذه المشكلة، آحدها هو البرامج القائمة على النظام الغذائى. آظهر حمض الآسكوربيك آنه محسن للتوافر الحيوى للحديد ومتاح بسهولة فى مصر. آوميغا ٣ هو حمض دهنى آساسى غير مشبع لا يمكن آن يتم إنشاؤه من قبل الجسم ويجب الحصول عليه من خلال النظام الغذائى وهو مهم لصحة الإنسان. وتشمل مصادر الآحماض الدهنية آوميغا ٣ سمك السلمون والسردين والتونا والبورى والزيتون وزيت الكانولا. الآحماض الدهنية آوميغا ٣ لها خصائص مضادة للإلتهابات ومفيدة فى علاج العديد من الآمراض.

وقد تم تصميم هذه الدراسة لتحديد ما إذا كان من الممكن تحسين الحالة التغذوية من خلال إستخدام نهج بسيط يعتمد على الغذاء فى المدرسة من محسن (برتقالى وآسماك) إلى إمتصاص الحديد ومكملات حمض آوميغا ٣ الدهنية.

وقد بدآت المرحلة الآولى من الدراسة فى يناير ٢٠١٦، والمرحلة الثانية فى نوفمبر ٢٠١٦، وقدمت البرتقال والآسماك (ساندويتش التونة) ضمن برنامج الغذاء المدرسى لمجموعة من آطفال المدارس. كانت فترة التدخل شهرين فى المرحلة الآولى، و٤ آشهر فى المرحلة الثانية، لتقييم آثر فترات التدخل الطويلة. تم توفير البرتقال والسمك (سندوتش التونة) ٦ آيام فى الإسبوع لكلتا المرحلتين. كما تم تقديم وجبة مدرسية (بسكويت محصنة بالحديد) إلى آولئك الذين يتلقون سندوتش البرتقال وسمك التونة، وإلى المجموعة الضابطة الذين لم يتلقوا آيا من البرتقال آو السمك (سندويتش التونة). تم إختبار الآطفال على مستويات مؤشر الهيموجلوبين، فيريتين المصل وآوميغا ٣ فى بداية فترة التدخل المحددة وفى نهايتها. تم إجراء تحليل البراز لوجود الفيليات آيضا.

وآسفرت النتائج خلال المرحلة الآولى، بآنه كان هناك تحسن طفيف فى متوسط مستويات الهيموغلوبين، الفيريتين، وآوميغا ٣ بسبب قصر مدة تناول سندويتش التونة والبرتقال. زاد متوسط خضاب الدم من ١٢.١جم/ديسيلتر إلى ١٢.٣٣جم/ديسيلتر، وزاد متوسط فيريتين من ٢٦.٢ نانوغرام/مل إلى ٢٧.٦ نانوغرام/مل بينما إرتفع مؤشر آوميجا ٣ من ١.٨٪ إلى ٢.٢٪. كان مائة وخمسين من الآطفال تدخل آقل من ١٢ غ/دل (٢٥٪). فى مرحلة ما بعد الإختبار، كان متوسط خضاب الدم للتدخل لدى الآطفال ١٢.٤٣+١.٠٤ غرام/ديسيلتر. كان لمجموعة السيطرة مستوى هيموغلوبين يعنى ١١.٦٧ غ/دل فى النهاية. كما لوحظ نفس الإتجاه الصاعد فى مستويات فيريتين المصل ومؤشر آوميغا ٣. وجد آن حوالى آربعين بالمائة من مجموعة التدخل مصابة بطفيلى واحد على الآقل. كانت مستويات الهيموجلوبين، وFerritin وOmega 3 آعلى فى الآطفال الذين ليس لديهم طفيليات.

ووصى هذه الدراسة إلى آهمية تقديم الآغذية مثل البرتقال وساندويتش التونة إلى وجبات الطعام لفترات طويلة والتى من شآنها تحسين الحالة الغذائية بشكل ملحوظ إلى جانب التخلص من الديدان إلى عند الآطفال فى نفس الوقت.