Iron and Zinc Serum Levels in Young Adult Cameroonians after Supplementation in Poor Vitamin A and Controlled Diets

Kana Sop Marie Modestine¹, Gouado Innocent¹, Schweigert Florian², Van Camp John³, Oberleas Donald⁴, Amvam Zollo Paul Henri⁵ and Tetanye Ekoe⁶

¹. Department of Biochemistry, Faculty of Science, University of Douala, Douala 24157, Cameroon
². Institute of Nutritional Science, University of Potsdam, Potsdam 114-115 D-14558, Germany
³. Department of Food Safety and Food Quality, University of Gent, Ghent 653, B-9000, Belgium
⁴. Food and Nutrition, University of Lubbock, Lubbock 79423, USA
⁵. Department of Biochemistry, University of Ngaoundéré, Ngaoundéré 454, Cameroon
⁶. Department of Paediatrics, Faculty of Medicine and Biomedical Sciences, University of Yaoundé, Yaoundé 2036, Cameroon

Abstract: Iron, zinc and vitamin A deficiencies co-exist in Cameroon in all age groups. However, natural sources of vitamin A are available and could be used to meet the need of the whole population in association with iron and zinc supplementation. This study aims at assessing the serum levels of zinc and iron after 11 days of supplementation. The study enrolled 26 men (18-33 years), distributed into five groups. From the first day, they were supplemented with 20 mg of zinc and iron, taken each alone, both either together or at two different times. The five last days, participants were put on free vitamin A diets. Serums were obtained at day 1, day 5 and day 11 for Zn and Fe levels determination by atomic absorption spectrophotometry. The highest serum iron and zinc concentrations were observed in groups either supplemented with zinc or with iron given alone. In those two groups, serum Zn concentrations increased from 0.69 μg/mL ± 0.02 μg/mL to 0.95 μg/mL ± 0.13 μg/mL (group 2), from 0.48 μg/mL ± 0.06 μg/mL to 0.97 μg/mL ± 0.11 μg/mL (group 3); and serum Fe concentrations from 1.49 μg/mL ± 0.54 μg/mL to 3.49 μg/mL ± 1.01 μg/mL (group 2); and from 1.42 μg/mL ± 0.45 μg/mL to 3.41 μg/mL ± 0.81 μg/mL (group 3), respectively. Supplementation with Fe or Zn alone increased both Fe and Zn serum levels of participants. Serum levels of iron and zinc when given together or at different time were not significantly different. Further studies on a larger population are necessary to confirm that supplementation with zinc or with iron alone could raise both zinc and iron levels in serum simultaneously.

Key words: Supplementation, iron, zinc, young-adults, Cameroon.

1. Introduction

Approximately two billion persons worldwide suffer from iron deficiency (ID) [1, 2]. ID is often accompanied by other nutrient deficiencies such as zinc deficiency, especially when the ID is caused by insufficient dietary intakes, as is often the case in developing countries [3].

Iron and zinc are essential for normal neurologic function. ID affects myelination, neurotransmitter metabolism and iron-containing enzymes [1]. Zinc is also important to both the structure and function of the brain [4, 5]. Zinc deficiency during rapid periods of brain growth can alter emotional behavior, decrease spontaneous activity and impair memory, attention and learning ability [6]. Infancy is a period of rapid brain growth [7, 8]. Zinc deficiency is associated with allergic, infectious and autoimmune diseases, and experiments in mice indicate that its epigenetic consequences could even persist for
Iron and Zinc Serum Levels in Young Adult Cameroonianis after Supplementation in Poor Vitamin A and Controlled Diets

several generations [9]. Zinc is required for the activity of more than 300 enzymes, covering all six classes of enzymes [10].

All the indicators of malnutrition and poverty have been increasing in Cameroon up to date. The most affected groups are young children of 0-59 months, pregnant and lactating mothers. Available data showed that the national prevalence of iron deficiency anemia (IDA) in young children under two years has increased from 58% to 82% [11]. The prevalence of anemia in pregnant women is also high (51%). Vitamin A deficiency was 38% in 2004, but even with immunization, the prevalence remains high. More than 70% of all childhood deaths worldwide are due to malnutrition [12]. Iron and zinc are important micronutrients for child growth and development [8, 13]. In Cameroon and many developing countries, micronutrients deficiencies are due to inappropriate feeding, poor knowledge in nutrition, inadequate access to flesh food rich in most available nutrients, health services and unhealthy environments. Zinc deficiency is usually associated to IDA. Any perturbations during early childhood, such as zinc deficiency and poor stimulation, can lead to long-term impairment of brain structure and function [14]. Diversification and appropriate use of available local foods combined with nutritional education could help to reduce the prevalence of vitamin A by half. However, minerals deficiency cannot easily be reduced by traditional feeding in developing countries, because, the most affected families are poor and have very limited access to animal foods that contain the best qualities of available nutrients. Even though the main affected groups are young children, pregnant and lactating mothers, the study group was constituted of young adult men. Children can not follow voluntary diet restriction. Pregnant and lactating mothers have mineral absorption influenced by their status as well as young girl.

A similar but independent study in Central Java, Indonesia, showed benefits on psychomotor development from iron but not zinc supplementation [15, 16]. Supplementation with iron or zinc or both during infancy does not lead to long-term cognitive improvement in nine-year-old children [8]. Zinc binding sites in proteins are often distorted tetrahedral or trigonal bipyramidal geometry, made up of the sulfur from cysteine, of the nitrogen from histidine and of the oxygen from aspartate and glutamate, or a combination all those atoms. Zinc in proteins can either participate directly in chemical catalysis or be important for maintaining protein structure and stability. In all catalytic sites, the zinc ion functions as a Lewis acid [17]. Many studies have assessed the effect of iron and zinc supplementation in infants on their iron and zinc status, growth, development, and morbidity [15, 18-21]. The positive influence of the zinc supplement nature was reported [22]. Supplementation with iron or zinc or both during infancy in another study does not lead to long-term cognitive improvement in nine-year-old children [8].

Studies in zinc intakes and status in Cameroon are scarce [23-25]. However, Cameroon is indicated among countries with 50% of zinc deficiencies. Data of iron and zinc intakes from supplements are needed for large scale supplementation with these two minerals. We carry this study to investigate if there is any influence in associating 20 mg of iron and zinc each or giving them separately during supplementation.

2. Materials and Methods

2.1 Choice of Participants

Twenty six adult men and young students were chosen and enrolled in supplementation studies with zinc, iron, zinc and iron taken together and iron and zinc taking at different time during 11 days under controlled diet. All participants gave their written informed consent for their participation. Inclusion factors including healthy status were approved by a medical doctor that examined each participant before
the starting of the study.

Before the enrollment of participants, information and sensitization campaigns were organized to raise awareness on vitamin A and minerals deficiencies with most attention on ID anemia, zinc and vitamin A deficiencies. In fact, those three deficiencies are of public health in Cameroon with high and increasing prevalence. Even if the most affected groups by micronutrient deficiencies in Cameroon are young children and women of child bearing age, in this study we chose the group of young adult men for three main reasons. Children are not able to follow diet restriction we included in the study. Pregnant and lactating mothers are expected to have extra-mineral needs for fetus and young children. This may influence the absorption of minerals of individuals, according to the levels of stores and the age of gestation or the age of the lactating baby. Young adult women were excluded because during the study they could get pregnancy or menstruation that will increase interindividual changes and differences in micronutrients absorption.

The information campaigns concern both sexes because whenever the study was design for young adult men, the girls and women of childbearing ages and young children are the most affected and the main beneficiaries for future interventions. Also, on campus mix men and women usually eat and share ideas together. The aim of the campaigns was to inform the participants about the importance of the study and explain why young adult men were chosen instead of young adult women who were really willing to participate to the study. During the seminars and information campaigns, the role of zinc, iron and vitamin A and their possible interaction in their specific metabolism was explained. Details on the main causes, consequences of deficiencies and food sources of the three micronutrients that were studied in this trial were discussed. They were clearly taught on the food sources of vitamin A, including vegetal sources of carotenoids provitamin A.

2.2 The Supplements

The study lasted 11 days. Since the first day, participant bloods, their anthropometric parameters (weights, sizes and ages) and their arterial tensions were took. Those that had to take the supplements of iron and zinc (iron, zinc, iron + zinc taking at the same time or zinc/iron with iron taking in the morning and zinc in the evening) began at the same day to take them till the 11th day. We wanted to verify if there is an influence when iron and zinc are given together or separately. The supplementation dozes of zinc and iron are 20 mg for both iron daily needs being 8 mg/d to 14 mg/d (men) and 15 mg/d to 18 mg/d for women; zinc needs being 11 mg/d to 15 mg/d for men and 8 mg/d to 12 mg/day (women) (Table 1). It is admitted to give in amount, one to two recommended daily allowances (RDA) in supplementation studies. The ingested iron or zinc is also linked to the form of mineral in the supplement. On the 6th day after the blood puncture and the registration of all others up listed parameters, the participants have been submitted to a diet poor or without vitamin A and in provitamins A carotenoids. We gave them the breakfast, the lunch and dinners at the faculty of science restaurant.

2.3 Distribution of the Participants According to Feeding and Supplementations Mod

After medical examinations by a medical doctor in our University Social Medico Center, the 32 participants were divided into six groups as indicated in Table 1.

Blood puncture and analyses were carried out at the Laboratory of the Department of Biochemistry of the University of Douala. All the groups apart for control group received zinc and iron supplements in 11 days either only one of each, or both together / both at two different moments. The innovation in this study is the administration of the same dozes of zinc and iron together (both zinc and iron in the morning) and also separately (iron in the morning and zinc in the evening).
Table 1  Distribution of groups according to supplementation.

| Groups  | No. of participants | Supplements given                          |
|---------|---------------------|--------------------------------------------|
| Group 1 | 5                   | Iron (Fe)                                  |
| Group 2 | 6                   | Zinc (Zn)                                  |
| Group 3 | 6                   | Zinc and iron once (Fe + Zn)               |
| Group 4 | 5                   | Zinc, iron (iron morning, zinc evening) (Fe/Zn) |
| Group 5 | 5                   | No supplement                              |

As indicated in Table 1, the first group constituted of five persons took 20 mg of iron during the 11 days. The second group (group 2) of six persons took 20 mg of zinc during the 11 days. Group 3 made of six persons took 20 mg of iron and 20 mg of zinc in the morning. Group 4 made of five persons received 20 mg of iron in the morning and 20 mg of zinc in the evening, after 12 h. The control group, group 5 made of five persons was put in the same conditions, but did not receive any supplement.

2.4 Anthropometric Measures

Anthropometric measures (weights, heights and blood pressures) were recorded at days 1, 6 and 11. The body masses indexes (BMI) were calculated. Weight and length were measured at the start and end of the supplementation. Weight was recorded to the nearest 0.1 kg, while participant were minimally clothed without shoes with a scale (SECA balance, Hamburg, Germany). Length was recorded to the nearest 0.1 cm, and BMI were calculated.

2.5 Supplementation Trials

Participants, all young adult men, selected after the medical examination according to our study criterias were enrolled in 11 days supplementation under free vitamin A diets. Each participant was dewormed by 500 mg vermox and took 600 mg of quinine to limit the influence of intestinal parasite and malaria on the lost and the absorption of minerals, especially iron. The supplements were distributed each morning and during the breakfast offered to the participants. For the first week of study, the groups supplemented with iron and zinc started receiving supplement according to the above group descriptions, and group 6 took vitamin A supplement at the 11th day. They were offered breakfasts and lunch at the restaurant of the faculty. At that time they were given supplements as in previous day. The aim of given those supplements under control to them were to prevent or to limit responses bias. We carefully prepared for them breakfast and lunch recipes equilibrated in energy and free of vitamin A sources. Because of the knowledge they acquired on food sources of vitamin A (animal) and carotenoids provitamin A (vegetal), they were allow to have free diners, but according to recommendation not to take any food rich in vitamin A or its sources. The supplementations continue in the second week following the same principle as in the first six days.

2.6 Blood Puncture and Analyses of Serum Zinc and Iron

Blood samples (5 mL) were obtained by venipuncture. Fasting blood samples were collected at day 1 (the beginning of the supplementation), at day 6 (the beginning of free vitamin A and carotenoids provitamin A diets) and at day 11 (the end of the supplementation). Serum was obtained by centrifuging the blood in the field at 3,600 × 3 g for 15 min. The serum was stored at -80 °C until analyzes of serum zinc and iron with a Varian Spectr AA-40 atomic absorption spectrometer (Varian, Inc., Palo Alto, CA, USA) [26]. It was decided not to measure specific indicator like ferritine and hemoglobin that indicate the influence of supplementation on anemia, because our subject were apparently healthy, but total iron in serum would rapidly indicate the iron level according to the mode of supplementation.
2.7 Ethics

Written informed consent was required for the inclusion of each participant. The protocol of this study was conformed to the International Guidelines for Ethical Review of Epidemiological Studies and was approved by the National Ethical Committee of Cameroon (Ethical Clairance Number: 032 / CNE / DNM / 07).

2.8 Statistics

One-way analysis of variance procedures were applied by using Sigma Stat (SPSS, Cary, NC, USA). All values are presented as means-standard deviations. A probability level of \( P \leq 0.05 \) indicated statistical significance.

Statistical analysis was done with SPSS 8.0 for Windows (1998). Means for each organ were compared by two-way analysis of variance. If the analysis for the group was significant \( (P \leq 0.05) \), the individual means were compared with Fisher’s protected least significant difference (PLSD) post hoc analysis.

3. Results

The results are presented in the forms of tables and figures, the numbers corresponding to the average \( \pm \) standard deviation of the average. They were summers analyzed by SPSS version 13.0 for Windows (SPSS Inc., Chicago, USA). The serum concentrations of zinc and iron were studied comparatively using one way analysis of variances \( t \) (one way ANOVA) followed by the test of Duncan. The multiple correlations of person made it possible to determine the relations between significantly different variables. The figures were traced by using the software Sigmat stud 9.0. The differences were considered significant when \( P < 0.05 \). Results of the statistical analyses are integrated in the form of later. In the same table, the numbers having the same later superscript are not significantly different at \( P < 0.05 \). Highest serum of iron and zinc concentrations were observed in groups either supplemented in zinc or in iron given alone. There was not improvement during the first week of supplementation when the students were on their regular diets.

3.1 Anthropometric Parameters of the Participants during the Study

Anthropometric measurements made the first day, the 6th and the 11th day enabled us to control the influence of feeding on the variation of the nutritional status of the participants during the study. Statistically appreciable or visible variations were not observed on the weights and the indices of body mass from the beginning to the end of the study. This reassures us that the mode applied negatively did not influence the statute nutritional of the participants during the 11 days of study. These conditions of studies were thus reproducible. The BMI were in majority in the normal range showing that the students were under better feeding conditions. Indeed, when the BMI are below the normal recommendations these proves that participant have a deficiency in energy and probably low stores in micronutrients. In this case, the absorption of minerals will be much larger than that under the normal conditions. Participants’ physical parameters (BMI and blood pressure (BP)) at the enrollment were not significantly different (Table 2).

All the body mass indices were lower than 25 kg/m\(^2\) and considered normal, overweight as a BMI between 25 kg/m\(^2\) and 30 kg/m\(^2\), because obesity is defined as a BMI above 30 kg/m\(^2\); morbid obesity as a BMI more than 35 kg/m\(^2\); No overweight was observed. BMI and PD are physical parameters that can influence nutritional status and are usually correlated with nutrients absorption.

Blood pressure (BP), blood pressure systolic (BPS) (mm Hg) and blood pressure diastolic (BPD) (mm Hg) were normal (normal \( < 120 < 80 \)). The values were comprised between 10.60 ± 0.55 and 11.67 ± 0.52 for BPS and from 7.00 ± 0.63 to 7.60 ± 0.55 for BPD. We did not observe any prehypertention or hypertension indicators (BP comprised between 120-139 and 80-89,
Iron and Zinc Serum Levels in Young Adult Cameroonians after Supplementation in Poor Vitamin A and Controlled Diets

Table 2  Anthropometric variables of participants during the study.

| Groups                        | Group codes | Height (m) | Ages (years) | Weight (kg) | IMC (kg/m²) |
|-------------------------------|-------------|------------|--------------|-------------|-------------|
|                               |             | 1.74 ± 0.07a | 23.6 ± 2.41a | 63.20 ± 5.59a | 21.06 ± 1.37a |
| Supplemented in iron          | 1           |            |              | Day 1       | Day 6       | Day 11      |
|                               |             | 1.80 ± 0.06b | 25.00 ± 1.26b | 65.60 ± 4.77b | 21.19 ± 1.44b |
| Supplemented in zinc          | 2           |            |              | Day 1       | Day 6       | Day 11      |
| Supplemented in zinc and iron once | 3       | 1.74 ± 0.09a | 23.83 ± 2.56a | 63.10 ± 5.03a | 21.19 ± 1.44b |
| Supplemented in zinc and iron (Fe.M, Zn.E) | 4       | 1.75 ± 0.05a | 24.00 ± 1.22ab | 63.10 ± 5.03a | 21.19 ± 1.44b |
| Control                       | 5           | 1.77 ± 0.08ab | 25.00 ± 4.63b | 63.10 ± 5.03a | 21.19 ± 1.44b |

| P values                      | 0.484       | 0.887      | 0.608        | 0.780       | 0.640       | 0.875       | 0.902       | 0.563       |

Values are means ± standard deviation for n participant; means in the same column with the same superscript (letter) are not statistically significant different at the 95% confidence level (P ≤ 0.05); Fe.M = iron in the morning; Zn.E = zinc in the evening.

Table 3  Blood pressures (BP) participant during the study.

| Groups                        | Group codes | BPD (mmHg) | BPS (mmHg) | BPD (mmHg) | BPS (mmHg) | BPD (mmHg) | BPS (mmHg) |
|-------------------------------|-------------|------------|------------|------------|------------|------------|------------|
| Supplemented in fer           | 1 (n = 5)  | 13.20 ± 1.64c | 7.60 ± 1.14ab | 11.00 ± 1.00a | 7.60 ± 0.55ab | 10.80 ± 0.84a | 7.40 ± 0.55a |
| Supplemented in zinc          | 2 (n = 6)  | 11.83 ± 0.75ab | 6.67 ± 0.52b | 11.33 ± 0.52ab | 7.17 ± 0.41a | 11.50 ± 0.55a | 7.00 ± 0.63a |
| Supplemented in zinc and iron once | 3 (n = 5) | 11.83 ± 0.75ab | 6.67 ± 0.52b | 11.33 ± 0.52ab | 7.17 ± 0.41a | 11.67 ± 0.52ab | 7.00 ± 0.89a |
| Supplemented in zinc and iron (Fe.M, Zn.E) | 4 (n = 6) | 11.80 ± 0.84ab | 6.80 ± 0.84b | 10.80 ± 0.84a | 7.40 ± 0.55a | 11.00 ± 1.00a | 7.60 ± 0.55ab |
| Control                       | 5 (n = 5)  | 11.40 ± 0.55a | 7.00 ± 1.00a | 10.60 ± 0.55a | 7.20 ± 0.45a | 10.60 ± 0.55a | 7.20 ± 0.45a |

| P values                      | 0.032       | 0.042      | 0.596       | 0.705       | 0.222       | 0.695       |

Values are means ± standard deviation for n participant; means in the same column with the same superscript (letter) are not statistically significant different at the 95% confidence level (P ≤ 0.05); BPD (diastolic blood pressure), BPS (systolic blood pressure); Fe.M = iron in the morning; Zn.E = zinc in the evening.

3.2 Levels of Serum Iron and Serum Zinc during the Study

The examination of the serum levels of zinc obtained by atomic absorption did not vary between the first and the 6th day. In addition, we noted that these rates increased at the 11th day. The study of the correlations shows that the concentrations of zinc were correlated with the groups and the days of blood puncture, whereas those of iron were correlated only with the days of blood puncture and not with groups. Indeed the days of blood puncture were the first day with the introduction of the supplementation, the 6th day, with 11th, the systolic blood pressures and diastolic were correlated just to the size, the body mass and the BMI. The correlations between the blood pressures in one and above 140 and 90, respectively for BPS and BPD). There were not significant differences between body max indices BMI ranged between 20.49 ± 0.92 and 22.66 ± 2.44 at the 11th day (Table 3). The diastolic and systolic blood pressures of the participants measured during the 1st, the 6th and the 11th days were in normal range. These measurements allowed us confirm that the participants were able to support the study and the blood puncture. Generally the variations between the various groups and the various days statistically did not show. However, the blood pressures of the first day were higher than those of the 6th and 11th days. This could be explained by the fact that many participants had a little stress for the first day, especially because it was their first time to take part in this kind of studies.
Iron and Zinc Serum Levels in Young Adult Cameroonians after Supplementation in Poor Vitamin A and Controlled Diets

Table 4  Serum levels of zinc and iron after supplementation.

| Groups                                | Group codes | Zinc (μg/mL) | Iron (μg/mL) |
|---------------------------------------|-------------|--------------|--------------|
|                                       |             | Day 1        | Day 6        | Day 11       | Day 1        | Day 6        | Day 11       |
| Supplemented in iron                  | 1 (n = 5)   | 0.69 ± 0.02c | 0.53 ± 0.09a | 0.95 ± 0.13b | 1.49 ± 0.54ab| 1.12 ± 0.27ab| 3.49 ± 1.01c |
| Supplemented in zinc                   | 2 (n = 6)   | 0.48 ± 0.06a | 0.56 ± 0.08a | 0.97 ± 0.11b | 1.42 ± 0.45ab| 0.84 ± 0.26a | 3.41 ± 0.81c |
| Supplemented in zinc and iron once    | 3 (n = 5)   | 0.53 ± 0.06ab| 0.57 ± 0.08ab| 0.79 ± 0.13a | 1.73 ± 0.53b | 0.94 ± 0.51a | 2.92 ± 0.80b |
| Supplemented in zinc and iron (Fe.M, Zn.E) | 4 (n = 6)   | 0.66 ± 0.04c | 0.52 ± 0.04a | 0.87 ± 0.12ab| 1.05 ± 0.36a | 0.93 ± 0.17a | 2.77 ± 0.61abc|
| Control                               | 5 (n = 5)   | 0.56 ± 0.08b | 0.52 ± 0.05a | 0.77 ± 0.12a | 1.60 ± 0.28ab| 1.64 ± 0.12b | 1.93 ± 0.10a |
| P values                              |             | 0.000        | 0.774        | 0.006        | 0.24         | 0.000        | 0.003        |

Values are means ± standard deviation for n participant; means in the same column with the same superscript (letter) are not statistically significant different at the 95% confidence level (P ≤ 0.05); Fe.M = iron in the morning; Zn.E = zinc in the evening.

Fig. 1  Comparison of zinc serum profiles of participants after supplementation in iron and zinc.
The graphs on day 1, day 6 and day11 with the same letter (a, b, c) show that zinc concentrations were not significantly different at 0.05 among the groups.

Fig. 2  Comparison of iron serum profiles of participants after supplementation in iron and zinc.
The graphs on day 1, day 6 and day11 with the same letter (a, b, c) show that iron concentrations were not significantly different at 0.05 among the groups.
Iron and Zinc Serum Levels in Young Adult Cameroonian After Supplementation in Poor Vitamin A and Controlled Diets

were lower than those of the study population reported by Granado et al. [27] in 2006, comprising between 22 and 25 (average 23.1 kg/m²).

The serum iron and zinc were previously reported to be influenced negatively by the stress and other environmental factors [28]. But in our study, we assume that, lower iron stores in the participants were influenced by their new diet the first week and not by the stress.

The medium values of serum iron concentrations ranging between 90 ug/dL and 110 ug/dL (or 0.9 g/mL and 1.1 g/mL) are age dependant. They are high in young adults. Sex seems not to have great influence on serum iron concentration. Total serum iron is influenced negatively during ID while total iron binding capacity increases. Low serum iron concentrations during ID anemia are correlated to low transferrin saturation level and high total iron binding capacity. Serum iron content is a measure of the number of atoms bound to the iron transport protein transferrin. It is assumed that each molecule of transferrin can be bond to one or two atoms or iron. However, both binding site are rarely occupied [29]. Serum iron concentration is influenced by many factors. Usually, overload of iron is generally due to hemochromatosis, hemolytic anemia, acute liver damage, excessive absorption of iron from transfusions and iron therapy. This results in elevated iron stores that may falsely increase serum iron concentrations. In contrast, chronic disease states like infections, inflammations and malignancy typically may also lead to low serum levels of iron [30]. This is why antimalarial was given to participants prior to the supplementation to limit the influences in serum iron linked to malaria state. We used fasting blood to limit biological variations of serum iron values that can be very high after some meals. Serum iron values tend to be elevated in the morning, decreasing in the afternoon and the evening. As a result, measurements of serum iron/zinc were preferably on fasting morning blood samples to minimize the effect of both recently dietary intake and diurnal variation. Day to day, variations in

4. Discussion

Antropometric parameters, particularly blood pressure of our participants were good, and in normal range (10.60 ± 0.55 and 11.67 ± 0.52 for BPS and from 7.00 ± 0.63 to 7.60 ± 0.55 for BPD) for the study. BMI hand, and the BMI in another hand, the size and the body mass were not significant. However, they significant with other parameters. In the two groups as observed in Table 4, serum concentrations of Zn increased from 0.69 ± 0.02 to 0.95 ± 0.13 (Fe group) and from 0.48 ± 0.06 to 0.97 ± 0.11 (Zn group); those of Fe increased from 1.49 ± 0.54 to 3.49 ± 1.01 (iron group); and from 1.42 ± 0.45 to 3.41 ± 0.81 (zinc group).

Apart from the reference group, the serum rates of iron and zinc significantly increased at the end of the study (at the 11th day). A fall of the serum levels of iron and zinc was also observed at the 6th day in all the groups no matter the supplementation status. The supplementation had, however, started since the first day. The levels of serum iron and serum zinc significantly increased between the 6th day and the 11th day. We observed two phenomena. In spite of the iron supplementation, in zinc, iron + zinc and with the iron/zinc, the serum levels of iron and zinc did not increase, but rather, they decreased the 6th day (Fig. 1).

We also observed an increase in the serum rates of iron and zinc in all the groups at the 11th day. We think that the recipes were a good sources of iron and zinc, especially because the serum rates of these two minerals also significantly increased in the not supplemented reference groups, but at the less extend than in the groups supplemented with iron and zinc. We observed an increase in the serum levels of iron and zinc when only one of each was taken (Fig. 2).

The iron levels were higher in the iron group, but not statistically significant, compared with the group supplemented in zinc and with those supplemented with iron + zinc (both iron and zinc taking at the same time in the morning) and with iron/zinc (iron taking in the morning and zinc taking in the evening).
Iron and Zinc Serum Levels in Young Adult Cameroonians after Supplementation in Poor Vitamin A and Controlled Diets

Iron and zinc concentrations also occur. Low birth weight was associated with maternal anaemia and, in some circumstances, with low iron and zinc status [10].

Zinc is an essential trace mineral. The human body has between 1.5 g and 2.5 g zinc, making it nearly as abundant as iron. It is highly concentrated in specialized areas of the brain, pancreas and adrenal gland, but is present in all cells, particularly in the nucleus. Zinc has structural, catalytic (enzymatic) and regulatory roles. About 1% of the human genome codes for zinc finger proteins, where zinc provides a structural role for regulatory functions. Over 200 enzymes require zinc for activity, including the RNA polymerases. Zinc is actively taken up by synaptic vesicles, supporting a role in neuronal activity and memory. Zinc metabolism is altered during disease and physical stress through hormones, cytokines and toxins, presumably as part of a host defense response. An early sign of zinc deficiency in animals is decreased food intake. It is a type II deficiency since a reduction in growth occurs without an apparent reduction in tissue zinc. Reduced immune function, involving lymphocyte B (B cell) and lymphocute T (T cell) depletion and/or reduced activity, and skin lesions associated with secondary infections are common findings. Chronic zinc deficiency in humans results in reduced growth (dwarfism) and sexual development which are reversible by raising zinc intake. Signs of zinc deficiency may reflect its involvement in cell proliferation and differentiation. Growth, behavioral abnormalities and cognition may respond to zinc supplementation in some populations. Many clinical findings that relate to depressed growth or immunity may have marginal zinc deficiency as a secondary cause. Zinc is not widely used as a therapeutic agent except as an ingredient of topical medication. Oral zinc may be used to treat idiopathic skin lesions, some inflammatory conditions and depressed immunity. Zinc is usually indicated in rehabilitation therapy from malnutrition and/or malabsorption in children and adults, used in feeding programs for premature infants and neonates and is also a component of therapeutic post and neonatal (TPN) solutions. Supplemental zinc reduces acute diarrhea and depressed immunity.

The recommended dietary allowances (RDAs) are infants, 5 mg/day; children < 10 years old, 10 mg/day; males > 10 years old, 15 mg/day; females > 10 years old, 12 mg/day; pregnancy, 15 mg/day; and lactation, 0-6 month, 19 mg/day; 7-12 month, 16 mg/day.

We observed that iron and zinc taken together were less effective to raise serum zinc and serum iron than iron or zinc taken alone. It was previously reported that, iron supplementation combined with zinc was less effective than iron supplementation alone in reducing the prevalence of anemia (20% vs. 38% reduction) and in increasing hemoglobin and plasma ferritin concentrations [19].

The importance of iron and zinc in the body is linked to their implications and their role in many functions. Iron and zinc are essential for normal neurologic function. ID affects myelination, neurotransmitter metabolism and iron-containing enzymes [31]. In addition, indirect mechanisms may link anemia to poor cognitive development, such as functional isolation, which leads to reduced exploration of the environment and reduced activity [4]. Zinc is also important to both the structure and function of the brain [32]. The activity of virtually all immune cells is modulated by zinc in vitro and in vivo [9]. Significant correlations were observed between zinc and iron serum concentrations in one hand, and in another hand, between experiment day, serum iron and zinc concentrations (Table 5). These means was a confirmations that iron or zinc supplementation both or alone increase iron and zinc serum concentrations, whether taken as single or together. There were also significant correlations between experiment day and blood pressure. However, blood pressures of participants were still in
Iron and Zinc Serum Levels in Young Adult Cameroonians after Supplementation in Poor Vitamin A and Controlled Diets

Table 5  Correlations between different parameters.

| Groups        | Pearson’s correlations | Experiment days | Zinc          | Iron          | Height        | Weight        | BMI           | Age           | BPS           | BPD           |
|---------------|------------------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|               |                        |                 | -0.222*       | -0.072        | -0.106        | 0.065         | 0.128         | 0.100         | -0.134        | 0.032         |
|               |                        |                 | 1.000         | 1.000         | 0.027         | 0.476         | 0.297         | 0.523         | 0.207         | 0.327         |
|               |                        |                 | 0.000         | 0.000         | 0.000         | 1.000         | 0.000         | 0.993         | 0.712         | 0.958         |
|               |                        |                 | -0.222*       | 0.661**       | 0.739**       | -0.019        | -0.126        | -0.088        | -0.022        | -0.048        |
|               |                        |                 | 1.000         | 1.000         | 1.000         | 1.000         | 0.993         | 0.712         | 0.958         | 0.000         |
|               |                        |                 | -0.072        | 0.553**       | 0.739**       | 1             | -0.026        | -0.038        | 0.031         | -0.015        |
|               |                        |                 | 0.476         | 0.000         | 0.000         | 0.789         | 0.711         | 0.763         | 0.880         | 0.982         |
|               |                        |                 | -0.106        | 0.000         | -0.019        | -0.026        | 1             | 0.568**       | 0.097         | -0.183        |
|               |                        |                 | 0.297         | 1.000         | 0.854         | 0.798         | 0.000         | 0.339         | 0.070         | 0.595         |
|               |                        |                 | 0.065         | 0.001         | -0.126        | -0.038        | 0.568**       | 1             | 0.813**       | -0.014        |
|               |                        |                 | 0.523         | 0.000         | 0.000         | 0.789         | 0.711         | 0.763         | 0.880         | 0.982         |
|               |                        |                 | 0.128         | 0.038         | -0.088        | 0.031         | 0.097         | 0.813**       | 1             | 0.118         |
|               |                        |                 | 0.207         | 0.000         | 0.000         | 0.789         | 0.711         | 0.763         | 0.880         | 0.982         |
|               |                        |                 | 0.128         | 0.038         | -0.088        | 0.031         | 0.097         | 0.813**       | 1             | 0.118         |
|               |                        |                 | 0.100         | -0.005        | -0.022        | -0.015        | -0.183        | -0.014        | 0.118         | 1             |
|               |                        |                 | 0.327         | 0.000         | 0.000         | 0.831         | 0.880         | 0.070         | 0.892         | 0.246         |
|               |                        |                 | 0.327         | 0.000         | 0.000         | 0.831         | 0.880         | 0.070         | 0.892         | 0.246         |
|               |                        |                 | 0.185         | 0.000         | 0.000         | 0.639         | 0.982         | 0.595         | 0.664         | 0.602         |
|               |                        |                 | 0.032         | 0.016         | 0.091         | 0.041         | 0.081         | 0.039         | -0.013        | -0.084        |
|               |                        |                 | 0.756         | 0.877         | 0.368         | 0.689         | 0.426         | 0.699         | 0.899         | 0.410         |

After considering all the parameters the correlations were significant at level 0.05 or 0.01; *the correlation is significant at the level 0.05 (bilateral); **the correlation is significant at the level 0.01 (bilateral).

Severe maternal zinc deficiency has a devastating effect on pregnancy outcome. Studies of experimental animals and humans show that maternal zinc deficiency can cause infertility, prolonged labor, intrauterine growth retardation, teratogenesis, or embryonic or fetal death. The effect of zinc on immune function is not based on a single mechanism; rather, zinc affects the expression of hundreds of genes in immune cells [33]. Neither is the effect of zinc limited to one part of the immune system. Functional consequences of zinc deficiency affect lymphopoiesis, virtually all types of mature immune cells, cytokine production, and the polarization of T helper subsets [34]. The functional consequences of zinc deficiency are as multifaceted as the effects of zinc on the immune system.

5. Conclusions

At the end of the study, we could affirm that in the presence of adequate food intakes, the supplementation by iron or zinc alone respectively improves absorption by increased serum zinc level on one hand, and that of iron on the other hand. We finally concluded that, there was a synergistic effect between iron supplementation and food zinc levels on the one hand, and between zinc supplementation and food iron content, respectively. The increase in the serum iron rate in all the groups
including the reference group at the 11th day proves that the food brought iron and zinc, more than in the usual diets of the participants. After observing the supplementation of iron and zinc taken together or isolated, we noted that the serum rates of the two minerals increased well when taken separately, but they have different influences according to groups. Zinc levels were significantly lower when iron and zinc were taken together than separately. For the supplementation, it would be thus better to advise to give iron or zinc while controlling the food and nutrients intakes rather than to give iron and zinc together. This would help to considerably reduce the cost of the large scale interventions at community level.

Acknowledgments

This work was done under the grant of the International Foundation for Science (IFS) under the project E-4328-1. We also thank all the participants and Dr. Demasse-Mawamba Adelaïde for their kind contribution to the study.

Conflict of interest: The authors declare no conflict of interest.

Kana Sop Marie Modestne contributed in the conception of the project and she is the grant winner, Gouado Inocent oriented the analyses and provide relevant documents, Van Camp John contributed in the project design, Amvam Zollo Paul Henri provided necessary advises, Schweigert Florian participated in some laboratories analyses, Oberleas Donald edited the document and oriented the analyses and Tetanye Ekoe help conceiving and checking the ethical and application aspects of the project.

References

[1] J.L. Beard, Iron biology in immune function, muscle metabolism and neuronal functioning, J. Nutr. 131 (2001) 568S-579S.
[2] E. Souganidis, The relevance of micronutriments to the prévention of stunting, Sight and Life 26 (2) (2012) 10-18.
[3] H. Haase, Functional significance of zinc-related signaling pathways in immune cells rink, Annu. Rev. Nutr. 29 (2009) 133-152.
[4] B. Lozoff, N.K. Klein, E.C. Nelson, D.K. McClish, M. Manuel, M.E. Chacon, Behavior of infants with iron-deficiency anemia, Child Dev. 69 (1998) 24-36.
[5] S. Bhatnagar, S. Tanjela, Zinc and cognitive development, Br. J. Nutr. 85 (2001) S139-S145.
[6] N. Solomons, M. Ruz, Zinc and iron interaction: Concepts and perspectives in the developing world, Nutr. Res. 17 (1997) 177-185.
[7] B. Lozoff, E. Jimenez, A.W. Wolf, Long-term developmental outcome of infants with iron deficiency, N. Engl. J. Med. 325 (1991) 687-694.
[8] P. Tippawan, A.M. Girolamo, U. Ramakrishnan, P. Winichagoon, R. Flores, R. Martorell, Long-term effects of iron and zinc supplementation during infancy on cognitive function at nine year of age in Northeast Thai children: A follow-up study, Am. J. Clin. Nutr. 93 (2011) 636-643.
[9] M.J. Salgueiro, M.B. Zubillaga, A.E. Lysioneck, R.A. Caro, R. Weill, J.R. Boccio, The role of zinc in the growth and development of children, Nutrition 18 (2002) 510-519.
[10] G.H. Lowrey, Growth and Development of Children, 8th ed., IL Year Book Medical Publishers Inc., Chicago, 1986, pp. 15-35.
[11] INS, Demographic and Health Survey in Cameroon (DHSC), ORC Macro 11785 Beltsville Drive Calverton, Maryland, USA 2004, pp. 3-66.
[12] INS, Demographic and Health Survey—Multiple Indicators (DHSC MICS) of 2011, Cameroon, 2012, pp. 28-39.
[13] C. Hotz, R.S. Gibson, Complementary feeding practices and dietary intakes from complementary foods amongst weanlings in rural Malawi, Eur. J. Clin. Nutr. 55 (2001) 841-849.
[14] S. Grantham-McGregor, Y.B. Cheung, S. Cueto, P. Glewwe, L. Richter, B. Strupp, Developmental potential in the first five years for children in developing countries, Lancet 369 (2007) 60-70.
[15] T. Lind, O. Hernell, B. Lonnerdal, H. Stenlund, M. Domellof, L.A. Persson, Dietary iron intake is positively associated with hemoglobin concentration during infancy but not during the second year of life, J. Nutr. 134 (2004) 1064-1070.
[16] T. Lind, B. Lonnerdal, H. Stenlund, A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: Effects on growth and development, Am. J. Clin. Nutr. 80 (2004) 729-736.
[17] K.A. McCall, C.C. Huang, C.A. Fierke, Zinc and health: Current status and future directions function and mechanism of zinc, J. Nutr. 130 (2000) 1437S-1446S.
[18] T. Lind, B. Lonnerdal, H. Stenlund, A community-based randomized controlled trial of iron and zinc
supplementation in Indonesian infants: Interactions between iron and zinc, Am. J. Clin. Nutr. 77 (2003) 883-890.

[19] M.A. Dijkhuizen, F.T. Wieringa, C.E. West, S. Martuti, Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth, J. Nutr. 131 (2001) 2860-2865.

[20] J. Berger, N.X. Ninh, N.C. Khan, Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants, Eur. J. Clin. Nutr. 60 (2006) 443-454.

[21] M.M. Black, A.H. Baqui, K. Zaman, Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants, Am. J. Clin. Nutr. 80 (2004) 903-910.

[22] N.W. Solomons, R.A. Jacob, Studies on the bioavailability of zinc in humans: Effects of heme and nonheme iron on the absorption of zinc, Am. J. Clin. Nutr. 34 (1981) 475-482.

[23] M.M.K. Sop, J. Kikafunda, J.K. Meli, I. Gouado, Z.P.H. Amvam, F.C. Oberleas, E. Tetanye, Young children feeding and zinc levels of complementary foods in Western Cameroon, African Journal of Food, Agriculture Nutrition and Development 4 (11) (2011) 4953-4967.

[24] M.M.K. Sop, I. Gouado, M.J. Mananga, W.A. Djuekeu, P.H.A. Zollo, D. Oberleas, Trace elements in foods of children from Cameroon: A focus on zinc and phytates content, J. Trace Elem. Med. Biol. 26 (2012) 201-204.

[25] M.M.K. Sop, I. Gouado, M.J. Mananga, L.D. Ekoule, A.P.H. Zollo, E. Tetanye, Evaluation of nutritional status of young children aged 0-2 years in the Douala city (Cameroon), survey of some practices during diversification of complementary foods, African Journal of Food Science and Technology 4 (2) (2013) 29-34.

[26] M.S. Clegg, C.L. Keen, B. Lönnerdal, L.S. Hurley, Influence of ashing techniques on the analysis of trace elements in tissues, Biol. Trace Element Res. 3 (1981) 107-115.

[27] F. Granado, B. Olmedilla, C. Herrero, B.N. Perez-Sacrista, I. Blanco, S. Blazquez, Bioavailability of carotenoids and tocopherols from broccoli: In vivo and in vitro assessment, Exp. Biol. Med. 231 (2006) 1733-1738.

[28] V.B. Grossie, J.W.R. Kennedy, D. Narins, Zinc, copper and iron in plasma and tissues after intestinal ischemia and reperfusion in the rat, Nutrition 19 (2003) 1003-1005.

[29] R. Gibson, Principles of Nutritional Assessment, Oxford University Press, New York, 1990, pp. 542-552.

[30] S.Y. Hess, D.I. Thurnham, R.F. Hurrell, Influence of Provitamin A Carotenoids on Iron, Zinc and Vitamin A Status, HarvestPlus, Washington DC and Cali, 2005, pp. 4-56.

[31] J.L. Beard, Iron deficiency alters brain development and functioning, J. Nutr. 133 (2003) 1468S-1472S.

[32] R.E. Black, Zinc deficiency and child development, Am. J. Clin. Nutr. 68 (1998) 464S-469S.

[33] F.W. Beck, Y. Li, B. Bao, A.S. Prasad, F.H. Sarkar, Evidence for reprogramming global gene expression during zinc deficiency in the HUT-78 cell line, Nutrition 22 (2006) 1045-1056.

[34] A.S. Prasad, Zinc: Mechanisms of host defense, J. Nutr. 137 (2007) 1345-1349.