Iron and zinc are essential micronutrients for human growth, development and maintenance of the immune system (1). Iron is needed for psychomotor development, maintenance of physical activity, work capacity and resistance to infections. Zinc is needed for growth and maintenance of the immune system, which enhances the prevalence of infections and delays recovery from it (2). The coexistence of multiple micronutrient deficiencies is increasingly recognized as a widespread public health problem in developing countries (3, 4). In many developing countries, as in Cameroon, most diets are cereal-based, low in animal products and high in phytates which lead to micronutrient deficiencies (1, 5, 6). Interactions between iron, zinc and vitamins influence the metabolism of these micronutrients (1, 7, 8). These deficiencies are common in geographically similar areas. It has also been shown that growing children and women of reproductive age are the most vulnerable to these deficiencies, which may occur simultaneously (9). Numerous studies have addressed the effect of vitamin A on iron metabolism, but very little is known on the effect of iron on provitamin A carotenoid (PAC) absorption (3, 10). In Cameroon, the most common nutritional challenges include: iron deficiency anemia, and zinc, vitamin A and iodine deficiencies with prevalence rates above the World Health Organisation (WHO) standards (3, 7).

National data showed that the prevalence of iron deficiency anemia in young children under 2 y increased from 58% (2004) to 82% (2008) and stunting from 24% (2004) to 36% (2008) (11, 12). A recent study on micronutrient status and fortifiable foods in Cameroon reported very high prevalences of stunting (33%), vitamin A deficiency (VAD) (34%), Iron deficiency (IDA)
(57.6%) and zinc deficiency (69.1%). These figures are higher than the prevalence in the world as reported by the WHO (12). The underlying causes of poor nutrition in some parts of Cameroon as reported by recent studies are lack of knowledge about nutrient content of food and balanced diet formulation, poor primary health care, food monotonry, poor mineral content of dishes and complementary foods, increasing food and nutrition insecurity, and poor access to animal food sources (4, 6, 13). It is probable that Cameroon will not attain the millennium development goals, and its people may continue to suffer from poor health with impaired cognitive development due to malnutrition in the younger generation (4). Poor content and bioavailability of iron and zinc in complementary foods (combined to blending, fermentation and germination) was reported as one of the underlying causes of deficiencies in these minerals (5, 6). Iron supplementation recommended for young children and pregnant women from the 3rd trimester is not followed up. However, many plant sources of provitamin A carotenoids exist and can be used to reduce the prevalence of vitamin A deficiency in Cameroon (14, 15). Food based approaches take a longer time to implement, but are considered a sustainable method of reducing malnutrition. Combined strategies of diet and supplementation may produce better overall results. In this case supplementation of iron and zinc to improve bioavailability of natural sources of vitamin A (4) is required.

Many sources of provitamin A carotenoids such as oranges, fresh fruits and tubers, and dark green leafy vegetables have been identified (15, 16). Papaya, one of best sources, is available throughout the year in Cameroon, and can easily be grown in most locations (17). This study is aimed at investigating the influence of iron and zinc supplementation on the bioavailability of provitamin A carotenoids from local papaya.

MATERIALS AND METHODS

Study design. Twelve young male students aged 18 to 31 y in the Douala University who participated in a nutrition seminar were recruited. They were informed about how to recognize different food groups, and distinguish dietary sources of vitamin A and provitamin A carotenoids. Inclusion criteria were excellent health as indicated by their health history and blood parameters with normolipidemia. Exclusion criteria were restrictive eating and with BMI 30 kg/m2 were also excluded. Participants were given Vernox 500 mg (mebendazole) against intestinal worms and they also took 600 mg quinine as single dose, against malaria. This was to limit infections that might negatively influence the absorption and bioavailability of provitamin A carotenoids or impact mineral metabolism. Participants were divided into 3 groups of 4 persons each: The iron group received an iron supplement (iron fumarate, 20 mg/d), the zinc group received a zinc supplement (zinc sulfate, 20 mg/d) and the iron+zinc group received a combined supplement of iron (Fe fumarate), and zinc (zinc sulfate) (20 mg of iron in the morning, and 20 mg of zinc in the evening). The study lasted for 11 d. The participants were themselves used as the control. Chylomicron levels of carotenoids were assayed using high performance liquid chromatography (HPLC), serum triacylglycerides using enzymatic kits and serum iron and zinc by atomic absorption spectrophotometry. These served as the auto control or baseline data. On the first day (at enrollment), blood samples were collected, anthropometric measurements (weight and height) taken and blood pressure measured. All the samples for the study were collected in trace element-free tubes. Supplementation with iron, zinc, or zinc+iron started immediately and continued until Day 11. Daily iron requirements were 8 to 14 mg/d (men) and zinc requirements were 11 to 15 mg/d. It is acceptable to provide up to twice the RDA recommendations in supplementation studies (3). On Day 6, blood samples were collected, participants weighed and blood pressure measured. On this day, the subjects were provided vitamin A- and provitamin A carotenoid-free diets. All meals were prepared at the Faculty restaurant. On Day 11, anthropometric measurements were recorded, fasting blood samples were collected and participants were given a test meal (breakfast, 7 h–7 h 30 min) that contained 550 g of papaya in small pieces as source of P Acs, skimmed milk, olive oil (5 g) and mineral water. Lunch contained white yam stew (cooked with olive oil), fat-free yoghurt, white bread and mineral water. The amount of each provitamin A carotenoid ingested in the 550 g of papaya was 2.13 mg β-carotene (177.55 retinol activity equivalent (RAE) or 355.09 retinol equivalent (RE)), 1.32 mg α-carotene and 0.19 mg β-cryptoxanthin (1.51 mg (6.29 RAE, 125.96 RE for α-carotene + β-cryptoxanthin)), respectively. The total amount of ingested provitamin A carotenoids in the test meal was 3.64 mg.

Determination of carotenoids and triacylglycerols. Blood samples were taken from every participant before the test meal (T0), and after 2 h (T2), 4 h (T4) and 7 h (T7) in test tubes with no anticoagulant. The serum was obtained after 30 min by centrifugation of blood samples at 3,000 rpm/min for 10 min, and distributed in 2 aliquots in brown Eppendorf tubes for determination of retinol and provitamin A carotenoids. Retinol was extracted from the serum and analysed. Prior to extraction, 500 µL of absolute ethanol containing retinyl acetate as an internal standard was used for precipitating protein in 200 µL of serum. The extraction was done using 1 mL of n-hexane twice and centrifugation at 3,000 rpm/min for 5 min at −5°C, and evaporated under nitrogen, recollected in acetoniitrile and passed through a Sonicator (Bioblock Scientifique 88169) at 10°C for 30 min). Samples for provitamin A carotenoids were frozen at −80°C until analyses. Chylomicrons were extracted from 1 mL of thawed serum layered under 1 mL NaCl 9 g/L (d=1.004 kg/L after ultracentrifugation (150,000 ×g for 1 h, at 20°C)) in the lipid
phase. The method had been previously described by Cardinault et al., using plasma (16). The chylomicron supernatant layer was collected, dried under nitrogen and stored at −80°C until analyses. Carotenoids were extracted from the chylomicrons using ethanol containing an internal standard (which precipitates the proteins) and hexane to solubilize the carotenoids. The hexane extract was evaporated to dryness under nitrogen and the residue was solubilized in an acetonitrile/dichloromethane mixture (1:1, v/v).

The carotenoids were quantified by reverse-phase HPLC on a Waters system (Waters SA, Saint-Quentin en Yvelines, France). This system comprised a Waters 660 pump, a Waters 717 plus cooled auto-sampler, and a Waters 996 UV-visible diode-array detector. The mobile phase was an isocratic acetonitrile/dichloromethane/methanol (containing 50 mmol/L ammonium acetate)/water (70:10:15:5 v/v) mixture. Carotenoids were detected at 450 nm and identified by comparison of their retention times (the retention times were between 35 and 45 min, that is, 33–34 min for β-cryptoxanthin, 36 min for α-carotene and 38–40 min for β-carotene) and spectral analysis (from 300–550 nm). These were compared to those of pure standards (Roche Vitamines France, Neuilly-sur-Seine, France).

Triacylglycerols were assayed by using an enzymatic colorimetric method with a commercial kit (Biomerieux, Craponne, France). The concentrations were measured spectrophotometrically at 490 nm using a Microplate Reader MR 700 (Dynatech Laboratories Inc., Guernsey, UK).

The study protocol was approved by the National Ethics Committee of Cameroon, according to the Declaration of Helsinki and International Conferences on Harmonization for Good Clinical Practice. Written informed consent was obtained from the participants before their enrollment.

Statistical analyses. Comparisons between groups were made by one-way analysis of variance (ANOVA) followed by Fisher’s LSD (least significant difference) multiple range test. The statistical analyses were performed using SAS (Statistical Analysis Software) software (SAS Institute, Cary, NC). Postprandial carotenoids in chylomicrons were expressed in concentrations and compared to the baseline values (18). Provitamin A carotenoid concentrations in the groups (supplemented with the different minerals) were compared using an unpaired Student’s t-test. p values ≤0.05 were considered significant. The data presented in the tables represent the mean values of groups mean with standard deviations (SD). The comparisons in the tables are in the form of letter superscripts. Means in the same columns with the same superscripts are not significantly different at p≤0.05. The ANOVA in this study splits the variance of the data into two components: a between-group component and a within-group component. The F-ratio represents the between-group estimate, and the within-group estimate is represented by the p-value.

| Supplements        | Age (y) | BMI (kg/m²) | SBP   | DBP   | Triacylglycerols (mg/dL) | Serum zinc (µg/mL) | Serum iron (µg/mL) |
|--------------------|---------|-------------|-------|-------|--------------------------|--------------------|--------------------|
| Iron               | 23.6±2.4 | 20.80±1.47  | 108.0±8.4 | 74.0±5.5 | 3.74±0.69               | 0.69±0.02          | 0.69±0.02          |
| Zinc               | 25.00±1.2 | 21.89±1.33  | 118.5±6.3 | 70.0±5.5 | 3.81±0.73               | 0.55±0.03          | 0.55±0.03          |
| Iron+zinc          | 24.06±1.2 | 20.49±1.59  | 110.0±6.1 | 76.0±5.5 | 4.11±0.34               | 0.68±0.04          | 0.68±0.04          |

BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

Values are means±standard deviations.
ces (BMIs) were lower than 25 kg/m², thus, in normal
in Tables 1 and 2 respectively. All the body mass indi-
eters of the participants were measured. These are found

Participants’ health parameters at enrollment

absorbed provitamin A carotenoids.

individual values added to newly formed retinol and
appearance because after the test meal, they represent
levels were expressed as

6 mean standard deviation of three analyses. The retinol

208

for prehypertension (BP between 120–139 mmHg and
SBP and 7.00–7.60 mmHg for DBP with no indicators
normal range (120 mmHg for SBP and

results in vivo and in vitro bioavailability of
carotenoids and tocopherols from broccoli. Blood pres-
sure (BP) (systolic, SBP and diastolic, DBP) were in the

20.49 ± 0.92 kg/m² and 22.66 ± 0.35 for the 1st
and 11th days respectively. These values were lower
than those reported by Granado et al. (19) in a similar
study assessing the in vivo and in vitro bioavailability of
carotenoids and tocopherols from broccoli. Blood pres-

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mean ± standard deviation of three analyses. The retinol
and provitamin A carotenoid levels were expressed as
appearance because after the test meal, they represent
individual values added to newly formed retinol and
absorbed provitamin A carotenoids.

Participants’ health parameters at enrollment

Before enrollment, anthropometric and serum param-
eters of the participants were measured. These are found
in Tables 1 and 2 respectively. All the body mass indices
(BMIs) were lower than 25 kg/m², thus, in normal
range with no significant difference. The averages were
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and 11th days respectively. These values were lower
than those reported by Granado et al. (19) in a similar
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6 the absorption peak at T2
and in the iron
zinc association were
Although the retinol levels were lower in the zinc group
higher in groups 1 and 2 than in the iron + zinc group.
Although the retinol levels were lower in the zinc group
and in the iron + zinc group the absorption peak at T2
was very specific in these two groups. The absorption
peak at T2 was significantly higher in the iron group (Fe)
than in the zinc group (p = 0.03). There was no significant difference in the
iron + zinc supplemented group (p = 0.13) (Tables 3–5).

Table 2. Baseline retinol and provitamin A carotenoid levels of participants (µg/mL).

| Groups | Retinol    | α-Carotene | β-Carotene | β-Cryptoxanthin |
|--------|------------|------------|------------|----------------|
| Iron   | 0.31 ± 0.04a | 0.34 ± 0.05b | 0.55 ± 0.08b | 0.26 ± 0.05a |
|        | (0.27–0.32) | (0.29–0.36) | (0.48–0.63) | (0.21–0.31) |
| Zinc   | 0.31 ± 0.07a | 0.33 ± 0.03ab | 0.54 ± 0.07a | 0.26 ± 0.08a |
|        | (0.26–0.35) | (0.29–0.36) | (0.46–0.61) | (0.15–0.37) |
| Iron+zinc | 0.34 ± 0.02b | 0.23 ± 0.01a | 0.55 ± 0.06b | 0.44 ± 0.11b |
|        | (0.32–0.35) | (0.2–0.26)  | (0.49–0.60) | (0.33–0.45) |

Values are means ± standard deviation for n participant. Values in the bracket are the minimal and maximal values for each analysis. Means in the same column with the same superscript (letter) have p-values of the F-test greater than or equal to 0.05, meaning that they are not statistically significant different at the 95% confidence level.

Table 3. Postprandial serum retinol and provitamin A carotenoid levels in chylomicrons (µg/mL) in the zinc group over 7 h.

| Zinc group | Retinol    | α-Carotene | β-Carotene | β-Cryptoxanthin |
|------------|------------|------------|------------|----------------|
| T0         | 0.30 ± 0.10a | 0.33 ± 0.10a | 0.55 ± 0.16a | 0.025 ± 0.012a |
|            | (0.24–0.35) | (0.29–0.37) | (0.54–0.64) | (0.02–0.046)   |
| T2         | 0.36 ± 0.1a  | 0.38 ± 0.1a  | 0.66 ± 0.2a  | 0.041 ± 0.042a |
|            | (0.30–0.41) | (0.33–0.45) | (0.54–0.74) | (0.035–0.055)  |
| T4         | 0.33 ± 0.10ab | 0.36 ± 0.1ab | 0.62 ± 0.23ab | 0.043 ± 0.015ab |
|            | (0.27–0.37) | (0.31–0.42) | (0.50–0.76) | (0.037–0.08)   |
| T7         | 0.29 ± 0.1a  | 0.35 ± 0.1ab | 0.60 ± 0.21a | 0.046 ± 0.019b |
|            | (0.24–0.35) | (0.30–0.40) | (0.48–0.71) | (0.036–0.09)   |
| Average    | 0.32        | 0.37        | 0.60        | 0.053          |
| p          | 0.83        | 0.90        | 0.93        | 0.68           |
| F          | 0.29        | 0.19        | 0.15        | 0.51           |

Values in brackets are minimal or maximal for analyses. Means in the same column with the same letter superscript are not significantly different at the 95% confidence level. T, time of blood draw; T0, baseline or 1st blood draw just before the test meal; T2, 2nd blood draw 2 h after the test meal; T4, 3rd blood draw 4 h after the test meal; T7, 4th blood draw 7 h after the test meal.

RESULTS

The values of analyzed parameters were presented as mean ± standard deviation of three analyses. The retinol and provitamin A carotenoid levels were expressed as appearance because after the test meal, they represent individual values added to newly formed retinol and absorbed provitamin A carotenoids.

Participants’ health parameters at enrollment

Before enrollment, anthropometric and serum parameters of the participants were measured. These are found in Tables 1 and 2 respectively. All the body mass indices (BMIs) were lower than 25 kg/m², thus, in normal range with no significant difference. The averages were 20.49 ± 0.92 kg/m² and 22.66 ± 2.44 kg/m² for the 1st and 11th days respectively. These values were lower than those reported by Granado et al. (19) in a similar study assessing the in vivo and in vitro bioavailability of carotenoids and tocopherols from broccoli. Blood pressure (BP) (systolic, SBP and diastolic, DBP) were in the normal range (<120 mmHg for SBP and <80 mmHg for DBP). This was between 10.60 and 11.67 mmHg for SBP and 7.00–7.60 mmHg for DBP with no indicators for prehypertension (BP between 120–139 mmHg and 80–89 mmHg), and for hypertension (>140 mmHg for SBP and 90 mmHg for DBP). The retinol, α-carotene, β-carotene and β-cryptoxanthin levels did not show any difference in any of the groups.

Retinol levels in the groups

The postprandial retinol levels were significantly higher in the iron group (Fe) than in the zinc group (p = 0.03). There was no significant difference in the iron+zinc supplemented group (p = 0.13) (Tables 3–5). The standard deviation between the individuals was higher in groups 1 and 2 than in the iron + zinc group.

The absorption peaks in the iron group and the iron+zinc group at T2 were not significantly different. This indicates that iron and iron+zinc association were good for optimal chylomicron appearance of retinol. The highest peak corresponds to the iron+zinc supplementation in absolute value. It was observed that chylomicron retinol levels were high in all the individuals of the iron+zinc group, while in the iron and in the

80–89 mmHg), and for hypertension (>140 mmHg for SBP and 90 mmHg for DBP). The retinol, α-carotene, β-carotene and β-cryptoxanthin levels did not show any difference in any of the groups.
Iron, Zinc Supplementation and Provitamins A of Papaya

zinc groups, individuals responded very differently. For example, in the iron group, individual 1 had a very high retinol concentration and individual 3 had very little. Total values in the zinc group were lower than those of the iron group and the iron + zinc group.

**Chylomicron α-carotene levels**

The chylomicron levels of α-carotene were higher in the zinc group than in the iron group and the iron + zinc group, as indicated in Fig. 1. There was no significant difference in the values in the zinc group and the iron + zinc group (p=0.28), but there was a significant difference in the iron group (p=0.04) (Tables 3–5). The absorption peaks were found at T2 in the 2 groups, although they were more visible in the iron + zinc group with very little difference between the individuals. This means that zinc and iron + zinc association improve chylomicron α-carotene levels better. The significantly lower absorption observed with the iron group still had the highest absorption peak at T2 (Fig. 1). Iron + zinc could therefore be recommended for better chylomicron intact appearance of α-carotene than iron alone.

**Chylomicron β-carotene levels**

The levels of β-carotene were significantly higher in the zinc group than in the iron group (p=0.04) but with no significant difference in the iron + zinc group (p=0.28). When compared to that of α-carotene, the chylomicron β-carotene levels showed significant differences at T2 in the iron group and the zinc group (p=0.05) and in the iron group and the iron + zinc group (p=0.04). For the zinc and iron + zinc groups, the absorption peak was observed at T2, even though, it was more pronounced pointed for the iron + zinc group with

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Table 4. Postprandial serum retinol and provitamin A carotenoids in chylomicrons (μg/mL) in the iron group after 7 h.

| Iron group | Retinol   | α-Carotene | β-Carotene | β-Cryptoxanthin |
|------------|-----------|------------|------------|-----------------|
| T0         | 0.44±0.04<sup>a</sup> | 0.29±0.08<sup>a</sup> | 0.49±0.11<sup>a</sup> | 0.022±0.007<sup>a</sup> |
|            | (0.38–0.47) | (0.24–0.34) | (0.42–0.55) | (0.018–0.023) |
| T2         | 0.51±0.05<sup>b</sup> | 0.34±0.09<sup>b</sup> | 0.50±0.11<sup>b</sup> | 0.031±0.005<sup>ab</sup> |
|            | (0.45–0.56) | (0.29–0.39) | (0.43–0.57) | (0.028–0.033) |
| T4         | 0.46±0.04<sup>ab</sup> | 0.31±0.10<sup>ab</sup> | 0.51±0.12<sup>ab</sup> | 0.041±0.009<sup>b</sup> |
|            | (0.42–0.52) | (0.25–0.36) | (0.44–0.58) | (0.035–0.046) |
| T7         | 0.40±0.03<sup>a</sup> | 0.30±0.10<sup>a</sup> | 0.50±0.12<sup>a</sup> | 0.047±0.003<sup>a</sup> |
|            | (0.36–0.47) | (0.24–0.35) | (0.43–0.57) | (0.046–0.049) |
| Average    | 0.45      | 0.308      | 0.5        | 0.039           |
| p          | 0.089     | 0.92       | 0.99      | 0.022           |
| F          | 0.34      | 0.18       | 0.02      | 5.05            |

Values in brackets are minimal or maximal for analyses. Means in the same column with the same letter superscript are not significantly different at the 95% confidence level. T: time of blood draw; T0, baseline or 1st blood draw just before the test meal; T2, 2nd blood draw 2 h after the test meal; T4, 3rd blood draw 4 h after the test meal; T7, 4th blood draw 7 h after the test meal.

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Table 5. Postprandial serum retinol and provitamin A carotenoids in chylomicrons (μg/mL) in the iron + zinc group over 7 h.

| Iron + zinc group | Retinol   | α-Carotene | β-Carotene | β-Cryptoxanthin |
|-------------------|-----------|------------|------------|-----------------|
| T0                | 0.38±0.002<sup>a</sup> | 0.29±0.002<sup>a</sup> | 0.49±0.004<sup>a</sup> | 0.056±0.002<sup>a</sup> |
|                   | (0.36–0.39) | (0.28–0.31) | (0.48–0.50) | (0.055–0.057) |
| T2                | 0.50±0.010<sup>b</sup> | 0.38±0.005<sup>b</sup> | 0.64±0.006<sup>b</sup> | 0.088±0.003<sup>b</sup> |
|                   | (0.48–0.51) | (0.27–0.39) | (0.63–0.65) | (0.066–0.089) |
| T4                | 0.43±0.019<sup>ab</sup> | 0.31±0.001<sup>a</sup> | 0.49±0.004<sup>a</sup> | 0.098±0.002<sup>c</sup> |
|                   | (0.42–0.46) | (0.29–0.31) | (0.48–0.51) | (0.097–0.101) |
| T7                | 0.36±0.004<sup>a</sup> | 0.30±0.001<sup>a</sup> | 0.49±0.002<sup>a</sup> | 0.092±0.001<sup>ab</sup> |
|                   | (0.35–0.37) | (0.29–0.31) | (0.48–0.51) | (0.09–0.093) |
| Average           | 0.42      | 0.31       | 0.52      | 0.083           |
| p                 | 0.001     | 0.001      | 0.001     | 0.001           |
| F                 | 96.81     | 604.22     | 1050.62   | 263.62          |

Values are means±standard deviation for n participant. Values in bracket are minimal and maximal for analyses. Means in the same column with the same superscript (letter) have p-values of the F-test greater than or equal to 0.05, meaning that they are not statistically significantly different at the 95% confidence level. T: time of blood puncture; T0, Baseline or first blood puncture just before the test meal; T2, 2nd blood puncture 2 h after the test meal; T4, 3rd blood puncture 4 h after the test meal; T7, 4th blood puncture 7 h after the test meal.
very little standard deviations between participants in the same group (Fig. 2).

The absorption was significantly lower in the iron group, with a flat peak at T4, indicating slow and low absorption. Hence, as in the case of \(\alpha\)-carotene, zinc and iron+zincc improve the absorption of \(\beta\)-carotene. The peak of absorption of the zinc group was flat at T4, showing a slower absorption. It was noted that more \(\beta\)-carotene was absorbed, but slowly until the end of the process.

The chylomicron intact appearance of \(\alpha\)-carotene and \(\beta\)-carotene in the zinc group within 7 h, faster than in the iron group and the iron+zincc group. It is important to note that the concentrations of zinc in the zinc group were higher than those of the other groups. When associated with iron (iron+zincc) some competition can occur as both minerals are bivalent cations and are absorbed by the same receptors (3). The results therefore showed that the best supplement to improve \(\beta\)-carotene absorption was zinc, followed by zinc+iron.

**Chylomicron intact appearance of \(\beta\)-cryptoxanthin**

The chylomicron intact appearance of \(\beta\)-cryptoxanthin was very different in the three groups. The appearance was significantly higher in the iron+zincc group than in the Fe group and Zn group at T2 (\(p=0.01\) for iron group and \(p=0.002\) for zinc group). These differences continued to be significant between the three groups, at T4 and at T7 (Fig. 3). The absorption peak of the iron+zincc group was at T4, indicating a very slow absorption rate, although higher than that of the two other groups. Between T0 and T7, the rates of absorption were low and were not significantly different between the iron group and the zinc group. Between the iron+zincc and the iron groups, the differences in absorption rates were significant during the whole process of absorption, from T0 to T7 (\(p=0.004\)) as observed in Table 5.

No peak was observed in groups 1 and 2 during the 7 h of absorption, indicating a very slow and continuous chylomicron appearance of intact \(\beta\)-cryptoxanthin in the two groups supplemented with iron and zinc. The absorption was very high in the iron+zincc group with a flat peak at T4 (usually seen at T2 in the case of other provitamin A carotenoids). In absolute value, the amount of \(\beta\)-cryptoxanthin absorbed by the iron+zincc group was very high and this led to the absorption of the highest quantity of the \(\beta\)-cryptoxanthin with that combination. Iron+zincc could therefore be recommended for higher \(\beta\)-cryptoxanthin absorption than iron or zinc taken separately.

**DISCUSSION**

All twelve participants, who were young men aged 18 to 31 y, were put on low- or no-vitamin A and pro-
vitamin A carotenoid diets for 6 d before the test meal, in order to better appreciate the influence of the supplements on provitamin A carotenoid absorption and chylomicron appearance of retinol. It had been reported that the conversion of β-carotene into vitamin A could not be estimated accurately in well-nourished humans by the assessment of changes in chylomicron retinol after supplementation with unlabeled β-carotene. The inability is because it is difficult to distinguish newly formed retinol from retinol derived from body reserves and blood retinol concentrations since they are homeostatically controlled (2). Women who are in a risky group were excluded from the study because their physiological status (menstruation or early pregnancy) could significantly influence the bioavailability of the micronutrients used within 11 d. BMI (body mass index) and BP (blood pressure), which are physical parameters that indicate nutritional status and are usually correlated with nutrient absorption and β-carotene especially, were in the normal range (20). In another study, conversion factors of the subjects who received synthetic β-carotene were significantly correlated with BMI, suggesting that subjects with more body fat have a lower ability to convert β-carotene to vitamin A. The bioconversion of dietary β-carotene to vitamin A may also be related to the genetic characteristics of the subjects. The enzyme responsible for β-carotene conversion into retinol is β-carotene 15,15'-monooxygenase (BCM01) with genetic polymorphism that may contribute to the poor converter phenotype (2, 19). The test meal in this study contained papaya as the only known source of provitamin A carotenoids in addition to 5 g of olive oil to facilitate provitamin A carotenoid absorption. Dietary fat (at least 2.4 g fat/meal) affects bioavailability and bioconversion of β-carotene, no matter the source (8, 21). The aim of using papaya as the only source of provitamin A carotenoids was to limit the influence of food (2).

**Influence of iron, zinc and iron+zinc on chylomicron appearance of retinol levels**

There were very significant differences between provitamin A carotenoid absorption and retinol chylomicron appearance among individuals in the same group. Human beings may have different abilities to absorb and convert provitamin A carotenoids to vitamin A. These may be due to genetic variability in β-carotene and other provitamin A carotenoid metabolism of individuals. This implies that provitamin A carotenoids might not be a good source of vitamin A for people with the poor converter phenotype (2). The present study did not screen participants to eliminate low respondents to provitamin A carotenoids. It was observed that iron and iron+zinc were linked to higher levels of serum intact appearance of retinol (Fig. 4). The interest of combining iron and zinc to the absorption of provitamin A carotenoids is due to the coexistence of high prevalences of vitamin A, zinc and iron deficiencies as public health problems in Cameroon (12). Vitamin A supplementation campaigns in Cameroon have not totally succeeded in reducing vitamin A deficiencies. Many sources of PACs are available in the country, but it is important to educate the population on the consumption of natural PACs to improve vitamin A status (9). On the other hand, the iron and zinc intakes of the people in areas with mostly vegetable-based diets are low, so there is a need for supplementation or fortification (7). The fact that intact appearance of retinol in the serum was very high with iron supplementation and that the iron group showed the lowest concentrations of β-carotene can be explained by the acceleration of its bioconversion to retinol. Ameny et al. in 2002 (3) demonstrated in their study that oral supplementation with iron improves vitamin A status. These results suggest that iron deficiency inhibits the mobilization of vitamin A stores in the liver. Iron may either affect the absorption and the availability of vitamin A from the diet or it may affect the mobilization of vitamin A from the main storage organ of vitamin A in the body, the liver. With regard to the first possibility, a recent study conducted in humans showed that the addition of vitamin A and α-carotene (the major provitamin A in the diet), improve nonhaem iron absorption. This positive effect might be due to the reduction of the inhibitory effect of phytates and polyphenols on iron absorption (17). A study on pregnant women in India showed that oral iron supplementation increased plasma retinol and lowered the number of cases with plasma levels of retinol below 300 ng/L (15).

In the zinc group, the highest chylomicron intact appearance of β and α carotenes was observed. In absolute value, β-carotene concentrations were higher, even if it is assumed that part of it was converted. This is also because the major provitamin A carotenoid in papaya is β-carotene.

**Influence of iron, zinc and iron+zinc on chylomicron intact appearance of provitamin A carotenoids**

The provitamin A carotenoids concerned in this study were α-carotene, β-carotene and β-cryptoxanthin from papaya (Carica papaya L). The major source of provitamin A carotenoids in papaya is β-carotene (15, 16). In this study, the highest chylomicron intact appearance of β-carotene was observed in the zinc group, indicating the positive influence of zinc on β-carotene absorption, but not on bioconversion. Zinc supplementation greatly improved postprandial levels of β-carotene and
α-carotene in chylomicrons, followed by iron+zinc supplementation. Previous studies showed that zinc plays a role in the conversion of β-carotene to retinol by affecting transport, storage, and subsequent mobilization of β-carotene (9, 21). Chylomicron intact appearance of provitamin A carotenoids is so called because it is assumed that part of it is directly cleaved to yield retinol. However, retinol levels were lower in the zinc group than in the iron group, showing the positive influence of iron on provitamin A conversion. Many studies in humans have reported the conversion efficiency of dietary β-carotene to retinol with factors ranging from 3.6 to 28 : 1 by weight according to the diets and many other factors (2, 22). The bioavailability of carotenoids is linked to many factors known as SLAMENGHI to summarize all the factors such as: the type of carotenoids, the molecular linkage, the amount of carotenoids consumed in a meal, the matrix in which the carotenoid is incorporated, the effectors of absorption and bioconversion, the nutrient status of the host, genetic factors and host-related factors. These factors have been extensively discussed (21, 23, 24).

In Cameroon, consumption of foods from animal sources is low. This reduces the consumption of preformed vitamin A. Although available fruits and vegetables can contribute to the daily vitamin A supply, the recommended β-carotene intake of 2–4 mg/d can only be achieved in a population with education on guidelines for appropriate uses of local or traditional food resources (24). In association with good guidelines on appropriate provitamin A food sources, it is also important to supplement with iron, for it has been shown to accelerate vitamin A metabolism and provitamin A bioconversion (25).

The chylomicron responses in β-carotene, α-carotene, lycopene and lutein were similar in the old and in the young groups (26–28). Zinc (α- and β-carotene) and iron+zinc (iron and zinc group) (β-cryptoxanthin) showed highest intact chylomicron appearance of provitamin A carotenoids. Very high variations in intact appearances of provitamin A carotenoids were observed between individuals. Zinc and iron have been reported to influence β-carotene metabolism as the major provitamin A carotenoid (29). Both β-carotene and zinc associated with iron supplements during pregnancy have been effective in improving the vitamin A, iron and zinc status of the mothers and infants (2, 9). Zinc has also been effective in converting β-carotene to retinol (9). Supplementation with zinc, iron, or both improved indicators of vitamin A status in a longitudinal study, suggesting their interaction in vitamin A metabolism (3, 10, 30). The high β-caroten content in papaya was associated to high concentration of serum retinol with iron supplementation. Papaya may be use as vegetal source to improve vitamin A status as observed by previous authors with spinach and carrot (31) and with spirulina (32).

β-Cryptoxanthin, which is the main pigment of many orange-fleshed fruits, such as peach, nectarine, orange-fleshed papaya, persimmon and tomato, was absorbed very slowly compared to carotenes (33). We observed that, in absolute value, the rates of β-cryptoxanthin absorbed by the iron+zinc group was much higher than that of the iron group and zinc group supplemented with iron and zinc taken separately. The effect of iron, zinc and iron+zinc on serum retinol intact appearance and provitamin A carotenoids in chylomicrons is summarized in Table 6. β-cryptoxanthin reduces the risk of lung cancer and rheumatoid arthritis (34).

In conclusion, iron supplementation was best for optimal appearance of the retinol in the serum. Zinc supplementation was better for the optimal intact appearance of α-carotene and β-carotene than iron alone. Iron+zinc was the best supplement for optimal intact β-cryptoxanthin chylomicron appearance. The fact that chylomicron intact appearance of all the provitamin A carotenoids was low in the iron group led to the conclusion that provitamin A carotenoids were rapidly converted into retinol in the presence of iron. Iron supplementation should be integrated in food-based approach strategies to combat vitamin A deficiency. It is important to improve the intake of provitamin A carotenoids in our

**Table 6. Serum retinol and chylomicron appearance of PACs according to groups and peak time.**

| Nutrients          | Maximal peak time (h) | Major appearance (Highest concentration) | Order of appearance per group (concentrations) |
|--------------------|-----------------------|-------------------------------------------|-----------------------------------------------|
|                    | Iron group            | Zinc group                                | Iron group (G1)                               |
| Retinol            | T2                    | T2                                        | G1>G3>G2                                     |
| α-Carotene         | T2                    | T2                                        | Zinc group (G2)                              |
| β-Carotene         | T4                    | T2                                        | G2>G3>G1                                     |
| β-Cryptoxanthin    | T4                    | T7                                        | Iron+Zinc group (G3)                         |

Table 6 summarizes the chylomicron intact appearance of retinol, α-carotene, β-carotene, and β-cryptoxanthin. Each individual was subjected to four blood punctures (T0, just before the test meal; T2, after 2 h; T4, after 4 h; T7, after 7 h for retinol and PAC analyses). The maximum of the peak appearance of retinol is at T2, meaning 2 h after supplementation. The same observation was done with α-carotene. A part for iron group, where β-carotene peak was observed at T4, the maximum peak is also seen at T2. For β-cryptoxanthin, the appearance was much slower, at T4 for Iron groups and T7 for zinc and iron+zinc group.
diet by increasing the consumption of fruits and leafy vegetables alongside supplementation with minerals. This not only improves the micronutrient status of the body, but also protects against damage by free radicals. Our study was done in young adults and we expect to extend the application to the most affected groups such as infants, young children, and pregnant and lactating women.

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Conflict of interest
The authors declare no conflict of interest. Kana-Sop MM contributed in the conception and bioavailability study design. Gouado I oriented the analyses and provided relevant documents. Van Camp J contributed in the project design. Amvam Zollo PH provided necessary documents and advised on sample handling. Schweigert FJ participated in some laboratory analyses. Oberleas D edited the document and orientated the analyses, and Ekoe T helped in the conception and checked the ethical and application aspects of the project.

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