

IMPACT OF DAILY CONSUMPTION OF MORINGA (*MORINGA OLEIFERA*) DRY LEAF POWDER ON IRON STATUS OF SENEGALESE LACTATING WOMEN

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ABSTRACT

A randomized study was conducted to test the efficacy of Moringa powder on iron status and weight gain in women. In an open-labelled randomized trial, 82 moderately anaemic, lactating women, aged 26.7 ± 6.5 years, received a weekly dose of either 100g of Moringa powder (Moringa group) or 120 mg iron sulphate with 0.5 mg folic acid (Control group). Data from 64 women (33 from Moringa group and 31 from Control group) were analyzed. Baseline parameters, socio-economic, anthropometry, haematology, plasma ferritin, and acute phase proteins were comparable in both groups. Low plasma ferritin ($< 12 \mu\text{g/l}$) indicating iron deficiency was found in 13 and 14 women from the Moringa and Control groups, respectively. After 3 months of treatment, mean haemoglobin concentrations significantly increased in both groups ($p < 0.001$) but iron stores were unchanged in the Moringa group while they significantly increased in the Control group indicating that consumption of Moringa leaves failed to restore iron stores in anaemic subjects. A slight improvement was observed in the prevalence of anaemia in both groups but anaemia still persisted due to other reasons than iron deficiency anaemia. None of the groups gained weight during the 3 months. However, the average weight lost was less important in the Moringa group (-0.8 ± 2.1 kg) compared to the control group (-1.2 ± 2.3 kg) but the difference was not significant ($p = 0.45$). The amount of digestible protein in the powder could suggest that the consumption of Moringa was beneficial to the rural women by preventing weight loss during the rainy season. Micronutrient status improvement of vulnerable people in developing countries like Senegal should combine diet-based strategies through production and consumption of animal derived food, vegetable, fruits and food fortification program. However, Moringa *Oleifera* is one example of local food that can be used in nutritional intervention program, but its use needs additional rigorous clinical trials to confirm its nutritional benefits.

Key words: Iron status, malnutrition, Moringa, Senegal

INTRODUCTION

Moringa Oleifera leaves have been widely consumed by rural families in Senegal for a long time. According to the book “the miracle tree”, the dried and powdered leaves can prevent malnutrition and restore iron reserves because of its high content of protein and micronutrients including iron. However, scientific studies were lacking to substantiate these statements. Outside of anecdotal evidence of benefits from *Moringa Oleifera*, no clinical trials or randomized studies in human have been completed to show the efficacy of *Moringa Oleifera* leaves consumption on iron deficiency anaemia or malnutrition treatment. This was recently related by Thurber *et al.* [1].

Anaemia associated with malnutrition remains one of the most significant public health problems in developing countries. In Senegal, according to the Demographic and Health Survey, the prevalence of malnutrition in women (BMI < 18.5 kg/m²) increased from 15% in 1993 [2] to 18% in 2005 [3] and, 59% of Senegalese women were anaemic in 2005. This prevalence was 34.1% for mild (10.0-10.9 g/dl), 8.7% for moderate (7.0-9.9 g/dl) and 0.9% for severe anaemic women in Ziguinchor area [3]. Because strategies to prevent malnutrition and anaemia are expensive for rural populations in developing countries, local approaches are commonly used. Thus, edible plants like *Moringa Oleifera* leaves are used to fight this burden. Belonging to the genus *Moringaceae*, *Moringa Oleifera* is a well known ancient plant recently acknowledged as a multi-purpose tree with a tremendous variety of potential uses. In Senegal, the plant is called ‘Nebeday’ (never die) due to its drought-resistance and rapid growth. The *Moringa* tree grows everywhere in Senegal and the leaves can be found in both rural and urban markets. Senegalese people use its leaves in different ways: in the form of dried leaf powder which is added to many porridges or in the preparation of a sauce called “Mbumm”, which is eaten with maize or millet. Apart from medicinal properties, nutritional properties are also attributed to *Moringa* leaves due to its high concentration of iron, protein, calcium, copper, various vitamins and essential amino acids [4]. According to the book “*Moringa Oleifera*: the miracle tree”, *Moringa* dry leaves added on a regular basis to an individual’s usual diet, could prevent or cure anaemia and malnutrition [5]. However, no scientific study has been undertaken to evaluate the efficacy of *Moringa* leaves on impacting malnutrition and iron deficiency anaemia prevention or recovery. The aim of this study was to evaluate the effect of daily consumption of *Moringa* dried leaf powder on nutritional and iron status of anaemic lactating women in Senegal.

SUBJECTS AND METHODS

Study area and subjects.

The study was conducted in a district of Ziguinchor (Region of Ziguinchor) in south-western Senegal between July and October 2001 during the rainy season. Another project, ‘Projet de Nutrition Communautaire’ (PNC), one of whose objectives was to improve nutritional status of lactating women, was implemented in the same area and its facilities (including 15 PNC centres in the area) were used for this study. Thus, the subjects of the study were selected from among PNC recruits. All lactating women participating in PNC who had infants aged 3-4 months were selected on the basis of

their nutritional monitoring card (women were included if they were anaemic but not severely anaemic). A sample size was calculated with a power of 80% and a 5% significance level. To achieve a difference in haemoglobin concentration of 6 ± 8 g/L between the beginning and the end of supplementation, a minimum of 28 women were required in each group [6]. The study was approved by the Ethics Committee of Dakar University and by the regional hospital of Ziguinchor and was conducted according to the guidelines set out in the Helsinki declaration. Before enrolment, a full explanation of the study was given to the mothers, and verbal informed consent was obtained from 94 eligible women. A selection of anaemic women was carried out by capillary blood screening according to WHO criteria for the definition of anaemia, which is haemoglobin concentration <120 g/L [7]. Women with severe anaemia (haemoglobin <70 g/L) were excluded from the study and referred to the hospital for appropriate clinical care. A total of 82 anaemic women were randomly assigned to 1 of 2 groups, Moringa ($n=41$) or Control ($n=41$), using a computer-generated list.

Intervention

Women received either Moringa dried leaf powder or iron tablets with both ferrous sulphate and folic acid during 3 months. Because of the different colour and consistency of the supplements, the study was open trial. Women in the Moringa group received 100g of dried leaf powder per week which represented the usual supplementation dose they received at local health centres. The powder which was supplied by AGADA/CHURCH WORLD SERVICE, an NGO in Ziguinchor area, was prepared by crushing the leaves which were dried in the traditional way, out of direct sunlight. The weekly dose was consumed three times daily at mealtimes by adding it to sauces or to porridges just before serving them. Regular home visits (3/week) were made by PNC fieldworkers to check actual Moringa powder consumption. Control group women received 2 ferrous sulfate/folic acid tablets weekly. Each tablet was equivalent to 130 mg of elemental iron and 0.5mg of folic acid in accordance with recommended dosages for weekly treatment of anaemia at the beginning of the study [6]. The iron supplement was administered at PNC centres or at home if the subject missed her appointment.

Socioeconomic and diet surveys

Socioeconomic status and food consumption were assessed by trained fieldworkers using a questionnaire. The socioeconomic questionnaire included individual characteristics of the mother, occupation and education, information on infant feeding practices, as well as frequency consumption of foods rich in iron, compliance, utilisation of the powder and any unpleasant effect encountered.

Anthropometric measurements

Bodyweight and height were measured at baseline and at 1, 2 and 3 months of the study. Body weight was measured to the nearest 100g by an electronic scale (TANITA BWB-800, Tanita Corporation, Tokyo, Japan). Height was measured to the nearest millimetre by using a wall-mounted stadiometer. Body mass index (BMI) was calculated from: weight (kg)/ height (m^2).

Haematological and Biochemical measurements

Blood samples (5ml) were drawn at baseline and at the end of the study (3 months) by venipuncture from all women in EDTA tubes. The samples were transported in a portable ice box to the laboratory within 3h. After the haematological indices were assayed, the remaining blood was immediately centrifuged at 1735g (4000 rpm, Sigma 3K20; B. Braun, Laboratory Centrifuges GmbH, 3360 Osterode/Harz, Germany) for 5min at 4°C. The plasma was divided in 2 aliquots and stored at -20°C until analysis.

At baseline, whole blood, complete blood count, haemoglobin (Hb), hematocrit (Hct) and red cell indices were determined using a counter (model Sysmex K-1000, France). Anaemia was classified as microcytic if Hb<120g/L and Mean Corpuscular Volume (MCV)<80fl; hypochromic if Hb<120g/L and Mean Corpuscular haemoglobin (MCH)<27pg [7]. For parasite counts, thin and thick blood films were stained with 10% *Giemsa* and examined for the presence of malaria parasites. Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBC) on a thick blood film assuming a total WBC count of 8000/ μ l. The following formula was used:

Parasites/ μ l = (Number of parasites found after counted 200 WBC \times 8000) / 200. Sickle cell disease was also determined by microscopic observation of red cells containing sickle haemoglobin under a cover slip by suspending the cells in a droplet of a 2% solution of sodium metabisulfite (reducing agent) which deprived red cells of oxygen.

Iron status was defined by haemoglobin, hematocrit, MCV, MCH and ferritin parameters. Plasma ferritin was measured using an automated quantitative test on a MINI VIDAS analyser (Biomerieux SA, France). The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA: Enzyme Linked Fluorescence Assay). A plasma ferritin value, less than 12 μ g/L indicates a depletion of body iron stores [6]. To rule out inflammation, which is known to increase plasma ferritin concentration, C-reactive protein (CRP) and α -1 acid glycoprotein (AGP) were measured by immunoturbidimetry assay using Dako Diagnostic reagents (Dako A/S, Glostrup, Denmark). Both determinations were done with a Cobas Mira Plus (Roche, France). Inflammation and/or infection states were defined by AGP concentration >1.20g/L and/or detectable CRP >5mg/L [8].

Statistical analysis

The data were analyzed using Excel 2000 and SYSTAT 8.0 (SPSS Inc., 1998, Chicago IL, USA). The results are expressed as means \pm SD or percentages. Comparison between the groups was done using the Mann-Whitney test. The Wilcoxon test was used for matched groups' comparison and the chi-square (χ^2) test was used for proportions. Because plasma ferritin had a skewed distribution, log transformation was applied to normalize the distribution before statistical analysis. The relationships between quantitative variables were analysed by Pearson's linear correlation test. The level of significance was set at p<0.05.

RESULTS

During the intervention phase (3 months), full data were obtained from 33 women in the Moringa group, and 31 in the Control group. Data for the remaining 18 women were missing for the following reasons: 6 women dropped out during the second week of supplementation (3 in the Moringa group and 3 in the Control group); 7 women were absent during the fifth week of supplementation (3 in the Moringa group and 4 in the Control group) and 5 were not present at the last blood sampling (2 in the Moringa group and 3 in the Control group) (Figure 1).

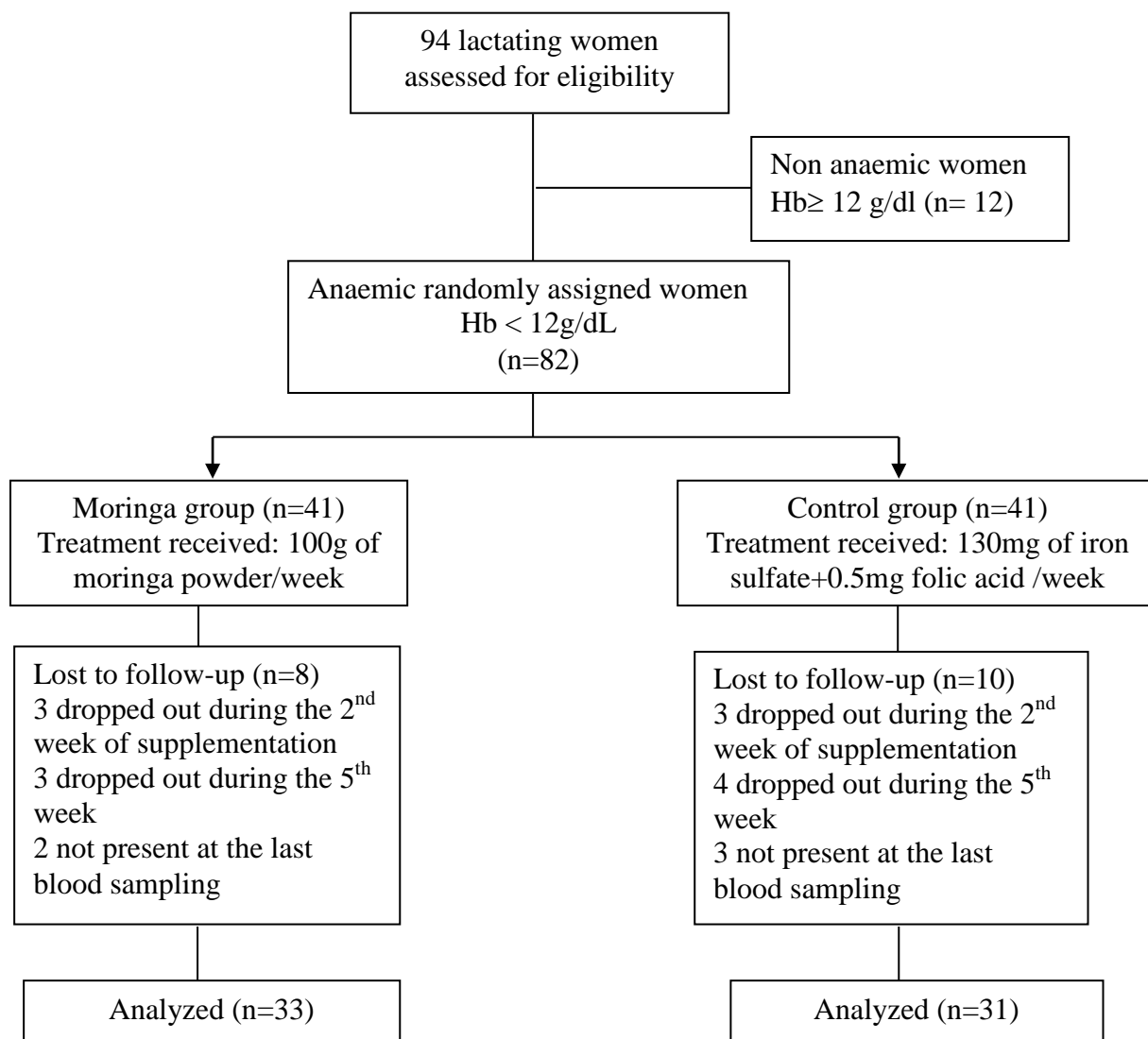


Figure 1: Study design

Baseline characteristics

Anthropometric and biological parameters of women in the Moringa and Control groups at baseline were summarised in Table 1. No significant difference was found between the 2 groups.

Likewise, both groups were not significantly different for iron status and acute phase proteins. 39% and 32% of women in the Moringa and Control groups, respectively, had haematocrits < 36%. Microcytic anaemia was found in three women in the Moringa group and hypochromic anaemia was found in 64% and 71% of the Moringa and Control groups, respectively. Low plasma ferritin concentrations (< 12 µg/L) were also found in Moringa (n=13) and Control group (n=14) at baseline.

Plasmodium falciparum test was positive in fifteen and nine women in the Moringa and the Control groups, respectively. The sickle cell trait was carried by 3 women in the Moringa and 4 women in the Control group. Differences in these baseline characteristics between the two groups were not statistically significant.

Socioeconomic status and treatment compliance

Socioeconomic status was comparable for both groups on marital status, education level, and husband's occupation. A significant difference has been found between the groups for women's occupation (p= 0.035). In the Control group, almost all of women had no jobs while 6 in the Moringa group had income generation activities and 2 were salaried.

Compliance to treatment was high as 57% of the women used a tablespoon of Moringa per meal and 43% used a teaspoon per meal. The powder was well accepted, but at the beginning of the supplementation, 1 case of vomiting and 3 cases of diarrhoea were recorded. Most of the women (84% and 87% in the Moringa and Control groups, respectively) claimed to take iron tablets before the study period, 26% and 36% of them had taken iron tablets during their pregnancy and after delivery.

Effects of supplementation on nutritional and iron status

At the end of the supplementation period (3 months), lactating women's weight was not significantly different between the groups. Women did not gain weight during the treatment period. The weight deficit was less important in the Moringa group (-0.8 ± 2.1 kg; p<0.05) compared to the control group (-1.2 ± 2.3 kg; p<0.01). The mean BMI indicated that in the Moringa group BMI was stable in contrast to the Control group in which there was a significant decrease (p<0.01) at 3 months (Table 2).

During the supplementation period, mean haemoglobin concentrations increased significantly in both groups (p<0.001), but this increment was higher in the Control group (p<0.05) than in the Moringa group. Thirty two percent of the women (n=10) in the Control group improved their Hb concentration (Hb ≥ 120 g/L) compared to 21% (n=7) in the Moringa group. Haematocrit levels also increased significantly in the Control group (p<0.01) but not in the Moringa group and the 2 groups were significantly different at 3mo (p<0.01). In the Control group, haematocrit levels below 36% (cut-off value for adults) were present in 16% of women compared to

45% of women in the Moringa group. MCV did not change, MCH significantly increased ($p < 0.001$) in both groups; however, 39% and 41% of the women in the Moringa and Control groups, respectively, still had MCH < 27 pg at the end of the study. At 3 months, plasma ferritin in the Control group was significantly higher ($p < 0.01$) compared to the Moringa group. The mean value increased significantly during the intervention period ($p < 0.001$) in the Control group unlike the Moringa group. Only two women (6%) had low plasma ferritin concentration in the Control group versus 13 women (39%) in the Moringa group. Inflammatory or infection status was not different at the end of the study between groups: 3 women in each group had acute infections (CRP > 5 mg/L).

DISCUSSION

Low iron bioavailability in Senegalese foods and typical diets of West African populations is known as one of the main causes of iron deficiency [9, 10]. Moreover, iron requirements for lactating women are more important than women of reproductive age as they usually have low iron stores due to high requirements during pregnancy, and blood loss during delivery and the post-partum period [11]. Also, women of reproductive age compliance with iron supplements were low during pregnancy and the post-partum periods. In our study, 88% of anaemic women claimed to have taken iron tablets but the number of tablets taken and the length of treatment were unknown.

Although haemoglobin concentrations were significantly increased in the Moringa and the Control groups ($p < 0.001$) at the end of 3 months supplementation, 68% of women in the Control group and 79% in the Moringa group had haemoglobin concentrations less than 120 g/L. In our study, presence of intestinal parasites was not determined but several studies carried out in rural and urban areas in Senegal indicate a prevalence of 60% of intestinal parasitosis in the Ziguinchor region [12]. The presence of malaria parasitemia and blood loss caused by other parasitosis such as schistosomiasis and ankylostomiasis could explain this high rate of anaemia. Infections by *Plasmodium vivax*, *Ascaris lumbricoides* and *Trichuris trichiura* have been identified as important causes of anaemia and iron deficiency in pregnant women in Nepal [13]. Since acute infection was not prevalent, intestinal parasitosis could likely explain the persistence of anaemia after 3 months of treatment. It is also possible that these women were deficient in other nutrients (vitamin B12) that could limit haematological responses. More research is needed to understand which nutrients are limiting.

Plasma ferritin was also found to be significantly increased in the Control group and 48% of women had improved their iron stores. This increase in plasma ferritin is consistent with findings of Imelda *et al.* [14] in female Indonesian adolescents supplemented with the same weekly dose of iron for 3 months. Several current studies show that weekly iron supplementation can decrease anaemia prevalence in pre-school children, adolescents and pregnant women [6, 15, 16]. Plasma ferritin did not change in the Moringa group during the intervention. This result was not surprising but highlighted the low iron bioavailability in plant food as noted by several authors [17, 18].

In a previous study, Moringa leaf powder's nutrient analysis, protein digestibility and iron bioavailability showed that the leaves contain important quantities of several minerals as well as protein [19]. The present study confirmed that despite the high amount of iron in the Moringa powder, the iron bioavailability was low (2.2%). Therefore, the amount of bio-available iron consumed per day in the meals mixed with Moringa powder was probably very low and unable to cover daily requirements.

As demonstrated by several authors, vegetable iron is non-heme iron with low absorption. In iron deficient rats fed with Moringa leaf powder, Ndong *et al.* [20] showed that Moringa did not improve haemoglobin or iron content of liver. Moreover, the food consumption questionnaire indicated that meat consumption was low in the study population. It is well established that muscle tissue like red meat is a strong enhancer of non-heme iron absorption, better than fish, poultry, ascorbic acid, retinol and carotenes [18]. Analysis of the nutritional value of *Moringa Oleifera* showed that Moringa powder contained polyphenols, which are known to have an inhibitory effect on iron bio-availability [19]. This inhibitory effect is related to phenolic structures associated with galloyl and catechol groups which chelate iron and form non-bio-available polyphenol-iron complexes [20, 21]. Nevertheless, slight improvements in haemoglobin concentration in the Moringa group could be explained by an increase in protein intake or by the presence of ascorbic acid and beta-carotene in Moringa leaf powder, which are also non-heme-iron enhancers [5, 18, 22]. The Moringa protein digestion products (amino acids) could also contribute to erythropoietin activity by providing amino acids to porphyrin, globin and transferrin synthesis [23]. Serum ferritin and other blood parameters of iron status were insensitive to dietary treatment according to Hunt *et al.* [24]. This could be explained by long-term adaptation in iron absorption to maintain iron stores.

At the end of the treatment, women in the Control group had lost more weight than those in the Moringa group. The Moringa group's BMI remained stable during the study while the Control group's BMI decreased significantly. Less weight loss in the Moringa group might be related to the high content of proteins in Moringa dried leaves.

In summary, contrary to the hypothesis of the book "Moringa Oleifera: the miracle tree", daily consumption of *Moringa Oleifera* dried leaf powder did not improve iron status in anaemic lactating women but prevented significant weight loss during the rainy season. Weekly supplementation with iron and folic acid is effective for correcting iron deficiency anaemia but not maintaining body weight. Moringa leaf powder contains iron but its bioavailability was low and could not cover iron requirements in the lactating women. But its wealth of proteins and other potential health benefit effect might be a great complement to the Senegalese diet [20]. Intervention programs to improve micronutrient status and recovery of vulnerable people should combine diet-based strategies through production and consumption of animal derived food, vegetable, fruits and food fortification program. The challenge is that several national iron/folic acid fortification programs undertaken by West African governments (like Senegal) will be successful and sustainable [25].

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Table 1: Baseline anthropometric measurements and biological parameters of subjects

	<i>Moringa group (n=33)</i>	<i>Control group³ (n=31)</i>
Age (y)	26 ± 7	27 ± 6
Weight (kg)	58.9 ± 10.7	58.8 ± 10.4
BMI (kg/m ²)	23.2 ± 4.2	22.8 ± 3.6
Hb (g/L)	105 ± 8	105 ± 9
Hct (%)	36.0 ± 2.5	36.5 ± 2.4
MCV (fl)	88.2 ± 7.2	89.0 ± 5.8
MCH (pg)	25.7 ± 2.7	25.8 ± 2.2
Ferritin ¹ (µg/L)	1.2 ± 0.4	1.1 ± 0.4
AGP (g/L)	0.7 ± 1.2	0.7 ± 0.2
² CRP	3	2

BMI: Body Mass Index; Hb: Haemoglobin; Hct: Hematocrit; MCV: Mean Corpuscular Volume;

MCH: Mean Corpuscular Haemoglobin; AGP: α-1 acid glycoprotein; CRP: C-reactive protein
 Values are mean ± SD; n: number of subjects

¹ Log concentration of ferritin

² Number of subjects who had CRP value > 5 mg/l

³ The mean values were not significantly different between the Moringa and Control groups (Mann-Whitney test)

Table 2: Body mass index and haematological status of women at baseline and 3 months

	Moringa group (n=33)			Control group ⁴ (n=31)		
	baseline	3 mo	p ³	baseline	3 mo	p ³
Weight (kg)	58.9±10.7	58.1±10.8	0.031	58.8±10.4	57.5±10.9	0.004
BMI	23.2 ± 4.2	23.0 ± 4.2	n.s	22.8 ± 3.6	22.3 ± 3.7	0.01
Hb (g/L)	105 ± 8	109 ± 9	0.001	105 ± 7	115 ± 9	0.001
Hct (%)	36.0 ± 2.5	36.1 ± 2.6	0.788	36.5 ± 2.4	38.1 ± 2.7	0.002
MCV (fl)	88.2 ± 7.2	88.6 ± 6.7	0.443	89.0 ± 5.8	90.3 ± 5.4	0.189
MCH (pg)	25.7 ± 2.7	27.0 ± 2.2	0.001	25.8 ± 2.2	27.5 ± 1.9	0.001
Ferritin ¹ (µg/L)	1.2 ± 0.4	1.2 ± 0.4	0.601	1.1 ± 0.4	1.4 ± 0.3	0.001
AGP (g/L)	0.7 ± 1.2	0.8 ± 0.4	0.809	0.7 ± 0.2	0.8 ± 0.2	0.209
² CRP	3	3		2	3	

BMI: Body Mass Index; Hb: Haemoglobin; Hct: Hematocrit; MCV: Mean Corpuscular Volume;

MCH: Mean Corpuscular Haemoglobin; AGP: α-1 acid glycoprotein; CRP: C-reactive protein Mean ± SD; n = number of subjects

¹Log concentration of ferritin

²Number of subjects who had CRP value > 5 mg/l

³Comparison of variables in paired samples, from baseline to the end of study (3 mo), was done with Wilcoxon test.

⁴Comparison of both groups at baseline and at 3 mo was done with Mann-Whitney test

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