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A Placebo-Controlled Study of the Impact of Dietary Salmon Protein Hydrolysate Supplementation in Increasing Ferritin and Hemoglobin Levels in Iron-Deficient Anemic Subjects

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Abstract

Iron deficiency anemia is the most common micronutrient deficiency in the world today. This placebo-controlled, iron-deficient anemic, patient study measured the changes in serum ferritin concentration, as well as circulatory hemoglobin concentration, after daily supplementation of their normal diet with 16 grams per day of salmon protein hydrolysate tablets. Salmon protein hydrolysate has a low iron content of 3.1 mg/kg versus 20 mg/kg for the whey protein isolate tested here. Yet, our results show that iron-deficient subjects treated with salmon protein hydrolysate for 6 weeks, showed a 14% increase in hemoglobin levels, while treatment with whey protein isolate showed only a 2% increase. Bioactive peptides in the salmon protein hydrolysate may be playing a significant role in increasing iron uptake from a normal diet.

Keywords: Salmon; Protein; Hydrolysate; Ferritin; Hemoglobin; Anemia

Introduction

Iron deficiency is the most common form of global malnourishment [1]. Iron deficient anemia, which is defined as an inadequate amount of red blood cells (hemoglobin) caused by a deficiency of iron, leads to poor cognitive development [2], increased maternal mortality [3] and decreased energy [4]. Iron deficient anemia occurs when iron stores are depleted thus interrupting essential supply of iron to organs and tissues. Several enzymes are involved in iron uptake and distribution such as ferritin, transferrin and erythrocyte protoporphyrin [5]. A low circulating level of serum ferritin is often used as an early indicator of iron deficiency and a change in this biomarker can often predict iron depletion or recovery, before actual levels of hematocrit and clinical signs of anemia are presented in patients [6].

Many interventions exist to correct iron deficiency anemia but they all have significant drawbacks that prevent their universal use. These strategies are primarily the following: improving the diet to introduce iron-rich foods [7], providing direct iron supplements [8], addressing endemic public health issues to prevent high iron-losses [9], and adding iron-fortified foods to the diet [10].

The first line of action in improving iron deficiency anemia is to increase the intake of iron-rich (heme) foods. However, this approach is unsuitable in most regions of the world since these foods are often unavailable or too expensive to include in the normal diet [11].

Iron supplementation is already an important part of anemia control programs. Recent research has shown that the use of iron supplements without appropriate changes in diet may not lead to any significant change in circulating iron or increases in iron stores [12]. Other research has also revealed instances of iron overdose due to indiscriminate use of iron supplements, and this is becoming a health issue, especially for children and pregnant women [13]. Thus, the use of iron supplements can only be viewed as one of several possible tools in the battle against iron deficiency anemia, to be used judiciously in conjunction with other approaches.

Public health issues, such as endemic helminth infestations, also lead to a high incidence of situational anemia in already at-risk populations. Worms cause excessive intestinal blood loss by feeding on the intestinal mucosa and the blood loss is directly proportional to the extent of infection. Even moderate infections can cause a doubling of iron losses daily [14]. Unscreened anthelmintic treatment programs into the general population is the current standard of care but recent research suggests that the most effective strategy for anemia control is to combine anthelmintic chemotherapy with increased iron uptake strategies [15].

Fortification of foods with absorbable forms of iron is also a desirable approach to controlling iron deficiency. But this approach is limited by the composition of the diet and the presence and quantity of substances that inhibit or increase dietary iron absorption. For example, it has been documented that ascorbic acid, when consumed together with a meal is an enhancer of iron absorption from non-heme containing foods [16] while, drinking tea inhibits iron absorption when consumed with or shortly after a meal [17]. Unfortunately, ascorbic acid is an expensive supplement for daily intake and drinking tea is a major dietary habit in much of the at-risk populations of the world. Other factors that enhance or inhibit iron absorption are also known [18] but their use in changing the amount of critical iron stores in at-risk populations remains in question.

A few reports have indicated that natural supplements, such as Vitamin C, can be effective in increasing iron uptake. Other studies have

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described that the consumption of meat muscle, which may stimulate the secretion of gastric acids and provide a more acidic environment in which to solubilize non-heme iron, leading to an increased iron uptake [19]. In particular, studies using fish muscle have shown an impact on increased iron uptake in human studies [20] and in CACO-2 *in-vitro* cell models [21]. Cysteine and cysteine-containing peptides have been studied in both in vitro and in vivo studies to play an important role in enhancing non-heme iron availability, since they can reduce ferric iron to the more bioavailable ferrous iron [22,23]. However, other studies have shown that other cysteine equivalent proteins did not enhance non-heme iron absorption [24,25]. The presence and levels of histidine in the protein mass have also been studied extensively [25,26] but with divergent results. Other research has shown that proteolytic digestion of muscle mass leads to enhanced iron absorption, potentially by reducing dietary iron or by forming soluble iron complexes for easier uptake [27,28].

These and other studies have shown that a potentially promising approach to alleviate iron-deficiency anemia is through the use of functional foods. This study will investigate the use of a naturally occurring, sustainably sourced, salmon protein hydrolysate to potentially increase iron uptake and absorption in iron-deficient anemia patients. It has the potential to be a new tool in the public health treatment arsenal for increasing iron uptake especially from the low-heme iron diets common in most of the world.

Material and Methods

Study design and subjects

The objective of this prospective, open, randomized study was to determine the dietary effects of salmon protein hydrolysate for increasing hemoglobin and circulatory ferritin levels in iron-deficient anemic subjects. This study was conducted in accordance with the principles of the Declaration of Helsinki guidance for good clinical practice, Seoul 2008 and Good Clinical Practices guidelines for clinical research in India, ICMR. The final approved protocol and all the study related documents were reviewed and approved by the ClinXXL Independent Ethics Committee before the start of the study.

Salmon protein hydrolysate tablets were sourced from Pharmatech AS, Rolvsoy, Norway and Whey Protein Hydrolysate powder was sourced from Protein Fabrikken AS, Stokke, Norway). 48 iron-deficient human subjects between the ages of 18-65 with hemoglobin levels between 8 g/dL and 11 g/dL were recruited for the study. The number of subjects was selected on the basis of a statistical review of the results of a safety and dose-finding study, carried out earlier. Subjects who were already on iron supplements or other iron enriched diet recommendations were excluded from this study. Subjects who had uncontrolled diabetes, coronary artery disease, cardiovascular disease and coronary atherosclerosis or uncontrolled hypertension were also excluded. Subjects with clinical signs and symptoms of liver, kidney or thyroid disorder and tuberculosis, alcoholics, smokers, pregnant and lactating women and subjects on caloric reduction diet or other special diets were also excluded from this study.

Experimental design and procedures

The total duration of this study was 42 days. A total of 48 subjects of which female (n=37) and male (n=11) were recruited in the study. The 48 subjects were split into two study groups. Group 1 (G1) consisted of 24 anemic subjects with haemoglobin levels between 8 g/dL and 11g/dL who received 16 x 1 gram tablets of salmon protein hydrolysate to be taken daily at breakfast while maintaining their routine diets.

Group 2 (G2) consisted of 24 anemic subjects with haemoglobin levels between 8 g/dL and 11g/dL who received 18g powder sachets of whey protein isolate to be taken daily at breakfast while maintaining their routine diets.

Dietary counseling was carried out to normalize the subject pool between the two groups so that there was no significant calorie and type of foods difference in diet between them. A detailed history of food consumption was taken based on a 24-hour dietary recall method. The complete dietary history which included consumption of vegetarian or non vegetarian food; frequency of consumption of fish/meat was obtained for each subject. Standardized containers were used to assess the amount of food consumed. The average daily intake of major food groups namely cereals, pulses, meat, milk, vegetables, fruits and oil was assessed and subjects who were consuming oily rich food regularly were advised to limit the same during the study. The subjects were instructed to follow similar diets during the course of the study and to note any significant deviations during the twice weekly telephone follow-up.

At visit 1 (Clinic Visit 1/Day-2), the pre-enrollment clinical safety assessment was carried out, which included informed consent signing, medical history review, systemic and physical examination, for all screened subjects. The subjects were only enrolled in the study after confirmation of study inclusion and exclusion criteria.

During the baseline visit (Clinic Visit 2/Day 0), the subjects were given their salmon protein tablets or their whey protein powder sachets for the study and dietary counseling was repeated by trained dieticians. Telephonic follow-up for routine diet monitoring and protein hydrolysate tablets/sachet intake compliance was performed twice weekly for the entire duration of the study.

On the final day of the study (Clinic Visit 3/Day 42), a systemic and physical examination was carried out for each subject and 10 ml blood sample taken for hemoglobin and ferritin analysis. The subjects also completed a standard questionnaire to assess their overall energy and attention levels from start to completion of the study, as qualitative indicators for reduced anemia.

Among the recruited subjects, 1 subject was prematurely discontinued from the study due to reasons not related to the protein powder. A total of 47 subjects completed the study as per the protocol (Table 1).

Results

All raw data from this study was analyzed using "Sigma Plot 11.0" statistical software (Supplied by Cranes Software International Ltd. Bangalore). The mean and standard deviation was calculated using Microsoft Excel Sheets and all data summarized in tabular form. Data describing quantitative measures were expressed as mean, SD with range. Changes in variables were estimated by analysis of variance. All p values were reported based on two sided significance test and all statistical tests were interpreted at 5% level of significance. No treatment related clinical signs or symptoms were observed in any of the subjects at the end of the study period.

Group	Treatment	Dose (g/day)	Protein Content mg/g powder	Number of Subjects	Iron Content mg/kg powder
G1	SPH in diet (test)	16	920	24	3.1
G2	WPI in diet (control)	18	820	24	19.9

WPI: Whey Protein Isolate.

Table 1: Experimental design SPH: Salmon Protein Hydrolysate.

All 23 subjects that completed the trial in G1 showed a significant increase in hemoglobin levels such that the mean hemoglobin level for G1 showed a statistically significant 14.9% increase from baseline at Day 42. Only 7 out of the 24 subjects in G2 showed a very modest increase in hemoglobin levels such that the increase in mean hemoglobin level for G2 was not statistically significant, as shown below in Table 2.

The serum ferritin levels were found to be significantly increased for the salmon protein hydrolysate treated group G1 versus the whey protein isolate treated group G2. At the end of the 42 day study, the salmon protein hydrolysate test group showed a statistically significant increase in ferritin concentration (from a low baseline of 22 ng/mL to a more normal 53 ng/mL), as compared to the whey protein isolate control group, which did not show a statistically significant shift (from 31 ng/mL to 37 ng/mL) (Table 3).

The hemoglobin and ferritin results are graphically summarized in Figure 1.

A quality-of-life questionnaire for self-reporting alertness and energy was administered to each subject at the start and end of the study. The salmon protein hydrolysate treated group showed a visible improvement in alertness and energy, as compared to the whey protein isolate treated group (Table 4).

Discussion

The standard of care for iron-deficiency anemia treatment is iron-supplements as iron salt tablets. Multiple studies have shown that such iron supplements are not readily absorbed, especially when combined with a poor diet [29]. Instead of direct iron-supplements, the use of functional foods to gently increase iron uptake and alleviate the symptoms of iron-deficient anemia has seen increased research attention [30,31]. Other studies have also shown the positive impact of animal and yeast protein hydrolysates on increasing hemoglobin [32,33].

One aspect of our results show that 6 weeks of daily administration of 16g of salmon protein hydrolysate powder in iron-deficient anemic subjects, led to significant increase in hemoglobin level and in the related biomarker, serum ferritin, as well as an improvement in energy and alertness, as compared to the whey protein isolate treated subjects. The increase in serum ferritin levels implies that the increase in hemoglobin may be related to an increase in iron uptake from a normal unchanged diet in the presence of bioactive peptides that may be present in the salmon protein hydrolysate. Such increases in uptake have been reported for other essential elements like zinc and calcium for example from casein peptide hydrolysates [34], but increases in iron uptake from fish protein hydrolysates has not been reported. It is also noteworthy to mention here, that although every subject in the salmon protein hydrolysate treated group G1 showed an increase in iron stores, not a single subject in this group showed elevated concentrations above 200 ng/m for serum ferritin, making the use of salmon protein hydrolysate a safe product for use, with reference to the potential for iron overload, that occurs with many other iron supplement therapies.

Another aspect of our results shows that this clinically relevant increase in serum hemoglobin and ferritin also leads to a qualitative improvement in energy levels and alertness. Other studies have shown the link between anemia and low energy and alertness and it is interesting to note here that the energy and alertness show an improvement with only a 1.5 g/dL increase in hemoglobin, as observed in this study. Quantifying such quality of life improvements with serum levels of biomarkers, such as hemoglobin and ferritin, is also an area of active research in our laboratory.

Another aspect of our results is to investigate the bioactive peptide fraction from the 641 peptides present as measured by MALDI-TOF analysis in the salmon protein hydrolysate tested here. We are planning fractionation of the peptides by both size occlusion column as well as ion exchange chromatography to further investigate the role of the different bioactive peptide fraction in the salmon protein hydrolysate that may promote the iron-uptake activity observed here. This remains a focus of research in our laboratory.

Our current results clearly show that dietary supplementation with 16 g of salmon protein hydrolysate per day increases serum ferritin and hemoglobin levels in iron-deficient anemia patients within only 8 weeks of treatment.

Mean Hemoglobin Levels (g /dL) (± SD)		
	G1 (N=23)	**G2 (N=24)**
Day 0	10.05 ± 00.72	10.15 ± 0.40
Day 42	11.54 ± 00.75	10.38 ± 0.45
Mean Diff. (p value)	01.49 ± 00.16 *(0.001)	00.23 ± 0.30 (0.31) NS
% change	**↑ 14.9%**	**↑ 02.2%**

Table 2: Comparison of Changes in Mean Hemoglobin levels by Student t Test. * Significant, NS: Non-significant.

	Mean Ferritin Conc (ng/ml) (± SD)	
	G1 (N=23)	**G2 (N=24)**
Day 0	22 ± 14	31 ± 19
Day 42	53 ± 21	37 ± 17
Mean Diff. (p value)	31 (0.009) *	6 (0.463) (NS)

Table 3: Comparison of changes in mean serum ferritin concentrations By two-tailed, paired Student t-Test: *Significant, NS: Non Significant.

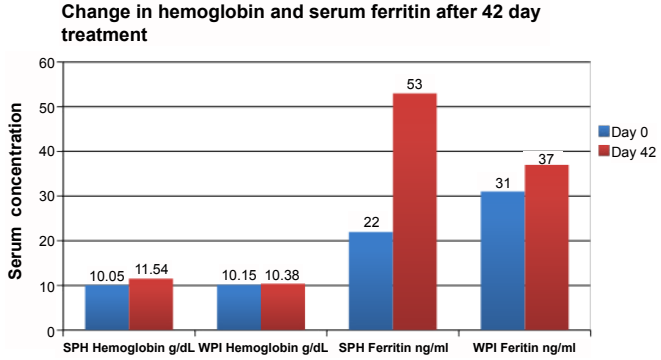

Figure 1: The hemoglobin and ferritin results are graphically summarized.

Assessment	SPH (N=23)		WPI (N=24)	
	Day 0 (N)	**Day 42 (N)**	**Day 0 (N)**	**Day 42 (N)**
Very Dissatisfied	02	0	01	01
Dissatisfied	09	04	10	09
Neither Satisfied Nor Dissatisfied	07	07	06	05
Satisfied	04	07	04	06
Very Satisfied	01	05	03	03

Table 4: Comparison of changes in improved alertness and energy.

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