Meat peptides enhance iron uptake from dietary supplements in a gut cell line model

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Abstract

In both developed and developing countries, iron deficiency anaemia is the most common of all nutritional deficiencies. One major cause is poor iron-bioavailability due to the presence of inhibitors (e.g., phytate) in the diet. Meat proteins promote iron uptake from phytate-rich vegetable diets in humans, probably by preventing chelation of iron by phytates. The objective of this study was to identify which fractions of meat proteins (sarcoplasmic, myofibrillar or stromal proteins) are responsible for maximizing iron uptake, and to determine the optimal conditions for iron uptake from iron supplements in combination with meat peptides by gut epithelial cells. The IEC-6 rat epithelial cell-line was used in this study, which is known as a good cell model of iron uptake in the gut. Results indicate that myofibrillar peptides enhance iron absorption most effectively. Iron uptake from a 40 µM iron concentration is maximal at pH 6.0, at and when the peptide size is less than 30 kDa compared to larger peptides. Future developments of this work will focus on creation of high-bioavailability iron-supplements.

Key words: Iron deficiency anaemia, food fortification, epithelial cells, meat, myofibrillar peptides.

I. Introduction

The World Health organization states that iron deficiency anaemia is the most common and widespread nutritional disorder in the world. It was estimated that 3.5 billion people in the world are iron deficient (Alleyne *et al*., 2008).

A main factor causing iron deficiency is poor dietary iron bioavailability due to the presence of iron absorption inhibitors in the diet (Cook *et al.,* 1981). Conventional iron supplements are inefficiently absorbed (Hurrell & Egli, 2010). Meat is not only the source of highly bioavailable heme-iron but also has a specific property that promotes the non-heme iron uptake in humans from phytate rich vegetables (Mulvihill *et al.,*1998). Regulation of iron absorption and body iron store occurs primarily in the proximal small intestine (Halleux and Schneider, 1991). Proteins such as DMT1 (Divalent Metal Transporter 1) and hephaestin are involved in iron absorption and transport in the human body (Kane and Miller, 1984). These proteins are expressed in IEC-6 cells. Which have been shown to be an appropriate cell model for iron uptake processes in the gut (Thomas and Oates, 2002). The aim of this work was to identify which fraction of peptides from digested meat proteins maximize iron uptake from a currently used iron supplement (ferrous gluconate) in the presence of phytates, with a view to developing nutraceuticals with added value to prevent iron deficiency.

II. Materials and Methods

Approximately 150 g of raw commercially-available porcine psoas muscle was blended with 500 mL precooled deionised water and was stirred for 2 h at 4^0 C. Water soluble proteins were recovered in the supernatant produced from centrifugation at 4000 x g for 20 min at 4^0C. The solid pellet from the centrifugation was rehomogenized in 500 mL of an ice-cold salt solution (0.3 M NaCl, 0.1 M NaH_2PO_4, 0.05 M Na_2HPO_4) for 2 h and centrifuged again as described above. Myofibrillar proteins were extracted from this supernatant by precipitation at low ionic strength (Savage *et al*., 1990). The remaining solid material after the extraction of salt-soluble protein was retained as the non-soluble protein fraction. Protein components in each fraction were identified by SDS-PAGE electrophoresis. Each protein fraction was first adjusted to a final protein concentration of 2 mg/ml concentration in 40 µM ferrous gluconate and digested by pepsin solution at 37^oC at pH 2.0 using a pepsin to protein ratio of 1:100 (Katho and Kubo, 1977).

IEC-6 cells were routinely maintained in the presence of Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum, 100U insulin and 50 mg/L streptomycin (Thomas and Oates, 2002). To study the uptake of iron, cells were washed 3 times with PBS to remove the culture medium and were incubated for 2 hours in the minimum essential medium (MEM) (control) or in MEM containing iron + peptide mixtures at 37^0C with 10% CO_2 and 90% air. In a preliminary experiment, iron uptake was found to be maximal at pH 6.0 in these cells, corresponding to the mildly acidic conditions in the first part of the duodenum. After treatment, cells in PBS-washed culture plates were counted by haemocytometer. The iron content of lysed cells was measured by means of a Ferine S assay using the Bio-vision Iron Assay kit. Iron content was normalised by the number of cells in each sample to calculate iron uptake. All experiments were conducted 3 separate times. Statistical analysis data was performed under SPSS using ANOVA with Tukey's post hoc test to compare the various means of each series of experiments. Means were considered significantly different if *p* values were less than or equal to 0.05. Variance within treatment groups was expressed as the standard error of the mean (SEM).

III. Results and Discussion

Peptides from myofibrillar proteins in the salt-soluble fraction are most effective in promoting iron uptake in gut epithelial cells. As Fig. 1 shows, only peptides from the salt-soluble protein (SSP) fraction resulted in iron absorption that is significantly higher than uptake from iron solution alone. Peptides from water soluble proteins have no enhancing effect, and peptides from insoluble (stromal) proteins actually have an inhibitory effect.

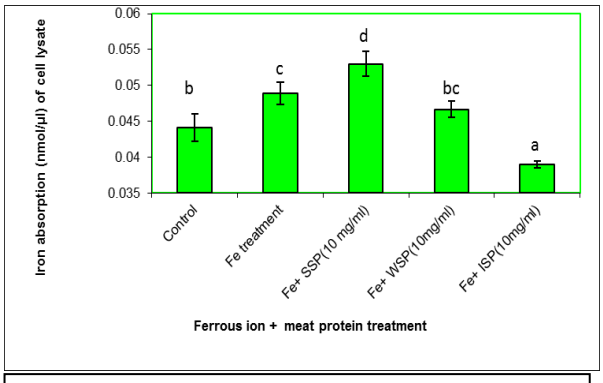

Fig.1. Effect of different protein fractions on iron absorption. Bars show mean (n=3) iron content of IEC-6 cell cultures normalized for cell number. Means with different letter are significantly different (P<0.05)

In the presence of phytates the cellular absorption of iron from both control (iron gluconate only) or iron plus SSP peptide treatments was diminished, as shown in Fig. 2. However, SSP peptides still caused a statistically-significant ($p < 0.05$) increase in iron uptake into the cells, compared to uptake from ferrous gluconate alone, in the presence of phytate.

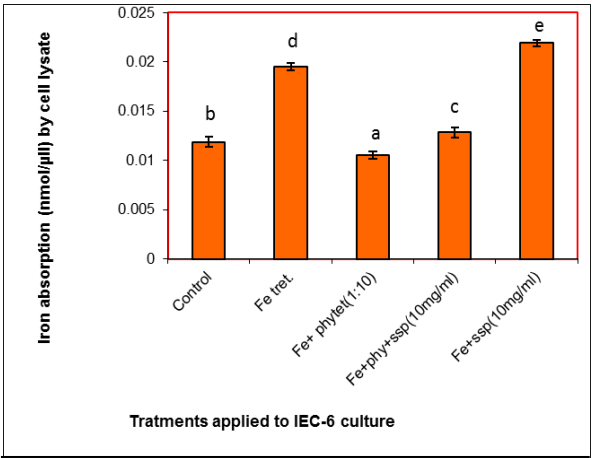

Fig.2. Effect of enhancer (salt soluble protein) and inhibitor (phytate) on iron absorption. Bars show mean (n=3) iron content of IEC-6 cell cultures normalized for cell number. Means with different letter are significantly different (P<0.05)

SSP peptides that are less than 30 kDa size are more effective than the larger peptides for enhancing iron absorption. A 30 kDa molecular weight cut off filter was used to separate peptides with MW<30kDa from the digested SSP+ferrous gluconate preparations. As shown in Fig. 3, this preparation results in a significantly greater cellular uptake of iron than the general digest of SSP proteins, indicating that smaller peptides have the highest bioactivity in promoting iron uptake.

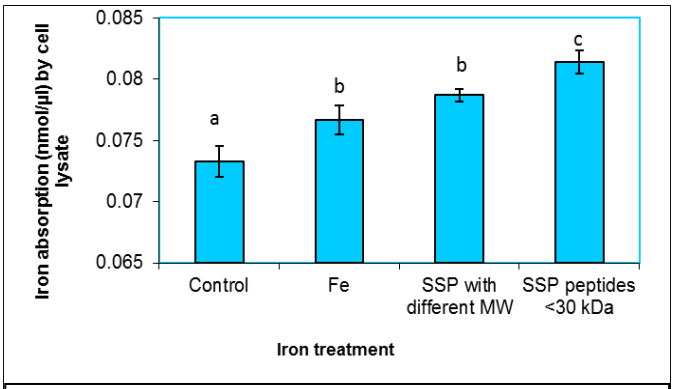

Fig.3. Effect of small (<30 kDa) peptides versus other peptides from SSP fraction on iron absorption. Bars show mean (n=3) iron content of IEC-6 cell cultures normalized for cell number. Means with different letter are significantly different (P<0.05)

IV. Conclusions and future work

The IEC-6 cell line was confirmed as a good model to study variations in iron uptake in the gut. Although not as effective as digests of beef muscle (Storcksdieck et al., 2007), small peptides from the myofibrillar proteins of porcine muscle were found to significantly promote the bioavailability of one of the ferrous salts conventionally used as an iron supplement for treatment of iron deficiency in humans. Admixtures of the peptides with iron supplementts offers the possibilty of increasing their absorption and so may allow a reduction of curent dose levels, which ofter have upleasant side effects (nasuea, headache). Mass spectroscopy revealed that the MW<30kDa SSP peptide mixture contained many hundreds of individual peptides (data not shown). Future work will therefore focus on more detailed separateion of the MW<30kDa SSP peptides in order to identify which peptide or group of peptides has the highest effect on iron uptake by intestinal cells.

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