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EFFECT OF MYOFIBRILLAR MUSCLE PROTEINS ON IN VITRO BIOAVAILABILITY OF IRON

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INTRODUCTION

The availability of food iron for absorption by humans depends on whether the predominant source of iron in the diet is from plant or animal sources. Humans absorb haem iron at a higher rate than non-haem iron. However, as foods contain more non-haem iron, it is this iron fraction that makes the major contribution to the body's iron pool. It is well established that most proteins are inhibitory to the absorption of non-haem iron (Berner and Miller, 1985). In contrast, cellular animal proteins appear to enhance the absorption of non-haem iron (Cook and Monsen, 1976). Approximately three-times as much non-haem iron is absorbed by humans when cellular animal proteins are substituted for egg albumin in semi-synthetic meals and the enhancing effect is dose related (Cook and Monsen, 1976). It has been suggested that amino acids and/or polypeptides arising from proteolytic digestion may chelate non-haem iron, thereby facilitating its absorption (Kane and Miller, 1984). Addition of a mixture of amino acids that mimic beef increased the absorption of non-haem iron (Monsen, 1988). However, when amino acids were administered individually, only cysteine was capable of reproducing the effect of meat (Layrisse *et al.*, 1984).

The objective of the present study was to use the in vitro dialysability technique of Miller *et al.* (1981) to identify the `meat factor' responsible for the enhancement of iron absorption.

MATERIALS AND METHODS

Whole muscle

The whole muscle fraction was prepared by homogenising ~10g muscle in ~100ml 0.6M KCl, 0.05M potassium phosphate buffer (pH6.5).

Preparation of muscle protein fractions

Rabbit skeletal muscle was used and was excised within 15 minutes of slaughter, coarsely minced and extracted immediately. The myofibrillar protein fraction (MP) was prepared by the method of Suzuki and Goll (1974). Myosin (M) was prepared by the method of Margossian and Lowey (1982). Heavy meromyosin (HMM), light meromyosin (LMM), the rod region (RR) and the head region (HR) were prepared according to the procedure of Margossian and Lowey (1982). Actin (A) was prepared using the method of Pardee and Spudich (1982).

All protein solutions were dialysed exhaustively at 4°C against a 0.6M KCl, 0.05M potassium phosphate buffer (pH6.5).

Mineral dialysability

Dialysable iron was determined according to the method of Miller *et al.* (1981). The homogenates were labelled with 59Fe and equilibrated prior to incubation. At the end of the incubation period the radioactivity in the dialysates and the original meals was determined by counting 5ml samples in an automatic LKB 1282 Compugamma Wallac Universal Gamma Counter. The per cent dialysable iron 59Fe was determined using the following equation :

$$\% \text{ diffusible iron} = \frac{\text{cpm 59Fe/ml dialysate} \times \text{ml dialysate}}{\text{cpm 59Fe/g meal} \times 20\text{g}}$$

RESULTS AND DISCUSSION

Effect of muscle protein fractions

The results in Figure 1 show the effect of whole muscle (WM) and the various myofibrillar fractions on the per cent dialysable iron. Egg albumin (EA) was used as reference protein. The results show that all the myofibrillar proteins, except for LMM, enhanced the in vitro bioavailability or dialysability of non-haem iron.

Substitution of EA by WM resulted in a 1.5-fold increase in iron dialysability, yielding a value of 8.1% dialysable iron. A 2.5-fold increase in iron dialysability was observed when MP was included in the semi-synthetic meal, with a value of 12.2% dialysable iron. Actin showed a similar enhancing effect to WM, yielding a value of 8% dialysable iron. A three-fold increase in dialysable iron (16%) was obtained when myosin was substituted for EA. All subfragments of myosin, with the exception of LMM, enhanced iron availability. Values of 16.1, 13.1 and 13% dialysable iron were obtained for HMM, HR and RR respectively. When LMM was substituted for EA, dialysable iron was reduced from 5.2 to 3.9%.

Hurrell *et al.* (1988) suggested that the differences in the facilitating effect on iron absorption between meat proteins and albumin is due to differences in the cysteine containing peptides. The results of the present study are consistent with this idea. Hofmann and Hamm (1978) concluded that ~65% of sulphydryl groups are present in myosin and 29% present in actin. Within the myosin molecule, HMM contains a relatively higher content of sulphydryl groups than LMM (Asghar *et al.*, 1985).

Effect of cysteine

In order to further clarify the role of cysteine residues on iron dialysability, sulphydryl groups in the form of free cysteine (0-30mM) were added to actin-containing meals. The presence of cysteine increased the dialysability by up to three-fold, i.e., from 7.9 to 25.9% (Figure 2). No significant ($P>0.05$) change in iron dialysability was observed at cysteine concentrations greater than 15mM.

Effect of N-ethylmaleimide

In order to further test the hypothesis that cysteine residues are major promoters of iron absorption, it was decided to evaluate the effects of specific SH-blocking agents on iron dialysability. N-ethylmaleimide (NEM) was added to semi-synthetic meals, containing myosin or actin, at concentrations up to 15mM and iron dialysability was determined. In the absence of NEM, myosin had a greater enhancing effect than actin (Figures 1 and 3). As the concentration of NEM was increased, there was little or no change in iron dialysability from the actin-containing meal. Dialysable iron decreased from 7.9 to 6.2%. The effect of NEM on iron dialysability from the myosin-containing meal was very pronounced. As the NEM concentration was increased, dialysable iron decreased from 16.0 to 8.3% at 15mM NEM. The presence of NEM had negligible effects on dialysable iron from the egg albumin meal. Myosin contains 40-45 cysteine residues per mol (Yates and Greaser, 1983). While actin and egg albumin contain only 5 and 1 cysteine

residues per mol, respectively. Thus, blocking sulphydryl groups of myosin eliminated most of the enhancing effect on iron dialysability. Other data (unpublished) show that the reduction of Fe(III) to Fe(II) under simulated gastrointestinal conditions was in the order : myosin > actin > egg albumin.

CONCLUSION

In conclusion, the present results show that the enhancing effect of meat on non-haem iron dialysability is associated with the main myofibrillar proteins, particularly the HMM region of myosin. The results further suggest that cysteine residues may constitute the `meat factor'.

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