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RAPID COMMUNICATION

Influence of the sulphydryl content of animal proteins on *in vitro* bioavailability of non-haem iron

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Meat is a known enhancer of non-haem iron bioavailability from foods. The exact mechanism by which this enhancement occurs remains unknown. This present study was designed to identify the 'meat factor'. The ability of different animal proteins to reduce non-haem Fe(III) to Fe(II) during an *in vitro* digestion was determined. The role of the -SH content of the proteins was also evaluated. When compared to egg albumin, meats from different species significantly enhanced dialysable ionic iron, D-(Fe(II) + Fe(III)), and dialysable Fe(II), D-Fe(II), ($p < 0.05$). In contrast, whey protein was inhibitory. Statistically significant linear correlations were established between the -SH content of the systems and their ability to reduce Fe(III) (r^2 0.451, $p < 0.02$) and dialyse the Fe(II) formed (r^2 0.524, $p < 0.01$). Incorporation of the -SH blocking agent, *N*-ethylmaleimide, significantly inhibited Fe(III) reduction and Fe(II) dialysability in a dose-related manner. These results suggest that the -SH content of animal proteins plays an important role in enhancing non-haem iron bioavailability and hence, may be related to the 'meat factor'.

INTRODUCTION

Reduction of ferric iron (Fe(III)) to the more soluble ferrous iron (Fe(II)) appears to be a prerequisite for iron uptake into the mucosal cell (Wollenberg and Rummel, 1987; Barrand *et al.*, 1990; Raja *et al.*, 1992). Fe(II) is more soluble and, therefore, it is less likely to hydrolyse under conditions present in the gastrointestinal lumen (Forth and Schafer, 1987). Moreover, complexes with Fe(II) are usually less stable than those with Fe(III) and hence, the iron is released more readily (Reddy *et al.*, 1986).

Cellular animal proteins are known enhancers of non-haem iron bioavailability (Hurrell *et al.*, 1988; Gordon and Godber, 1989; Kapsokefalou and Miller, 1991, 1993, 1995; Reddy and Cook, 1991). This enhancement is commonly known as the 'meat factor', but it remains to be elucidated. However, there is growing scientific interest linking the ability of meat to reduce iron in the gastrointestinal tract with the 'meat factor'.

It is established that the sulphydryl (-SH) groups of cysteine and glutathione are capable of reducing Fe(III) to Fe(II) at low pH (Hamed *et al.*, 1983). Recent research in our laboratory suggests that the unidentified 'meat factor' may be a component of the -SH-rich myofibrillar fractions of meat, in particular, the heavy meromyosin fraction (Kirwan *et al.*, 1993). Results indicate that the ability of the different myofibrillar fractions to enhance non-haem iron dialysability coincide with a known distribution of -SH groups within these fractions. Previous studies also showed that meat digestion products containing cysteine significantly enhanced iron absorption in humans (Taylor *et al.*, 1986).

In the present study, the ability of different meat systems to reduce non-haem Fe(III) to the more soluble Fe(II) during a simulated digestion process was evaluated. The role of the -SH groups in this process was also determined.

MATERIALS AND METHODS

In vitro estimation of dialysable ionic iron and dialysable ferrous iron

The method used to determine the relative iron bioavailability of the different protein systems was determined

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according to the *in vitro* method of Miller *et al.* (1981), as modified by Kapsokefalou and Miller (1991). Dialysable ionic iron, D-(Fe(II) + Fe(III)), and dialysable ferrous iron, D-Fe(II), were used as indicators of non-haem iron bioavailability.

Materials

- *Non-haem ferric iron*—A $FeCl_3$ solution, 1000 ppm in Fe (Atomic Absorption Standard), was used as a source of Fe(III) in all treatments.
- *Pepsin*—4.0 g pepsin powder was suspended in 0.01 N HCl and diluted to 100 ml with 0.1 N HCl.
- *Pancreatin-bile mixture*—0.5 g porcine pancreatin and 3.0 g bile extract were suspended in 0.01 N $NaHCO_3$ and diluted to 250 ml with 0.1 N $NaHCO_3$.
- *PIPES buffer, 0.15 N*—PIPES (piperazine-*N,N'*-bis-(2-ethane-sulfonic acid)) disodium salt was dissolved in water and adjusted to pH 6.1 or 6.3 using concentrated HCl. The pH 6.1 PIPES solution was used for the control. The pH 6.3 PIPES solution was used for the other test meals, to compensate for their higher buffering capacity.
- *HEPES buffer, 0.3 N*—HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulfonic acid) sodium salt was dissolved in water and adjusted to pH 9.9.
- *Reducing protein precipitant solution*—100 g trichloroacetic acid and 50 g hydroxylamine monohydrochloride were dissolved in water, 100 ml concentrated HCl were added and the volume was diluted to 1 litre with water.
- *Non-reducing protein precipitant solution*—This was the same as the reducing protein precipitant solution except that hydroxylamine monohydrochloride was omitted.
- *Ferrozine chromagen solution (5 mg ml^{-1})*—Ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine), disodium salt, was dissolved in water.
- *Dialysis tubing*—Dialysis tubing with a molecular weight cut off of 6000–8000 was soaked in water overnight.

Methods

Preparation of test meal

Top quality meat was purchased from a local butcher and trimmed of visible fat before use. An amount containing 4.0 g protein, based on protein content from The Composition of Foods (McCance and Widdowson, 5th edn, 1991) was homogenised in water; 0.05 mmol $FeC1_3$ was added and blended. The pH of the treatments was adjusted to 2.0 using 1 N HCl and the volume was adjusted to 100 ml with 0.01 N HCl. Treatment blanks were prepared as above, but $FeCl_3$ addition was omitted. $FeCl_3$ (0.50 mmol) in 0.01 N HCl was used as a control. Egg albumin and whey protein were also prepared as above.

In vitro digestion

Ten ml aliquots of each of the test meals and their respective blanks were transferred to plastic bottles, in triplicate, and mixed with 10 ml 0.01 N HCl. Pepsin suspension (1.0 ml) was added to each bottle. The mixture was incubated at 37°C in a shaking water-bath for 2 h. At the end of the pepsin incubation, a dialysis bag containing 20 ml PIPES buffer was placed in each bottle. After a further 30 min incubation, 5.0 ml of the pancreatin–bile mixture was added to each bottle, and incubation continued for another 2 h. At the end of the pancreatin–bile incubation the dialysis bags were removed and rinsed by dipping in water. Bag contents were transferred to tared beakers and weighed. The pH of each dialysate and retentate was measured. The experiment was conducted on a timed schedule so that all samples were incubated for the same period of time.

Iron assay

Iron concentrations in dialysates (Fe(II) and total) and retentates (Fe(II) only) were measured using the method proposed by Reddy *et al.* (1986) as modified by Kapsokefalou and Miller (1991). For total iron (Fe(II) + Fe(III)) measurement, reducing protein precipitant solution (1.0 ml) was added to 2.0 ml aliquots of each dialysate, in triplicate. For Fe(II) measurement, non-reducing protein precipitant solution (1.0 ml) was added to 2.0 ml aliquots of each dialysate and retentate, in triplicate. Samples were held overnight at room temperature. Subsequently they were centrifuged in a bench-top centrifuge at 2575 ***g*** for 10 min. Aliquots of the supernatants (1.0 ml in duplicate) were transferred to separate tubes. Ferrozine solution (0.25 ml) and HEPES buffer (2.0 ml) were added to each tube. Absorbances (562 nm) were measured, using a Shimadzu UV-120 Spectrophotometer, immediately after chromagen addition for Fe(II) determination and 1 h after chromagen addition for total iron determination.

Iron concentrations were calculated from the absorbance readings using a regression equation derived from data generated from standards. The standards were prepared from 1000 ppm $FeCl_3$ and were diluted with 0.1 N HCl. The standards used were 3.5, 7, 14, 20, 28, and 35 ppm Fe.

Calculations

Dialysable ferrous iron (D-Fe(II)), dialysable total iron (D-(Fe(II) + Fe(III))), total ferrous iron (D-Fe(II) + non-D-Fe(II)) and non-dialysable ferrous iron (non-D-Fe(II)) were expressed as percentages of total Fe(III) which was added to each bottle. It was assumed that dialysable iron had equilibrated across the dialysis membrane by the time the dialysis sacs were removed at the end of the digestion.

D-Fe(II) %:

$$\frac{(\text{Fe(II)})_{\text{D}}(\mu\text{g ml}^{-1}) \times \text{total volume(ml)}}{\text{Fe(III) in original sample } (\mu\text{g}) \times 100\%}$$

where—$(\text{Fe(II)})_{\text{D}}$:ferrous iron concentration in the dialysate.
—total volume: dialysate volume + retentate volume.

$$\text{D} - (\text{Fe(II)} + \text{Fe(III)})\% :$$
$$\frac{(\text{Fe(II)} + \text{Fe(III)})_{\text{D}}(\mu\text{g ml}^{-1}) \times \text{total volume(ml)}}{\text{Fe(III) in original sample } (\mu\text{g})} \times 100\%$$

where—$(\text{Fe(II)} + \text{Fe(III)})_{\text{D}}$: total iron concentration in the dialysate.

D-Fe(II) + non-D-Fe(II) %:

$$\frac{(\text{Fe(II)})_{\text{D}}(\mu\text{g ml}^{-1}) \times \text{dialysate volume (ml)} + (\text{Fe(II)})_{\text{R}}(\mu\text{g ml}^{-1}) \times \text{retentate volume (ml)}}{\text{Fe(III) in original sample } (\mu\text{g})} \times 100\%$$

where—dialysate volume: weight of the dialysate assuming a density of 1 g ml^{-1}.
—$(\text{Fe(II)})_{\text{R}}$:ferrous iron concentration in the retentate.
—retentate volume: total volume minus volume of dialysate.

Blank values for D-Fe(II), D-(Fe(II) + Fe(III)), D-Fe(II) + non-D-Fe(II) and non-D-Fe(II) were subtracted from sample values.

In the final part of the study, *N*-ethylmaleimide (NEM) (100–400 μmol g^{-1} protein), a known -SH blocker, was added to liver homogenate prior to *in vitro* digestion; Fe(II) formation and dialysable Fe(II) were determined as above.

Sulphydryl (-SH) group determination

The -SH content of the different systems was measured prior to *in vitro* digestion according to the method of Ellman (1959) as modified by Beveridge *et al.* (1974). Sodium dodecylsulphate (SDS) (0.5%) in Tris-glycine buffer (pH 8.0) was added to the test protein. The absorbance at 412 nm was read using a Shimadzu UV-120 Spectrophotometer. The -SH concentration was calculated using the molar extinction coefficient (ε) = 13 600 M^{-1} cm^{-1}.

Statistical analysis

The significant differences between the means were determined using Student's *t* test. Statistical analysis was performed using the Statsworks™ (Version 1.2) statistical package (Cricket Software, Inc., Philadelphia, USA).

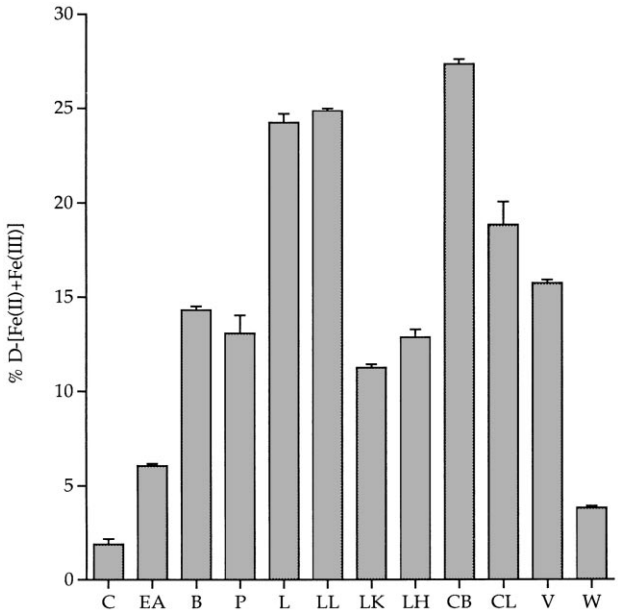

Fig. 1. The sum of ferrous and ferric iron, % D-(Fe(II) + Fe(III)), dialysed during *in vitro* digestion. Dialysable-(Fe(II) + Fe(III)) is expressed as a percentage of the total Fe(III) added to the samples prior to the incubation period. Each value is the mean ± SE of three analyses performed in triplicate. Abbreviations: C—control, EA—egg albumin, B—beef, P—pork, L—lamb, LL—lambs liver, LK—lambs kidney, LH—lambs heart, CB—chicken breast, CL—chicken leg, V—venison, W—whey. All systems were significantly different from egg albumin ($p < 0.05$).

RESULTS

The results presented in Figs 1–4 are expressed as percentages of the Fe(III) added to samples at the beginning of the incubation period. In Figs 1 and 2, egg albumin was used as the standard reference protein and the control sample did not contain protein.

The influence of different protein systems on the percentage dialysable ionic iron, % D-(Fe(II) + Fe(III)), is shown in Fig. 1. These results indicate that, with the exception of whey protein, all protein systems significantly ($p < 0.05$) enhanced dialysable ionic iron, D-(Fe(II) + Fe(III)), when compared with the egg albumin reference protein. The average dialysable ionic iron for egg albumin was 6.1%. Substitution of egg albumin by lamb, lambs liver or chicken breast, resulted in an approximate 4.2-fold increase in dialysable ionic iron, yielding values of 24.2, 24.8 and 27.3%, respectively. An approximate 2.2-fold increase in dialysable ionic iron was observed when egg albumin was substituted by beef, pork or venison, yielding values 14.3, 13.1 and 15.5%. Values of 18.9, 11.3 and 12.9% dialysable ionic iron were obtained for chicken leg, lambs kidney and lambs heart, respectively. An approximate 40% reduction in

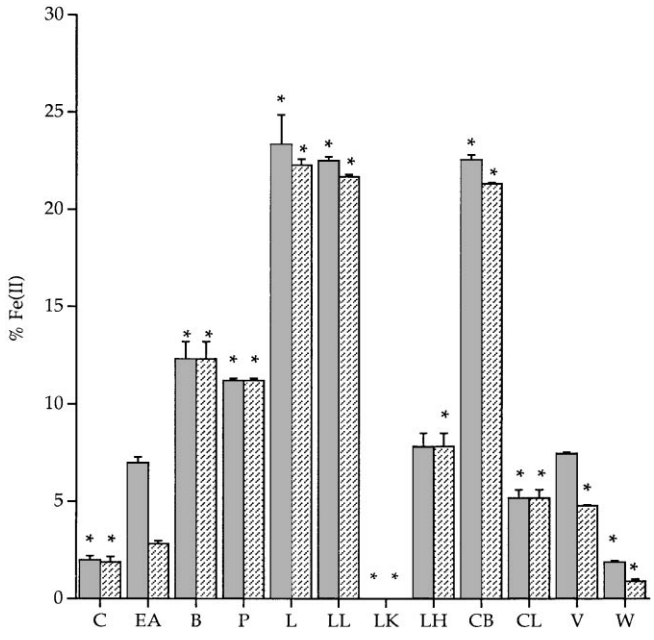

Fig. 2. The percentage of total ferrous iron, % D-Fe(II) + non-D-Fe(II), and dialysable ferrous iron, % D-Fe(II), formed during *in vitro* digestion. Fe(II) is expressed as a percentage of the total Fe(III) added to the samples prior to incubation. Each value is the mean ± SE of three analyses performed in triplicate. ▭, % D-Fe(II) + non-D-Fe(II); ▨, % D-Fe(II). For abbreviations see Fig. 1. *Significantly different from egg albumin ($p < 0.05$).

dialysable ionic iron was observed when whey protein was substituted for egg albumin.

The percentage of Fe(III) reduced to Fe(II), % D-Fe(II) + non-D-Fe(II), by the different protein systems is presented in Fig. 2. Dialysable Fe(II), % D-Fe(II), is also shown in Fig. 2. These results show that lambs kidney was the only system which did not reduce Fe(III) during *in vitro* digestion. Furthermore, with the exception of lambs kidney, all meat systems significantly enhanced dialysable Fe(II) when compared to the egg albumin reference protein ($p < 0.05$). In contrast, whey protein was inhibitory.

The reference protein, egg albumin, reduced 7.2% Fe(III) to Fe(II) during *in vitro* digestion. However, less than 40% of the Fe(II) formed was dialysed, yielding a value of 2.8% dialysable Fe(II). In contrast, lamb, lambs liver and chicken breast reduced the highest percentages of Fe(III) during *in vitro* digestion, yielding values of 23.4, 22.5 and 22.1% D-Fe(II) + non-D-Fe(II), respectively. Furthermore, over 90% of the Fe(II) formed by these systems was dialysed, yielding dialysable Fe(II) values, % D-Fe(II), of 22.3, 21.0 and 21.3%, respectively. These values were ~8-fold higher than the dialysable Fe(II) value for the standard egg albumin system. The dialysable Fe(II) values for beef and pork were 12.3 and 11.2%, representing an approximate 4-fold increase over the standard egg albumin. Substitution of egg albumin by lambs heart, venison or chicken leg yielded dialysable Fe(II) values of 7.8, 4.8 and 5.2%, respectively. Whey protein significantly inhibited Fe(III) reduction (1.9%) and Fe(II) dialysability (0.9%) when compared to egg albumin. Values of dialysable Fe(II), % D-Fe(II), and total ferrous iron formation, % D-Fe(II) + non-D-Fe(II), for beef, pork, lambs heart and chicken leg, were the same. This indicates that all of the Fe(II) formed by these systems was dialysed. In contrast, egg albumin had the highest percentage of non-dialysable Fe(II) among the systems.

Statistically significant linear correlations were established between the -SH content of the different systems and their ability to reduce Fe(III) (r^2 0.451, $p < 0.02$) (Fig. 3(a)) and dialyse the Fe(II) formed (r^2 0.524, $p < 0.01$) (Fig. 3(b)) during *in vitro* digestion.

The effect of added NEM (0-400 μmol g^{-1} protein) on total ferrous iron formation, % D-Fe(II) + non-D-Fe(II), and on dialysable ferrous iron, % D-Fe(II), is shown in Fig. 4. Addition of NEM (0-200 μmol g^{-1} protein) reduced both ferrous iron formation (Fig. 4(a)) and dialysable ferrous iron (Fig. 4(b)) in a dose related manner. Furthermore, values for ferrous iron formation (Fig. 4(a)) and dialysable ferrous iron (Fig. 4(b)) were

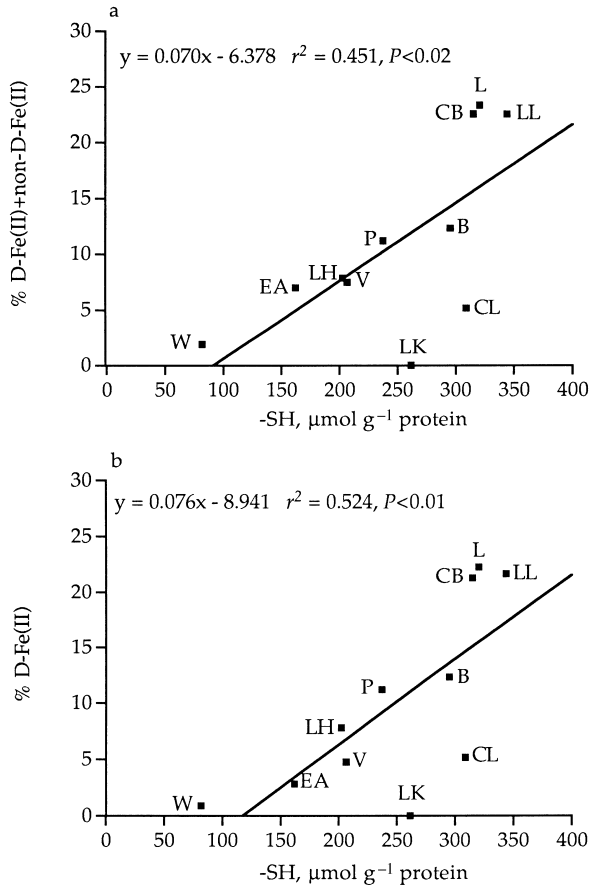

Fig. 3. Influence of the sulphydryl (-SH) content (μmol g^{-1} protein) of the different protein systems on: (a) total ferrous iron, % D-Fe(II) + non-D-Fe(II), and (b) dialysable ferrous iron, % D-Fe(II), formed during *in vitro* digestion. The values are expressed as percentages of Fe(III) added to samples prior to incubation. For abbreviations see Fig. 1.

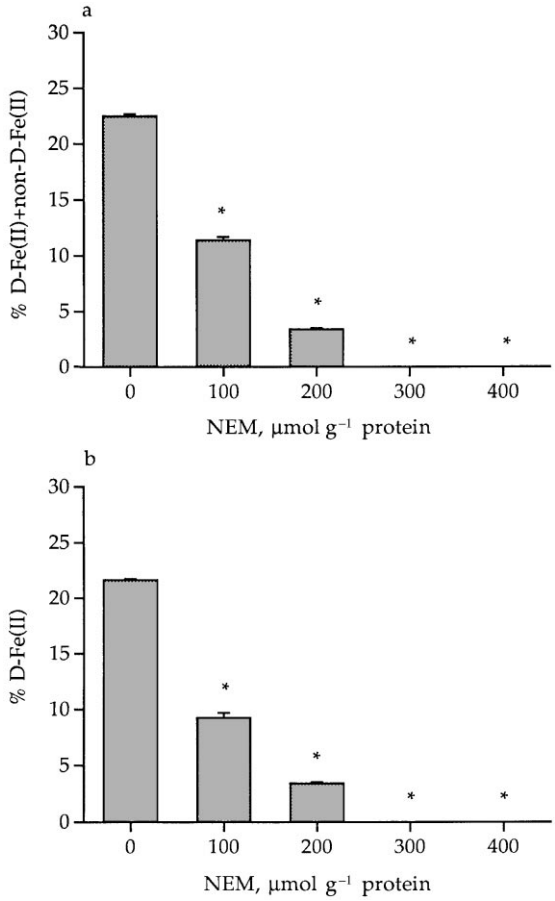

Fig. 4. The effect of *N*-ethylmaleimide (NEM) addition (μmol g^{-1} protein) to lambs liver system on (a) total ferrous iron, % D-Fe(II) + non-D-Fe(II), and (b) dialysable ferrous iron, % D-Fe(II), formed during *in vitro* digestion. The values are expressed as percentages of Fe(III) added to samples prior to incubation. *Significantly different to control (no added NEM).

negligible when 300 and 400 μmol NEM g^{-1} protein were incorporated into the lambs liver system.

DISCUSSION

The results highlight the importance of the -SH content of meat in enhancing non-haem iron dialysability. The results also show that the reducing influence of the -SH groups present in the meat digestion products plays an important role in the 'meat factor'.

The results presented in Fig. 1 clearly demonstrate that all meat systems significantly enhanced dialysable ionic iron when compared to the egg albumin reference protein ($p < 0.05$). In contrast, whey protein was inhibitory. These observations correlate well with earlier results which showed that meat protein enhances non-haem iron bioavailability, whereas, egg and milk protein are inhibitory (Cook and Monsen, 1976; Hurrell *et al.*, 1989). Furthermore, our results agree with those of Kane and Miller (1984) who observed that the type of protein does indeed influence the *in vitro* estimated iron bioavailability.

Kapsokefalou and Miller (1991) determined the reducing influence of different food components *in vitro* and noted that enhancers of non-haem iron bioavailability (ascorbic acid, cysteine, glutathione and beef) reduced high amounts of Fe(III), whereas, inhibitors (casein, bovine serum albumin and egg albumin) were ineffective at reducing Fe(III). The data presented in Fig. 2 correlate well with their findings. Egg albumin and whey protein, known inhibitors of non-haem iron bioavailability, reduced little Fe(III) during their digestion. In contrast, most of the meat systems reduced high amounts of Fe(III). For instance, lamb, lambs liver and chicken breast, the three meats which dialysed the greatest amounts of ionic iron (Fig. 1), also reduced the highest amounts of Fe(III) to Fe(II) during digestion (Fig. 2). Moreover, these three meats dialysed a very high proportion of the Fe(II) formed (Fig. 2). In fact, they dialysed approx. 8-times more Fe(II) than the egg albumin reference protein. It is of interest to note that, with the exception of lambs kidney, all the meat systems studied significantly enhanced dialysable Fe(II) when compared to egg albumin ($p < 0.05$). Indeed, Kapsokefalou and Miller (1991) suggested that dialysable Fe(II), D-Fe(II), is a reasonable predicator for non-haem iron bioavailability. Therefore, Fe(III) reduction appears to be an important prerequisite in the enhancement of non-haem iron dialysability by meat. This suggestion is plausible as *in vivo* studies have shown that iron must be reduced before it can be absorbed (Wollenberg and Rummel, 1987; Barrand *et al.*, 1990; Raja *et al.*, 1992). In a recent publication, Kapsokefalou and Miller (1995) showed that the intestinal digesta from rats fed beef contained approximately twice as much Fe(II) as did the digesta from rats fed egg or milk protein. They also observed a strong correlation between soluble Fe(II) in the intestinal digesta and iron absorption ($r = 0.980$, $p < 0.001$). Therefore, our results provide further evidence that the reducing influence of meat digestion products on Fe(III) and the enhancement of Fe(II) dialysability is likely to play a significant role in promoting non-haem iron bioavailability and may, in fact, be the 'meat factor'.

Notable differences in non dialysable Fe(II) values were observed among the different systems (Fig. 2). For instance, non dialysable Fe(II) values for beef, pork, lambs heart and chicken leg were negligible. This result implies that all of the Fe(II) formed by these systems, during the simulated digestion, was dialysed. In contrast, egg albumin had the highest amount of non dialysable Fe(II). The reason for this effect by egg albumin remains unknown. However, Monsen and Cook (1979) suggested that during the digestion of egg albumin iron-bioavailability-inhibiting-substances are released which masks the enhancing influence of animal proteins. It appears that these 'inhibiting-substances' may be related to the release of partially or non-digested high molecular weight peptides, which tightly bind the Fe(II)

formed, thus inhibiting its transport across the membrane. If this is the explanation it would suggest that the digestion products of beef, pork, lambs heart and chicken leg are small molecular weight peptides which are capable of promoting Fe(II) dialysis. Hence, the rate and degree of protein digestion may be an important factor influencing Fe(II) dialysability. The importance of protein digestibility in promoting iron bioavailability has been addressed by many investigators (Monsen and Cook, 1979; Kane and Miller, 1984; Slatkavitz and Clydesdale, 1988). However, Kane and Miller (1984) cautioned that protein digestibility *per se* is not the only 'protein related factor' affecting non-haem iron bioavailability. Therefore, we suggest that the rate and degree of protein digestion, the reducing influence of these digestion products and the presence of high/low molecular weight ligands all appear to be important factors affecting non-haem iron dialysability.

In the final part of this study, the role of the -SH content of the different systems in promoting Fe(III) reduction and Fe(II) dialysability was investigated. It is well established that the -SH groups of cysteine and glutathione are capable of reducing Fe(III) to Fe(II) at low pH (Hamed *et al.*, 1983). Indeed, Kapsokefalou and Miller (1991) observed the reducing influence of cysteine and glutathione *in vitro* and suggested that the -SH groups of cysteine may play a role in the 'meat factor'. Similarly, other studies showed that cysteine containing peptides released during the digestion of meat are capable of enhancing non-haem iron absorption in humans (Layrisse *et al.*, 1984; Taylor *et al.*, 1986). In Fig. 3, statistically significant linear correlations were established between the -SH content of the different systems and their ability to reduce Fe(III) (Fig. 3(a)) and moreover, to dialyse the Fe(II) formed (Fig. 3(b)). Whey protein, which inhibited ionic iron dialysability (Fig. 1) and Fe(II) dialysability (Fig. 2), contained the lowest levels of free -SH groups (Fig. 3). In contrast, the three major promoters of ionic iron dialysability (Fig. 1) and Fe(II) dialysability (Fig. 2), namely lamb, lambs liver and chicken breast, contained the highest concentrations of free -SH groups (Fig. 3). The equation of the best fit line in Fig. 3(a) gave a statistically significant linear relationship (r^2 0.451, $p < 0.02$) between the -SH content of the different systems and their ability to reduce Fe(III) during an *in vitro* digestion

$$\%\text{D}-\text{Fe(II)} + \text{non}-\text{D}-\text{Fe(II)} = 0.070(-\text{SH}) - 6.378 \quad (1)$$

Similarly, the equation of the best fit line in Fig. 3(b) gave a statistically significant linear correlation (r^2 0.524, $p < 0.01$) between Fe(II) dialysability and the -SH content of the systems

$$\%\text{D}-\text{Fe(II)} = 0.076(-\text{SH}) - 8.941 \quad (2)$$

where -SH = μmol -SH g^{-1} protein.

Therefore, eqns 1 and 2 may be used as a guide, to forecast the level of Fe(III) reduction and Fe(II) dialysability as a function of the -SH content of the systems. It appears from these results that proteins with high -SH group content should be capable of enhancing non-haem iron dialysability better than proteins with low -SH content. Previous work performed in our laboratory has shown that the ability of the different myofibrillar fractions to enhance non-haem iron dialysability coincided with a known distribution of free -SH groups where heavy meromyosin > myosin > actin (Kirwan *et al.*, 1993).

Figure 4 further strengthens the hypothesis that the -SH group is the key constituent in the 'meat factor'. Addition of NEM (100 and 200 μmol g^{-1} protein) to the lambs liver homogenate, prior to the *in vitro* digestion, significantly ($p < 0.05$) decreased Fe(III) reduction (Fig. 4(a)) and Fe(II) dialysability (Fig. 4(b)) in a dose-related manner. When the -SH groups of lambs liver were completely blocked with NEM (300 and 400 μmol g^{-1} protein), Fe(III) reduction (Fig. 4(a)) and Fe(II) dialysability (Fig. 4(b)) values were negligible.

Whey protein significantly inhibited ionic iron dialysability (Fig. 1) and Fe(II) iron dialysability (Fig. 2) when compared to the egg albumin reference protein. These results agree with those of Hurrell *et al.* (1989). This inhibitory effect may be related to the low -SH content of whey protein compared with the meat systems (82 μmol -SH g^{-1} protein).

Lambs kidney was the only system which did not reduce Fe(III) during the simulated gastrointestinal digestion (Fig. 2). No explanation for this effect is available at this time. However, the effect is probably not related to the free -SH content.

In summary, the results presented clearly show the importance of free -SH groups in promoting Fe(III) reduction and Fe(II) dialysability. The results also suggest that the 'meat factor' may be a -SH-rich proteolytic digestion product.

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