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Iron-solubilizing Isolate of Meat: Physiological, Compositional and Physicochemical Characteristics

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Abstract

Studies were performed to 1) investigate if ferric iron bound in complex with iron-solubilizing meat components is absorbable, 2) compare the relative iron-solubilizing capacity of meats, and 3) investigate the physicochemical and compositional characteristics if meat has iron-solubilizing components. Iron-solubilizing components of beef were isolated from pH 2 HCl homogenates into dialysis bags(MWCO of 6~8K). Radiolabelled iron complexes were then generated using ferric iron and the isolated low-molecular-weight components(ILC) from undigested beef or ascorbate. The bioavailabilities of radioiron in these complexes or as ferric iron were measured as radioiron absorption into the blood one hour after injection into ligated duodenal loops of rats. Iron absorptions were ferrous-ascorbate complexes(18.8±2.2%)>ferric-ILC complexes(4.9±0.6%)>ferric iron (2.2±0.3%)($p<0.05$). ILC from 0.1g of beef, pork, chicken, fish, or egg white were added to 400μg ferric iron in pH 2 HCl, the pH raised to 7.2, and soluble iron determined in the supernatant after centrifugation at 2,500g for 10min. Iron solubilizing capabilities of ILC were pork(99.9±0.1%)>beef(93.6±3.5%)>chicken(75.8±1.8%) >fish(64.6±3.6%)>egg white(50.9±0.9%)($p<0.05$). The compositional and physico-chemical characteristics of the ILC from the above dietary protein sources were investigated.

Key words: iron, iron solubility, meat, iron absorption

INTRODUCTION

Availability of dietary iron is affected by several enhancing and inhibiting factors present in foods. Meats, especially the red meats such as beef and pork are dietary components that enhance iron absorption via a presently obscure mechanism termed "the meat effect"(1-6). Studies employing single food items tagged biosynthetically with radioiron have shown that inorganic iron absorption from vegetable or cereal foods is usually less than 5% as compared with 15~20% absorption from animal sources such as beef, liver, and fish.

The factor(s) responsible for this meat enhancement has not been identified, but has been hypothesized to be a combination of meat influences on gastric functioning and chelation of dietary iron by a meat component(7). The amount of iron solubilized during *in vitro* digestion of meat-containing meals correlated with *in vivo* determinations of iron bioavailability and led to the suggestion that amino acids or peptides released from meat during digestion may be the meat factor(8-10). Soluble iron was defined for these *in vitro* digestions as iron that is dialyzable across a membrane with a molecular weight cut off(MWCO) of 6~8K. Other evidence has demonstrated the presence of an iron-solubilizing factor(s) in meat independent of digestion(11). These components are also dialyzable across a membrane with MWCO of 6~8K and referred to in the remainder of this paper as isolated low-molecular-weight components(ILC). Our studies suggest these iron-chelating components play an important role in meat enhancement of iron absorption(12). However, 1) the bioavailability of iron bound in such complexes has not been established, nor 2) has the digestion-independent iron-solubilizing capacity of various meats been examined, nor 3) has the compositional and chemical characteristics of the meat components responsible for iron solubilization been investigated. These studies were intended to address these deficiencies.

MATERIALS AND METHODS

Experimental design

Bioavailability of iron from a beef ILC-iron complex Thirty-six, six-week-old iron-deficient(7.9±0.7g Hb/

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dl) rats were used. Iron status of the rats was determined by measuring hemoglobin concentration in duplicate samples of fresh blood using the cyanmethemoglobin method(13). One hour after injection of radiolabelled test solutions(ferrous-ascorbate, ferric-ILC complex and ferric chloride) into the ligated loops of samll intestine, blood samples were taken and radioiron absorption measured. Three rats of each treatment were used in the experiment with four replicate trials on different days. Rats were randomly assigned for each treatment.

Iron solubilizing capacity of meat_

Beef(round eye steak), pork(rib loin), chicken(breast), fish(trout), and egg were purchased at a local market. Four separate retail packages of each protein source were selected. One retail sample for each protein source was used for measurement of iron solubilizing capacity on each of four separate days. Iron solubilizing capacity was measured using ILC prepared from the undigested meats.

Physicochemical and compositional characteristics of ILC_

ILC from beef, pork, chicken, fish, or egg white were prepared from undigested meats and analyzed for protein, amino aicd, phosphorus, iron, carnosine, and inosine monophosphate.

Isolation of ILC

Beef(round eye steak), pork(rib loin), chicken(breast), fish(trout), and egg were trimmed of visible fat. Weighed meat, 12.5g, was homogenized with 70ml 0.01mol HCl/L using a polytron homogenizer(Kinematica® GMBH, Switzerland). The pH was adjusted to 2.0 with 0.1mol HCl/L, and the total weight was raised to 100g with 0.01mol HCl/L. A dialysis bag with a MWCO of 6~8K(Spectrum, Medical Industries, Inc., Los Angeles, CA) containing 25ml 0.01mol HCl/L was put into the homogenized meat slurry. The mixture was incubated at 37°C with shaking for 3h. The dialysis bag from the meat slurry was rinsed with demineralized water, and the contents(ILC) were assayed for dialyzable iron. Amounts of ILC are expressed either 1) in volume units, which is how the liquid ILC was actually measured for the experiments, or 2) in weight units corresponding to the wet-weight of the protein source from which a specific amounts of ILC was produced(g PS: gram wet-weight of protein source).

Table 1. Formulation of iron complexes injected into *in situ* ligated loop

Treatment	Ferrous-ascorbate (ml)	Ferric-ILC[1] (ml)	Ferric (ml)
$FeCl_3$[2]	0.25	0.25	0.25
ILC	-	0.05	-
0.01mol HCl/L	0.05	0.05	0.01
Ascorbic acid(100mmol/L in 0.01mol HCl/L)	0.05	-	-
Demineralized water	0.605	0.615	0.615
0.25mol/L Tris buffer(pH 8.5)	0.045	0.035	0.035
Total volume(ml)	1.00	1.00	1.00

Final pH: 7.2

[1]Isolated low-molecular-weight components

[2]80mg Fe/L in 0.01mol HCl/L & $^{59}FeCl_3$(0.5μCi/ml)

Preparation of iron complexes

The formulations used to prepared the iron solutions to be injected into rat intestinal loops are given in Table 1. Iron standard $FeCl_3 \cdot 6H_2O$ in dilute HCl(Ricca Chemical Co., Arlington, TX) and ILC(contents in the dialysis bag), 0.01mol HCl/L, or 100mmol/L ascorbic acid(in 0.01 mol HCl/L) were mixed and incubated at 37°C for 30min. Demineralized water and pH 8.5, 0.25mol/L Tris buffer were added and mixed. The final pH of test solutions was 7.2. After preparation, all iron complexes were incubated at 37°C for 1h.

Animals

Sprague-Dawley weanling male rate(Simonson Laboratories, Gilroy, CA) were fed low-iron diet(15mg Fe/kg diet). The rats were individually housed in stainless steel cages with wire-mesh bottoms and fronts. Housing was in a temperature-controlled room(72°F) with a 12h light: dark cycle. Rats were fed the diet and deionized water *ad libitum* for 3wk.

Iron absorption from ligated intestinal segments

Animals were starved for one day before onset of the *in situ* iron absorption measurements. Animals were anesthetized by intraperitoneal injecton of 4mg sodium pentabarbital(Anthony Products Co., Arcadia, CA) per 100g of body weight. The animals were laparatomized, and 20cm of the duodenum, beginning 1cm below the pyloric valve, was ligated using cotton string. Test solutions(ferrous-ascorbate, ferric-ILC complex, and ferric chloride), 0.5ml prepared using radiolabelled iron, were injected into the ligated loops and the abdomen closed

using Michel clips. The animals were then maintained in a quiet room at 32°C for 1h and the animals exanguinated by decapitation. Blood samples were obtained for determination of radioiron absorption.

Determination of radioiron absorption

Duplicate 20μl blood samples were placed in test tubes with 5ml of demineralized water. The ^{59}Fe activity of blood samples was counted in a gamma counter(Packard Auto-Gamma Model 2000 Series, Meriden, CT). All ^{59}Fe counting data were corrected for decay and counting efficiency. The percentage of the ^{59}Fe administered that was absorbed into blood was calculated as follows:

^{59}Fe absorbed in blood(%)=[(μCi ^{59}Fe/0.02ml blood × g body weight × 0.067ml blood/g body weight)/μCi ^{59}Fe administered] × 100.

Determination of iron solubilizing capacity

ILC, 0.2ml, was mixed with 1ml of $FeCl_3$(from 80 or 160mg Fe/L stock solution) and then kept at room temperature for 20min. Demineralized water(2.4ml) and 0.25 mol/L pH 8.5 Tris buffer(0.2ml) were added to the mixture and shaken. Final iron concentration was 20 or 40mg Fe/L and final pH was about 7.2. The mixture was incubated at 37°C for 1h. Supernatant after centrifugation at 2,500 × g for 10min was assayed for soluble iron. Soluble iron was determined spectrophotometrically by ferrozine assay(14).

Effect of pH on iron solubility

Various ratios(0.2~0.8) of ILC and $FeCl_3$ in 0.01mol HCl/L were mixed and held at room temperature for 20min. The pH was adjusted by adding 0.25mol/L pH 10 Tris buffer and raised to 10ml volume with demineralized water. Soluble iron was determined using the procedure for determination for iron solubilizing capacity.

Phosphorus determination

ILC(25ml) was lyophilized and then wet-ashed by adding H_2SO_4 and HNO_3. Phosphorus was detected by the AOAC(15) method using molybdate and aminonaphtol sulfonic acid to form a colored complex.

Nonheme iron content

ILC was concentrated ten-fold by lyophilization and examined by ferrozine for presence of nonheme iron. Duplicate 0.5ml samples were used for ferrozine assay.

Heme iron contents

ILC was concentrated ten-fold by lyophilization and then used for absorption spectra from 400 to 700nm to determine presence of heme iron. The Hornsey method was used to quantify heme iron(16).

Total iron determination

ILC(25ml) was wet-ashed by adding H_2SO_4 and H_2NO_3. Wet-ashed ILC was added to 2.5ml demineralized water, and then 0.5ml samples, in duplicate, were applied to ferrozine assay.

Carnosine determination

ILC, 0.1ml, and 0.9ml OPA(*o*-phthalaldehyde) reagent (Sigma, St. Louis, MO) were mixed and filtered through 0.2μm syringe and then applied to HPLC(Beckman, Somerset, NJ). Test parameters were as follows: column, 5μm ultrasphere ODS high resolution end-capped column (250mm × 4.6mm); column temperature, ambient; detection, fluorescence 310nm for excitation and 375nm for emission; flow rate, 0.7ml/min; mobile phase, 50% B(methanol-acetonitrile(60:40, v/v)) in A(0.4% triethylamine in 0.06mol KH_2PO_4/L)(17,18). Reference compound, L-carnosine(N-β-alanyl-L-histidine), was purchased from Sigma.

Inosine monophosphate determination

ILC was filtered through 0.2μm syringe filter and then applied to HPLC(Beckman Instruments). Test parameters were as follows: column, 5μm ultrasphere ODS high-resolution end-capped column(250mm × 4.6mm); column temperature, ambient; detection, UV 254nm; flow rate 1.5ml/min; mobile phase, linear gradient from 0% to 25% of high-concentration eluent(methanol-water(60:40,v/v)) by volume in 30min and low-concentration eluent(20mmol KH_2PO_4/L)(19). Reference compound, inosine 3′,5′-cyclic mono phosphate(sodium salt), was purchased from Sigma.

Statistical analysis

Data were analyzed using ANOVA in a randomized block design. When F was significant($p<0.05$), means were compared by Fischer's least significant difference test.

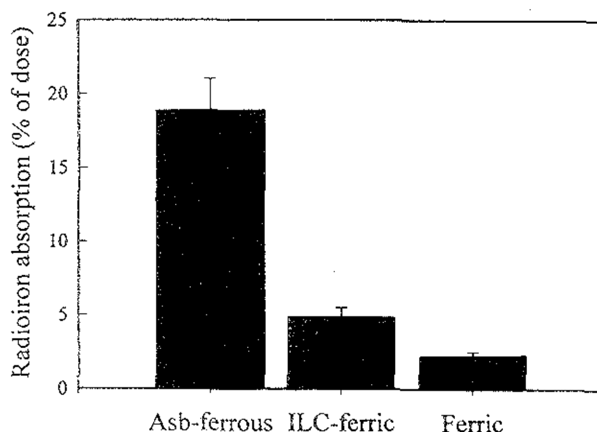

Fig. 1. Radioiron absorption from iron solutions injected into ligated segments of rat small intestine. Error bars represent one standard error mean. Iron absorption were Asb-ferrous(Ascorbate-ferrous)>ILC-ferric>ferric($p<0.05$).

To obtain a prediction equation for iron solubilizing capacity from analysis of ILC or from amino acid composition of ILC, multiple regression was done using Statview, software on Macintosh, at confidence interval 95%. To observe relationship between of iron solubilizing capacity and each components of ILC, simple regression was also done.

RESULTS AND DISCUSSION

Iron absorption from ligated loop of small intestine of rat

Iron bound in complexes with beef ILC was available for absorption from ligated segments of small intestine (Fig. 1). Ferrous-ascorbate was absorbed significantly ($p<0.001$) more than any other iron complex. Absorption rate was 18.8±2.2%(mean±SEM) of the dose. Although not as available as ferrous-ascorbate complexes, ILC-iron (4.9±0.6%) was more available($p<0.05$) for absorption than uncomplexed ferric iron(2.2±0.33%).

Iron solubilizing capacity of meats

There were significant differences in iron solubilizing capacity among meats($p<0.001$). Iron solubility from pork, beef, chicken, fish, and egg white was 99.9±0.1%(mean ±SEM), 93.6±3.5%, 75.8±1.8%, 64.6±3.6%, and 50.9± 0.9% respectively(Table 2). Iron solubilizing capacity of ILC from protein sources showed consistent(overall CV= 5.42%) value over different animal samples and different times.

Table 2. Precision of *in vitro* method to evaluate iron solubilizing capacity of meat

Protein source	n	ISC[1)](mean±SD)	CV(%)
Beef	8	$93.7^{a}\pm3.45$	3.68
Pork	8	$99.9^{b}\pm0.16$	0.16
Chicken	8	$75.8^{c}\pm3.66$	4.83
Fish	8	$64.6^{d}\pm6.10$	9.40
Egg white	8	$50.0^{e}\pm1.89$	3.78
Overall			5.42[2)]

[1)]Iron solubilizing capacity
[2)]CV for pork was excluded due to indications that capacity of assay was exceeded with pork

pH effect on iron solubility

Iron solubility of ILC-iron complexes was observed under different pH conditions(Fig. 2). Minimun solubility was observed approximately from pH 4 to pH 6.2 for all iron concentrations used. By increasing pH above 6.2, iron solubility was increased for 20 and 15mg Fe/L with ILC treatment. In contrast, the 32mg Fe/L with ILC treatment showed nearly zero solubility above pH 6.2. Solubility of ferric iron, a reference, was zero at above pH 4.5.

Effect of ratio of ILC and iron on solubility

For both iron concentrations used, 20 and 40mg Fe/L, maximum solubilities of 90 and 9%, respectively, were observed when the ratio of ferric(mg) : ILC(g PS) source was 0.8(Fig. 3). With 20mg Fe/L concentration, solubility was increased by increasing the ratio of ferric : ILC from 0.2 to 0.8. Iron solubility decreased when the ratio increased

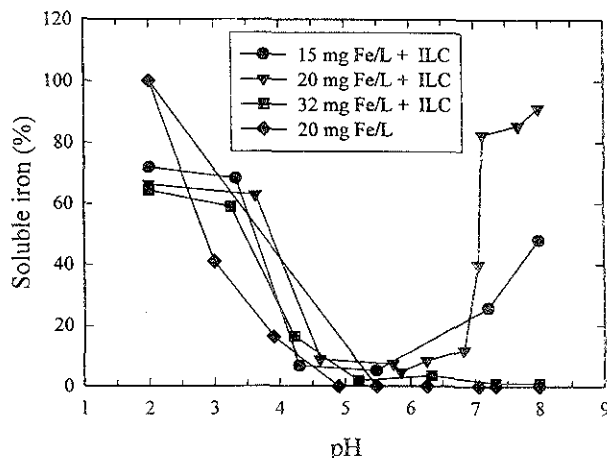

Fig. 2. Iron solubility of ILC from undigested beef and iron complex at various pH conditions. Amount of ILC was fixed to 0.5ml(0.25g wet wt. protein source) and 2.5ml $FeCl_3$ from stock solution of different concentrations were used to vary iron concentration.

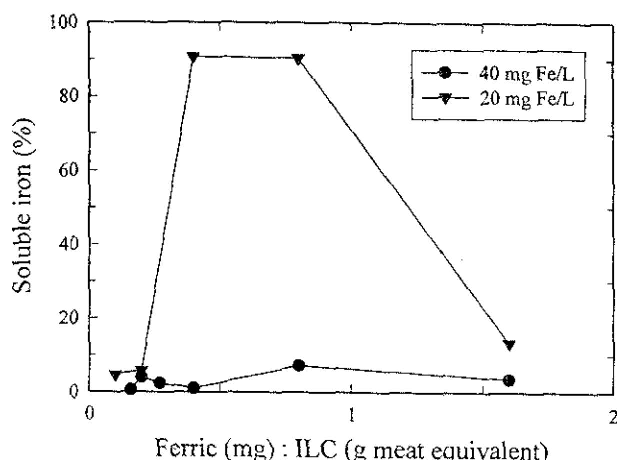

Fig. 3. Effect of iron : ILC ratio on solubility at pH 7.2. Volume of test solution(10ml) and amount of iron were fixed. Amount of ILC was varied to give different ratio of iron and ILC.

above 0.8. When iron concentration was 40mg Fe/L, percent iron solubility was much less than in 20mg Fe/L.

Composition of ILC

Beef ILC(512μg protein/g PS) showed more protein content than other ILCs(Table 3). ILC from pork, chicken, fish, and egg white contained 414, 440, 452, and 92μg/g PS respectively.

The phosphorus content of chicken ILC was 17.8μg/g PS which was 1.6 times more than other muscle meats. ILC from beef(11μg/g PS), pork(11.2μg/g PS), and fish (11.8μg/g PS) contained similar amounts of phosphorus. ILC from egg white had the lowest phosphorus content at, 12.5g of egg white, generated 3.2μg phosphorus/g PS.

Heme iron was not detected in ILC since no peak was observed from the absorption spectra from 400 to 700nm. No nonheme iron was detected in any ILC before ashing.

Table 3. Identified components of isolated low-molecular-weight components(ILC) from various undigested animal proteins

ILC	Protein (μg/PS[1])	Phosphorus (μg/PS)	Carnosine (μg/PS)	IMP[2] (μg/PS)	Heme iron (μg/PS)	Tatal iron (μg/PS)
Beef	512	11.0	6.6	26.0	ND[3]	0.44
Pork	414	11.2	12.2	84.0	ND	0.64
Chicken	440	17.8	3.2	ND	ND	0.38
Fish	452	11.8	4.4	ND	ND	0.38
Egg white	92	3.2	ND	ND	ND	ND

[1] g wet-weight protein source
[2] Inosine monophosphate
[3] Not detectable

Table 4. Amino acid composition of isolated low-molecular-weight components(ILC) from various undigested animal proteins

Amino Acid	Beef	Pork	Chicken	Fish	Egg white
	(Molar % of the total)				
Asp	3.0	4.1	5.2	2.7	6.2
Thr	2.5	2.8	3.9	3.8	5.3
Ser	3.6	3.6	5.5	3.9	7.9
Glx[1]	21.8	16.7	13.7	6.0	10.6
Pro	2.4	3.0	2.9	2.1	12.7
Gly	17.5	21.7	18.2	34.8	6.9
Ala	17.2	11.9	9.2	12.7	6.2
Cys	0.3	0.8	0.4	0.3	-
Val	3.2	3.3	3.4	3.9	7.0
Met	1.0	1.1	1.6	1.2	3.6
Ile	1.7	1.9	2.2	1.4	6.6
Leu	3.4	3.7	4.2	3.3	10.6
Tyr	1.2	1.2	1.6	2.1	5.0
Phe	1.5	1.7	1.7	2.1	5.0
His	14.7	16.8	18.1	13.8	1.9
Lys	2.9	3.6	5.1	5.6	1.1
Arg	2.1	2.1	3.1	1.6	2.9
Total	100.0	100.0	100.0	100.0	100.0

[1] Glx=Glu+Gln

Total iron contents in ILCs were measured after wet-ashing ILCs. The total iron content of beef, pork, chicken, and fish ILC were 0.44, 0.64, 0.38, and 0.38μg/g PS, respectively. No iron was detected in egg white ILC.

Carnosine(N-β-alany-L-histidine) was detected in ILC from muscle protein. It was not detectable in ILC from egg white. Pork ILC contained 12.2μg carnosine/g PS which was higher than ILC from other proteins used in this study. Carnosine contents of ILC from beef, chicken, and fish were 6.6, 3.2, and 4.4μg/g PS, respectively.

Inosine monophosphate(IMP), one of the nucleotides in muscle, was detected in beef(26μg/g PS) and pork ILC (84μg/g PS). It was not detectable in ILC from chicken, fish, or egg white.

The four major amino acids(glutamic acid, glycine, alanine, and histidine) had similar molar percentages among ILC from beef, pork, and chicken(Table 4). Overall, amino acid composition of ILC from beef, pork, and chicken was similar. Fish and egg white showed a different pattern from other muscle meats.

Regression study

Carnosine(r=0.904) and total iron content(r=0.908) were as highly correlated to iron solubilizing capacity(Table 5). Among amino acids, glutamic acid(r=0.919), alanine

Table 5. Associations between iron solubility and identified components of isolated low-molecular-weight components(ILC) from beef, pork, chicken, fish and egg white[x: concentra-tion of components(μg/g wet-weight protein source), y: iron solubility(%)]

Component	Correlation(r)	Intercept(β_0)	Slope(β_1)
Protein	0.717	42.8	0.087
Phosphous	0.497	69.8	0.301
Carnosine	0.904	54.4	4.060
IMP[1)]	0.811	65.8	0.454
Total iron	0.908	46.5	81.5

[1)]Inosine monophophate

Table 6. Associations between iron solubility and molar percentage of amino acid of isolated low-molecular-weight components(ILC) from beef, pork, chicken, fish and egg white(x: amino acid, y: iron solubility)

Amino Acid	Correlation(r)	Intercept(β_0)	Slope(β_1)
Asp	0.602	58.5	2.94
Thr	0.521	58.1	3.46
Ser	0.587	57.1	2.70
Glx[1)]	0.919	51.9	0.91
Pro	0.805	44.9	7.00
Gly	0.638	53.4	0.60
Ala	0.923	45.7	1.29
Cys	0.851	57.5	28.0
Val	0.774	46.5	5.38
Met	0.698	51.9	11.4
Ile	0.780	50.7	8.14
Leu	0.743	49.8	4.31
Tyr	0.594	54.4	9.66
Phe	0.731	46.4	10.3
His	0.936	45.0	1.34
Lys	0.451	61.6	2.17
Arg	0.655	57.2	5.30

[1)]Glx=Glu+Gln

(r=0.923), and histidine(r=0.936) were highly correlated to iron solubilizing capacity(Table 6).

To predict iron solubilizing capacity from these various components of ILC, which were individually correlated with iron solubilizing capacity, a predictive equation was obtained by multiple regression. The best fittng model was as follows; iron solubilizing capacity(%)=49.306+2.833[ala]+0.142[his]+3.926[glu]+1.75[carnosine]+0.077[IMP];R^2=0.967.

DISCUSSION

Iron solubility is a critical physicochemical factor controlling iron absorption since it is generally agreed that only soluble iron can be absorbed(20-22). Unfortunately, the neutral pH within the intestine will cause the precipitation of ferric iron, the predominant form of inorganic dietary iron, thereby precluding iron absorption. Dietary factors that can form complexes with ferric iron during the gastric phase of digestion and subsequently maintain ferric iron soluble at the neutral pH within the intestine, have the potential to enhance iron absorption, provided the iron is not bound so tightly so as to exclude uptake. Therefore, absorption of complexed iron is expected to be dependent not only on solubility, but also on the stability of the iron chelate. First, the chelate must have a stability constant for iron greater than the iron hydroxides in order to prevent hydroxide formation and maintain solubility. Second, the constant must be low enough that it allows the release of the iron to mucosal acceptors for absorption(23). Previous observations have documented that components from beef fulfill the first requirement in that they are capable of maintaining ferric iron in solution at neutral pH(11,24). However, it had not been determined whether ferric complexes formed with the meat components would be released to the intestine for uptake. *In situ* absorption by rats(Fig. 1) showed that iron bound in complexes with beef ILC was available for absorption. Thus, beef ILC contains components that have the characteristics necessary to enhance iron absorption via effects on iron solubility.

The content of these iron-solubilizing components in various source of dietary protein were measured in *in vitro*. Ability to solubilize iron *in vitro* has been suggested to be predictive of enhancement of iron absorption(25-29). With this in mind, the iron-solubilizing capacity of ILC from various sources of dietary protein were measured *in vitro*. Significant differences in iron-solubilizing capacity were found among the various sources of dietary protein used in this study(Table 2). The highest iron-solubilizing capacity was found for the red meats(beef and pork) while the lowest iron-solubilizing capacity was found in egg white. These *in vitro* measurements approximate previous reports of *in vivo* enhancement of iron absorption(1).

Kane and Miller(8) measured dialyzable iron(MW<6,000~8,000) after pepsin digestion. Slatkavitz and Clydesdale (24) reported that pepsin digestion products with MW< 10,000 solubilized significantly more iron than those with MW>10,000. In contrast, pancreatin digestion products of MW<10,000 were not effective iron-solubilizing agents. Politz and Clydesdale(23) reported that molecular rage

of 6,200~2,500 solubilized significantly more iron than all the other peptide fractions after pepsin digestion. Carpenter and Mahoney(11) have observed that iron chelation by meat components does not depend on nor is inhibited by proteolytic digestion. They concluded that meat contains a factor(s) that solubilizes iron independent of proteolytic digestion. In most cases, solubility is measured under intestinal conditions, usually after exposure to a lower pH environment similar to that of the stomach. *In vitro* approaches to the estimation of iron availability rely on the assumption that iron must be in solution to be absorbed. This is a weakness of *in vitro* estimation because soluble iron is not always absorbed depending on characteristics of binding with a chelator.

In order to better understand the functioning of ILC in possibly enhancing iron absorption via effects on iron solubility, studies were undertaken to identify various physicochmical characteristics of ILC under conditions which appear during the digestive process. A number of observations have been made about iron binding capacity of gastric juice(30-32). Rudzki and Deller(33) extracted a specific iron binder from gastric juice and characterized it as a glycoprotein. The iron binding characteristics of ILC over pH 2 to pH 8(Fig. 2) were similar to observations on gastric juice by Wynter and Williams(34). They also observed similar binding in various protein solutions such as trypsin, egg albumin, alkaline phosphatase from intestinal mucosa and phosphatase from wheat germ and urine, and concluded that the iron binding phenomenon was a nonspecific ionic effect. Parallel with our observations of ILC-iron complexes, solubility of gastric juice-iron complexes was decreased by increasing pH from 2 to 4, but it was increased again around neutral pH.

The effect of food additives such as EDTA on iron absorption depends on its molar ratio to iron(35). When present in small amounts, it actually enhances iron absorption, but as the molar ratio exceeds 2 : 1, absorption of iron progressively declines. Similarly, Spiro et al.(36) demonstrated that citrate ion, when present in equimolar concentration, chelates ferric iron at low pH with loss of its hydroxylic proton. As the solutions is neutralized, the characteristic brown color of polymerized iron appears. The brown color was observed in some solutions of iron-ILC complexes. Iron concentration was observed as a critical factor determining solubility of iron in the presence of ILC(Fig. 2, 3). On the other hand, low ratio (<0.4) of ferric iron(mg) : ILC(PS), which means excess ILC in solution did not increase iron solubility either. Both the ratio of ferric : ILC and total amount of iron were important. This is similar to observations with other iron chelators such as EDTA and citrate.

To identify the components of ILC that may be contributing to iron solubilization, ILC was examined for the presence of iron-binding components. Some of the components that ILC was examined for have previously been suggested to play a role in meat enhancement of iron absorption via effects on iron solubility. Diets low in protein are associated with a low iron uptake, and the amount of iron absorbed is roughly dependent on the amount of protein in the diet(37,38). Kroe et al.(39) suggested that one of the iron enhancing factors of protein could be the availability of increased amounts of amino acids derived from high dietary protein. It has since been shown that amino acids have a chelating effect upon iron(40,41). Van Campen(42) have observed that histidine increased ^{59}Fe retention when added to the dosing solution, but did not when added to the diet. Kroe et al.(43) have found that amino acids have an influence on iron absorption independent of pH, and that, for any given pH, greater iron absorption occurs if the iron is administered with an amino acid such as glutamine or histidine. It has been proposed that certain amino acids and intermediary products of cellular meat digestion chelate with soluble iron and deliver the iron to the gut mucosa. Non-cellular animal proteins do not appear to form chelates that enhance nonheme iron absorption.

The amount of these components in the ILC of various dietary proteins was correlated with their *in vitro* iron solubilizing capacity. Amino acid composition of ILC from beef, pork, and chicken was similar, although fish and egg white showed differences from other muscle meats(Tabel 4). Among the amino acids found in ILC, ala(r=0.923), his(r=0.936), and glx=glu+gln(r=0.919) were highly correlated with iron solubility(Table 6). From analysis of amino acid composition of ILC, histidine was suspected to play a role in iron solubilizing as a component of a certain polypeptide or as a free amino acid because of high molar percentage in ILC from cellular animal protein. There is a very small portion in egg white ILC. Histidine and alanine, which are components of carnosine, were highly correlated with iron solubilizing capacity of ILC. The ratio of the histidine dipetides which are present in skeletal muscle has been proposed as a useful aid to

the identification of the species or origin of meat used in processed meats(44,45). Carnosine(β-alanylhistidine) is a histidine-containing dipeptide and should chelate copper in tissues where this dipeptide is persent in high concentration(46). Although the biological role of carnosine has not been established, a proposed function is action as a defense mechanism against oxidative stress through a variety of mechanisms, including chelation of metal ions(46,47). Correlation of known iron-coordinating groups with compounds present in meat suggested the possibility that nucleotides, specifically inosine monophosphate and carnosine, were the functional components of ILC (Table 5). Carnosine has two tertiary amine groups(one is cyclic) that can act as iron-coordinating groups, while inosine monophosphate contains three tertiary amines and a phosphate that can act as iron-coordinating groups. Due to their multiple coordination sites, each nucleotide could act as links between iron molecules thereby promoting the formation of the large iron complexes we have observed.

CONCLUSIONS

Iron complexes formed with ILC from beef were available for absorption in the *in situ* ligated small intestine of rats. Iron absorption was ferrous-ascorbate complexes> ferric-beef ILC complexes>ferric iron($p<0.05$).

Iron solubilizing capacities of animal proteins in the *in vitro* system were pork>beef> chicken>fish>egg white ($p<0.05$). These results support the hypothesis that meat enhancement of iron absorption may be due to its iron solubilizing ability.

Prediction equation for iron solubility of protein sources obtained was: iron solubility(%)= 49.306+2.833[ala]+0.412 [his]+3.926[glx]+1.75[carnosine]+0.077[IMP]; R^2=0.967.

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