

# Maillard Reaction Products from Glucose-Methionine Mixtures Affect Iron Utilization in Rats

C. DELGADO-ANDRADE\*, I. SEIQUER and M. P. NAVARRO

*Unidad de Nutrición, Estación Experimental del Zaidín, CSIC, Granada, Spain,*

*\*E-mail: cdelgado@if.csic.es*

**Abstract:** The influence of Maillard reaction products from glucose-methionine on iron bioavailability was investigated, and compared with those from glucose-lysine (both 40% moisture, 150°C, 90 min). Iron balance was carried out in rats fed diets containing 3% of the different samples and a control diet (AIN93-G). After the balance period, rats were sacrificed, haemoglobin and hematocrit were measured and some organs were removed to analyze iron content. Consumption of the diet containing glucose-methionine heated mixtures increased iron digestibility and bioavailability with respect to animals fed the glucose-lysine diet, although values of net absorption and retention did not reach significant differences between groups. Haemoglobin, hematocrit and iron in liver were unaffected with the different diets, but higher values of iron concentration in spleen were found among animals fed the glucose-methionine diet.

**Keywords:** Maillard reaction; methionine; lysine; iron; bioavailability

## INTRODUCTION

Iron deficiency, that actually affects approximately 20% of the world population, is frequently due to poor bioavailability of dietary iron rather than low intake. Therefore, the adoption of balanced diets and suitable food processing to maintain or improve iron bioavailability warrants attention.

The Maillard reaction products (MRP), formed during the processing and conservation of foods containing reducing sugars and protein, are widely consumed in the human diet. The reaction causes losses in nutritional quality of foods and the MRP may interfere in mineral utilization, as these products behave like anionic polymers capable of complex minerals [1], being iron one of the most affected [2, 3]. MRP from lysine are frequently present in food, as this amino acid is one of the most sensitive to thermal processing; thus, derivatives from the glucose-lysine model system are frequently used to study diverse aspects of MRP [2]. Methionine, although less reactive than lysine, is also involved in the Maillard reaction, generating volatile compounds and becoming less available. These losses could be of considerable importance in nutritional terms because the sulphur amino acids, together

with lysine, are normally the first limiting amino acids in many food proteins. A previous paper describes that MRP from glucose-methionine affects iron solubility [4].

In this study, the influence of MRP from glucose-methionine on iron bioavailability was investigated, and compared with MRP from glucose-lysine.

## EXPERIMENTAL

**Samples and diets preparation.** Glucose (Merck, Darmstadt, Germany), lysine and methionine (Sigma Chemical Co., St. Louis, Mo., U.S.A.) were used to prepare samples Equimolar mixtures of glucose-lysine-HCl or glucose-DL-methionine (40% moisture) were heated in open recipients in a 150°C oven (Selecta 2000210, Barcelona, Spain) for 90 min to obtain the GL and GM samples, respectively. The samples were characterized by the determination of brown colour developed during the heat treatment, measuring the absorbance at 420 nm in the sample solutions with a Milton Roy Spectronic-1201 spectrophotometer (Rochester, N.Y., U.S.A.). Moreover, the free amino acid content in the samples was measured by high performance liquid chromatography (HPLC), using the Water

Pico-Tag method after derivatization with phenyl isothiocyanate, without the hydrolysis step.

The AIN-93G purified diet for laboratory rodents was used for the control group. 3% of the lyophilized samples were individually added to the control diet (AIN-93G), to prepare the GL-D and GM-D diets, respectively.

**Biological assay.** Thirty weanling Wistar rats weighing  $41.7 \pm 0.4$  g (mean  $\pm$  SE) were housed individually in metabolic cages in an environmentally controlled room kept at 20–22°C, with a 12 h light-dark cycle and 55–70% humidity. The rats were randomly distributed into 3 groups of 10 animals and assigned to one of the three dietary treatments. Animals had *ad libitum* access to their diets and demineralized water (Milli-Q Ultrapure Water System, Millipore Corp., Bedford, Mass., U.S.A.). The assay involved a preliminary 14-day period during which solid food intake and body weight were monitored, followed by a second period lasting 7 days in which solid intake and body weight were monitored and Fe balance was determined collecting faeces and urine daily, stored separately as a 1-week pool. Faeces were weighed before and after lyophilization and then homogenized; urine was collected on 0.5% HCl (vol/vol), filtered (Whatman Filter Paper No. 40, ashless, Whatman, England) and diluted. After the balance period, animals were anaesthetized and sacrificed by total bleeding by cannulating the carotid artery. Hematocrit and haemoglobin were determined, and the spleen and kidney were removed, weighed and frozen at –20°C until iron analysis.

**Analytical techniques.** Iron analysis in all samples were performed with flame AAS in a Perkin-Elmer Analyst 700 Spectrophotometer (Norwalk, Conn., U.S.A.). Previously, aliquots of diets, feces and organs were dry-ashed in a muffle furnace (Selecta, Mod.366, Barcelona, Spain) at 450°C until

the obtention of white ashes, which were dissolved with HCl/HNO<sub>3</sub>/H<sub>2</sub>O (1:1:2), and iron concentration in urine was determined directly. Bovine liver standard (certified reference material BCR No. 185, Community Bureau of Reference, Brussels, Belgium) was simultaneously used to quantify iron recovery: measured value  $215 \pm 5$  µg/g (mean  $\pm$  SD of 10 determinations), certified value  $214 \pm 5$  µg/g.

From the data of the iron intake and faecal and urinary excretion, apparent absorption (ingested Fe – faecal Fe); apparent retention or balance (apparent absorption – urinary Fe); absorption efficiency or digestibility (%A/I) = apparent absorption/ingested Fe  $\times$  100; retention efficiency (%R/A) = apparent retention/apparent absorption  $\times$  100; and utilization efficiency or bioavailability (%R/I) = apparent retention/ingested Fe  $\times$  100, were calculated.

**Statistical treatment.** The results were tested statistically by one-way analysis of variance (ANOVA), followed by Duncan's test to compare means with significant variation ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

No significant changes in food intake or body weight were observed in animals throughout the experimental period (data not shown).

During the balance week, no differences in iron intake were observed between groups (Table 1). Values of faecal and urinary iron excretion tended to decrease among animals fed the GM-D diet and, consequently, this group showed higher values of absorbed and retained iron, although differences were not significant in any case (Table 1). However, these differences led to significant higher efficiency of the absorption process (%A/I) and of the utilization efficiency (%R/I) in the methionine group, with respect both to the control and to the GL group (Figure 1).

Table 1. Iron balance in rats fed the different diets

Groups <sup>a</sup>	Fe (µg/day)				
	Ingested	Faecal	Urinary	Absorbed	Retained
Control	787 $\pm$ 39	431 $\pm$ 16	16.7 $\pm$ 1.1	356 $\pm$ 26	339 $\pm$ 26
GL90	750 $\pm$ 41	420 $\pm$ 26	18.5 $\pm$ 2.7	323 $\pm$ 27	305 $\pm$ 28
GM90	763 $\pm$ 39	375 $\pm$ 17	13.5 $\pm$ 2.3	388 $\pm$ 33	375 $\pm$ 34

<sup>a</sup>Control, group of rats fed the AIN-93G diet; GL90, group of rats fed the GL90-D diet; GM90, group of rats fed the GM90-D diet. Values are mean  $\pm$  SE. No significant differences were found between groups

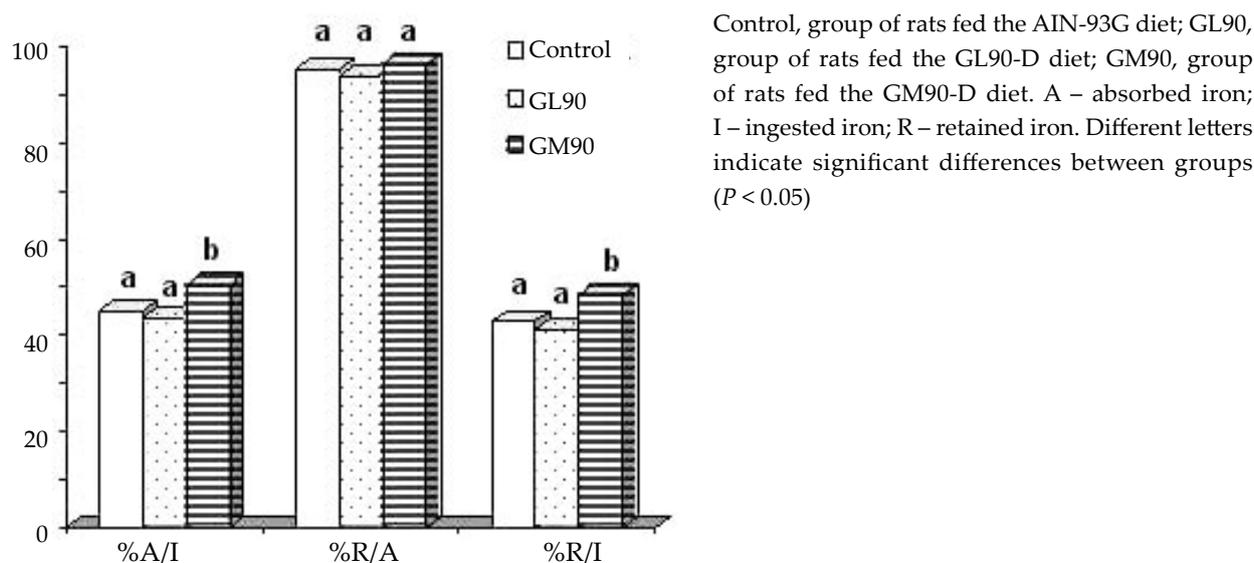


Figure 1. Utilization efficiency of iron in rats fed the different diets

Increases in Fe absorption in rats fed sterilized infant formula that developed MRP have been shown [5], but the bibliography usually describe stability of the iron balance after the inclusion of Maillard products in the diet [6], when the protein source is casein heated in the presence of glucose [7] and after the inclusion of toasted cereals in the diet of humans [8]. The higher digestibility and availability of iron found after the consumption of the GM-D diet could be due, in part, to the formation of soluble complexes between iron and free residual methionine (30.4% of the initial amino

acid, in the GM sample), that has been reported by other authors [9]. Moreover, of several amino acids, only methionine and cysteine are able to mimethize the positive effect of the “meat factor” in iron absorption [10]. The formation of iron chelates with the MRP from methionine should be taken as well in consideration. Previously, we have been shown that the presence of the GM sample promote iron insolubilization [4], and, according with some investigators, the insoluble iron fraction bound to some MRP is capable of being absorbed, after intestinal transformation [11].

Table 2. Iron concentration in liver and spleen. Haemoglobin and hematocrit values<sup>a</sup>

	Group <sup>b</sup>		
	Control	GL90	GM90
<b>Liver</b>			
Weight (g)	6.79 ± 0.62	6.02 ± 0.51	6.69 ± 0.57
Fe (µg/g)	103 ± 11	137 ± 14	134 ± 7
<b>Spleen</b>			
Weight (g)	0.54 ± 0.04	0.42 ± 0.04	0.42 ± 0.05
Fe (µg/g)	204 ± 7a	254 ± 23a	360 ± 51b
Haemoglobin (mg/dl)	13.2 ± 0.4	12.0 ± 0.3	12.1 ± 0.5
Hematocrit (%)	42.1 ± 1.9	43.0 ± 0.9	45.1 ± 2.1

<sup>a</sup>Different letters in the same line indicate significant differences ( $P < 0.05$ ); <sup>b</sup>Control, group of rats fed the AIN-93G diet; GL90, group of rats fed the GL90-D diet; GM90, group of rats fed the GM90-D diet. Values are mean ± SE

Higher values of digestive and metabolic iron utilization in GM group did not lead to enhanced iron functionality, as haemoglobin and hematocrit values were unchanged (Table 2). Iron concentration in liver was not increased among animals fed the GM-D diet, in spite that the liver is considered the main reservoir for iron. However, the iron concentration in the spleen was significantly higher in this group, which could indicate a higher catabolism.

### CONCLUSIONS

Consumption of MRP from glucose-methionine enhanced iron absorption and utilization efficiency in our experimental conditions, when compared with MRP from glucose-lysine. However, functional iron does not seem to improve, as haemoglobin and hematocrit values did not change.

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