EFFECTS OF SOME CARBOHYDRATES ON IRON ABSORPTION

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SUMMARY

Two experiments were performed to examine the effects of various carbohydrates (fructose, lactose, corn starch, wheat starch and potato starch) on the utilization of iron, on Fe-depleted rats. These received a single meal that contained the test carbohydrate at a 60o/o level, labelled with ^{59}Fe. The rest of the experiment the rats were fed a diet which contained glucose at a 60o/o level as the carbohydrate source.

In both experiments rats were fasted overnight, and the dose was offered in the form of a morning meal. To assay for ^{59}Fe, the animals were counted in a "Whole body counter" between two and four hours after dosing, and every day for the following 10 days. Percentage retention and absorption, as well as hemoglobin values were determined.

In the first experiment, the replacement of glucose by fructose at a 60o/o level enhanced significantly iron absorption and retention. An increase in absorption and retention also occurred when glucose was replaced by lactose at a 60o/o level, but the difference was not statistically significant. Administration of ^{59}Fe as an ^{59}Fe-fructose chelate did not seem to have a significant effect on retention and absorption when compared to the effect of dosing with ^{59}Fe adsorbed onto a fructose diet. This not rule out the possibility that chelation is the mechanism responsible for the enhancing effect of fructose on iron utilization. The complex could have been formed in the stomach, resulting in a significant absorption for both the ^{59}Fe-labelled meal and the ^{59}Fe-carbohydrate complex-labelled meal.

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In the second experiment, administration of a meal that contained either of the starches resulted in a reduction on retention and absorption of ^{59}Fe. The decrease, however, was statistically significant only for cooked corn starch, wheat starch and cooked wheat starch. The effect of cooking was to reduce even more the retention and absorption of ^{59}Fe, but this reduction was statistically significant only for corn starch.

The depressing effects of starches on iron retention and absorption are quite relevant to human nutrition. In the developing countries, diets are generally high in cereal products -often whole grain cereals- and low in animal products. The inhibitory effects of cereals on iron absorption have been traditionally attributed to the presence of phytates and fiber, but the data herein presented suggest that high intakes of starches may be inhibitory as well.

INTRODUCTION

Iron deficiency is considered one of the most common and widespread human nutritional deficiencies in the world. Unlike other nutritional deficiencies this problem is relevant to highly industrialized countries as well as to developing countries (1).

The total amount of iron in the human body is regulated through intestinal absorption (2). The form of iron in the diet can have major influence on availability for absorption. Heme and non-heme iron are absorbed by different mechanisms. The absorption of heme iron, unlike that of inorganic iron, is not affected by dietary components. Consequently, the amount of iron absorbed from the diet is dependent not only of the individual nutritional status, but also of the dietary factors that affect non-heme iron absorption. Some of these compounds promote iron absorption by maintaining the iron in a soluble form, either by formation of soluble chelates or by reduction of the ferric to the more soluble ferrous form (3).

Ascorbic acid (4), succinic acid (5), citric, tartaric and malic acid (6), cysteine and its peptides (7) and some proteins (8), increase iron absorption.

Phosphates (9), phytates (10), bicarbonate (11), tannins (6, 12) fiber (13) and soy protein (14) decrease iron absorption. An increase in iron absorption has been reported by fructose (15) and lactose (16), and a reduction of absorption with starches (17), although there are some discrepancies.

The work described in this paper was aimed at assessing the influence of some of the common dietary carbohydrates, on iron absorption in iron-deficient male rats.

MATERIAL AND METHODS

Two experiments were performed using weanling male rats (Sprague Dawley strain) with an average weight of 40 g, which were individually housed in stainless steel cages with wire mesh bottoms. Food and water were provided *ad libitum*, and weights were recorded weekly and at the end of the experiments.

Initially the rats were fed an iron-deficient glucose diet (17) (Table 1) during 25 days for Experiment 1, and during 35 days for Experiment 2. At the end of this period, hemoglobin values were determined (18) for each rat, and rats were assigned randomly to their respective treatment on the basis of hemoglobin values, with 10 rats per treatment. For each treatment, rats were fasted overnight and 2 g of a meal labelled with $^{59}FeCl_3$ was offered. The meal contained the carbohydrate at a 60o/o level (dry basis).

TABLE 1

COMPOSITION OF BASAL IRON-DEFICIENT GLUCOSE DIET

Ingredient	Concentration (g/kg)
Casein	200
Corn oil	50
Glucose monohydrate (cerelose)	686.5
Vitamins[a]	10.0
Minerals[b]	46.3
DL-methionine	5.0
Santoquin (preservative)	0.2
Choline chloride (70o/o)	2.0
Vitamin B_{12}	20.0 µg

a Supplies per 100 g of diet: retynil palmitate, 46.8 retinol equivalents; cholecalciferol, 500 µg; dl-alpha-tocopheryl acetate 8.9 tocopherol equivalents; vitamin K (k_5), 152 µg; (in mg) biotin 0.02; D-calcium pantothenate, 2; folic acid, 0.4; inositol, 25; niacin, 5; pyridoxine. HCl, 0.45; riboflavin, 1.0; thiamine. HCl, 1.0.

b Supplies per 100 g of diet (in g): $CaHPO_4.2H_2O$, 3.43; NaCl, 0.25; $KHCO_3$, 0.64; $MgSO_4$ (anhydrous), 0.27; (in mg) $MnSO_4.2H_2O$, 23; $CuSO_4.5H_2O$, 4; $ZnCO_3$, 8.8; KI, 0.065.

To assay for ^{59}Fe, the rats were placed in cylindrical plastic containers Each rat was then counted in a "whole body counter" (Packard Multichannel Analyzer, Model A9011) for 50 seconds between two and four hours after consumption of the meal and every day for the following 10 days. At the end of the experiment, hemoglobin values were again determined.

The per cent retention and per cent absorption were calculated from the body counts by the method of VanCampen and House (19).

Carbohydrates used in each experiment were as follows:

Experiment 1	Experiment 2
Raw corn starch	Fructose
Cooked corn starch	Lactose
Raw potato starch	Fructose-complex
Cooked potato starch	
Raw wheat starch	
Cooked wheat starch	

Cooking of the Starches

The gelatinization point of the starches was determined in a Brabender/ visco/amylograph (Model VA-VE) so that the same degree of gelanization could be reached for each starch. Starch slurries of a concentration of 8o/o were cooked slightly below the peak viscosity to avoid breakage of the starch granule. The time required to reach this point is shown in Table 2.

TABLE 2

TIME REQUIRED FOR GELATINIZATION OF STARCHES

Starch	Cooking time (min)
Corn	48
Wheat	42
Potato	33

Preparation of the Complex

The complex fluctose-^{59}Fe was prepared according to the method of Stitt *et al.* (20). The ratio of iron to carbohydrate was 1:100 mole basis. The final pH of the solution was 10.0. Even though the complex was not characterized, the absence of a precipitate assured its existence.

Statistical Tests

The retention and absorption data were subjected to an analysis of variance (21). Individual means were compared in a multiple range test (22).

RESULTS

Experiment 1

The Fe concentration of the iron-deficient glucose diet and of the raw starches used in the two experiments is presented in Table 3. Although the Fe concentration of the starches was different, they were not likely to have much effect since they were fed only as a single 2 g meal which contained 60o/o of the carbohydrate.

TABLE 3

IRON CONCENTRATION OF GLUCOSE DIET AND OF RAW STARCHES USED IN EXPERIMENT 1[a,b]

Treatment	Iron (ppm)
Glucose	12.4
Potato starch	17.2
Corn starch	1.1
Wheat starch	2.1

a Average of three determinations.
b Iron determined by atomic absorption.

No differences in average daily weight gain were observed for rats dosed with a meal containing the different carbohydrates. The average daily weight gain for all rats was 5.3 g ± 0.3 from the time they were dosed until the end of the experiment.

The hemoglobin values prior to dosing and at the end of the experiment are shown in Table 4.

Figure 1 depicts the retention curves for rats subjected to the different treatments. As the data reveal, rats dosed with a glucose meal had higher retention and absorption percentages than rats dosed with a meal containing either of the starches (raw or cooked). The difference in retention between glucose and the starches was statistically significant for rats dosed with a meal containing raw or cooked wheat starch (Table 5). Cooking had a depressing effect on iron retention and absorption in rats dosed with a meal containing corn starch ($p \leqslant 0.05$).

Experiment 2

Table 6 illustrates the average daily weight gain of the rats included in this experiment. Rats dosed with a glucose meal gained significantly less weight than those fed a meal containing either of the other carbohydrates. The initial and final hemoglobin levels are shown in Table 7. The ^{59}Fe retention curves for rats dosed with different carbohydrate meals labelled with ^{59}Fe are shown in Figure 2. Animals receiving a fructose meal

TABLE 4

HEMOGLOBIN VALUES FOR RATS DOSED WITH A MEAL CONTAINING DIFFERENT CARBOHYDRATES[a, b]

Treatment	Hemoglobin (g/100 ml)	
	Predosing	Terminal
Glucose	6.1 (10)	6.8 (10)
Raw potato starch	6.1 (9)	6.6 (9)
Cooked potato starch	6.1 (10)	6.7 (10)
Raw corn starch	6.1 (9)	6.9 (9)
Cooked corn starch	6.1 (10)	6.2 (10)
Raw wheat starch	6.1 (10)	6.4 (10)
Cooked wheat starch	6.1 (10)	6.8 (10)
Standard error[c]	0.2	0.2

a Values are means. Number of observations are given in parentheses.
b Means in the same column did not differ significantly ($p \leqslant 0.05$).
c Pooled standard error based on error mean square from analysis of variance.

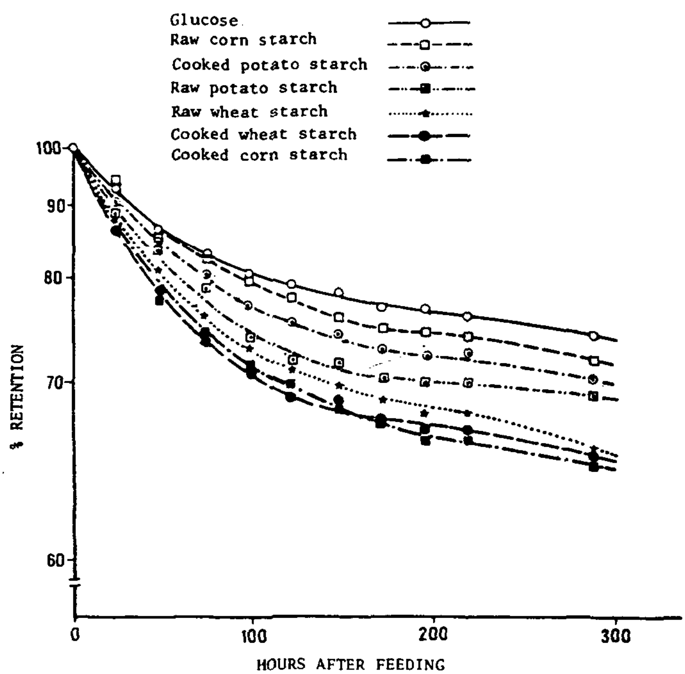

FIGURE 1

Effect of carbohydrates on ^{59}Fe retention by iron-deficient rats (Experiment 1)

TABLE 5

EFFECT OF CARBOHYDRATES ON THE RETENTION AND ABSORPTION OF ^{59}Fe BY IRON-DEPLETED RATS[a,b]

Treatment	Retention[c] (%)	Absorption (%)
Glucose	73.7 (10) (a)	83.4 (10) (a)
Raw potato starch	67.4 (10) (abc)	75.8 (10) (bc)
Cooked potato starch	69.8 (9) (ab)	79.4 (9) (ab)
Raw corn starch	71.4 (9) (c)	81.7 (9) (ab)
Cooked corn starch	64.2 (10) (c)	73.5 (10) (c)
Raw wheat starch	65.1 (10) (bc)	74.6 (10) (c)
Cooked wheat starch	64.5 (10) (c)	72.6 (10) (c)
Standard error[d]	2.1	2.2

a Values are means. Number of observations per treatment are given in parentheses.
b Numbers in any column followed by the same letter in parentheses are not statistically significant ($p \leqslant 0.05$).
c Percentage of iron retained ten days after oral administration.
d Pooled standard error based on error mean square of analysis of variance.

TABLE 6

BODY WEIGHT GAINS OF IRON-DEPLETED RATS DOSED WITH A MEAL CONTAINING VARIOUS CARBOHYDRATES[a,b]

Treatment	Weight gain (g/day)
Glucose	3.2 (6) (a)
Fructose	3.7 (8) (b)
Fructose-Cx[c]	3.6 (8) (b)
Lactose	3.8 (10) (b)
Standard error[d]	0.2

a Values are means. Number of observations per treatment are given in parentheses.
b Values followed by the same letter in parentheses are not significantly different ($p \leqslant 0.05$).
c Rats dosed with a ^{59}Fe-fructose complex.
d Pooled standard error, based on error mean square from analysis of variance.

labelled with ^{59}Fe and those fed a fructose meal labelled with a ^{59}Fe complex, exhibited a significantly higher per cent absorption than rats fed a glucose meal labelled with ^{59}Fe (Table 8). Rats fed a lactose meal had a significantly higher absorption and retention of ^{59}Fe than rats fed a glucose meal ($p \leqslant 0.05$).

TABLE 7

HEMOGLOBIN VALUES OF RATS DOSED WITH A MEAL CONTAINING DIFFERENT CARBOHYDRATES[a,b]

Treatment	Hemoglobin (g/100 ml)	
	Predosing	Terminal
Glucose	6.0 (6) (a)	8.0 (6) (a)
Fructose	5.9 (8) (a)	8.0 (8) (a)
Fructose-Cx[c]	6.5 (9) (a)	8.0 (8) (a)
Lactose	6.1 (10) (a)	8.0 (10) (a)
Standard error[d]	0.3	0.2

a Values are means. Number of observations are given in parentheses.
b Numbers in any column followed by the same letter in parentheses are not significantly different ($p \leqslant 0.05$).
c Rats dosed with a ^{59}Fe-fructose complex.
d Pooled standard error, based on error mean square from analysis of variance.

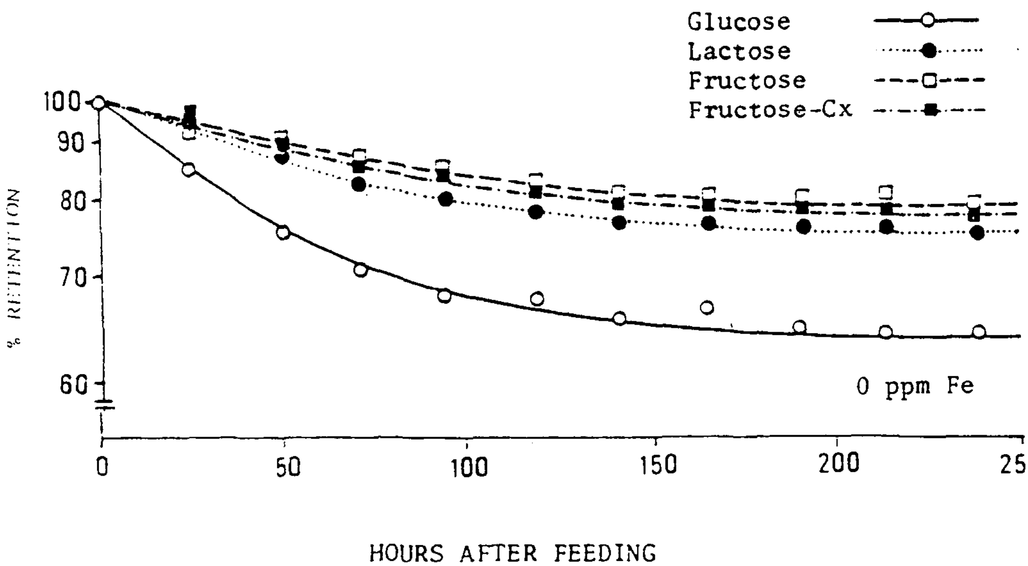

FIGURE 2

Effect of carbohydrates on ^{59}Fe retention by iron-deficient rats (Experiment 2)

TABLE 8

EFFECT OF CARBOHYDRATES ON THE RETENTION AND ABSORPTION OF ^{59}Fe BY IRON-DEFICIENT RATS[a,b]

Treatment	Retention[c] (°/o)	Absorption (°/o)
Glucose	67.4 (6) (ab)	72.9 (6) (a)
Fructose	77.6 (7) (c)	84.8 (7) (b)
Fructose-Cx[d]	76.7 (8) (c)	83.4 (8) (bc)
Lactose	74.0 (10) (ac)	81.8 (10) (bc)
Standard error[e]	2.4	2.7

a Values are means. Number of observations per treatment are given in parentheses.
b Numbers followed by the same letter in parentheses are not significantly different ($p \leq 0.05$).
c Per cent retention after 10 days of oral administration of ^{59}Fe.
d Rats dosed with a ^{59}Fe-fructose complex.
e Pooled standard error, based on error mean square from analysis of variance.

DISCUSSION

In Experiment 1, a reduction in per cent absorption and per cent retention of ^{59}Fe was observed in rats fed a meal containing the starches at a 60°/o level when compared to those fed a 60°/o glucose diet. This depression was statistically significant ($p \leq 0.05$) for cooked corn starch, raw wheat starch and cooked wheat starch.

Cooking of the starches seemed to depress further the iron absorption and retention, but differences were not significant when compared to raw starches.

Corn starch has been reported by some researchers to be a negative factor in iron absorption (23,24). Nevertheless, Miller and Landes (25) informed a positive effect of corn starch on iron absorption by rats. Binding of iron to modified corn starch has been demonstrated *in vitro* (17), and this may be the mechanism by which it reduces iron availability *in vivo*. The additional depression in iron retention and absorption observed with cooked corn and cooked wheat starch –but not present with potato starch– is possibly due to differences in the structure of the granules. Since the amylose/amylopectin ratio of the three starches assayed is similar, the difference cannot be attributed to this factor (26).

Cooking, however, produces a greater absorption surface and therefore, a higher capacity to adsorb cations.

The additional depression in iron retention and absorption when cooked starch is fed to iron-deficient rats deserves further investigation.

The effects of starches on iron absorption is quite relevant to human nutrition. In developing countries, diets are generally high in cereal products, often whole grain cereals, and low in animal products. The inhibitory effects of cereals on iron absorption have been traditionally attributed to the presence of phytates (10) and fiber (10,13,27) but the data herein presented suggest that high intakes of starch may be inhibitory as well.

The enhancing effect that fructose had on iron absorption, as found in our experiments, is in accordance with some literature reports (24,28, 29).

Even though the formation of an ^{59}Fe-fructose chelate *in vitro* was evident in our experiments, we cannot draw any conclussions as to whether this is the mechanism by which fructose increases iron absorption as has been proposed (28). Other mechanisms such as enhancement of iron absorption by fructose metabolites, pyruvate and lactate have been suggested (29), and it may well be that both mechanisms play a role. The inability of the ^{59}Fe-fructose complex to further increase iron absorption when compared to ^{59}Fe-fructose does not rule out the possibility of the chelate formation as mechanism to facilitate iron absorption. It may well be that an iron-fructose complex is formed in the stomach, and this could lead to similar absorption measurements with both ^{59}Fe-fructose complex labelling and ^{59}Fe labelling of fructose meals.

In comparison to rats dosed with a glucose meal, a significant increase in iron retention was observed for rats fed with a lactose meal. There was a significant increase in absorption but the increase in retention was not significant.

The enhancing effect of lactose on calcium absorption has been widely reported (30,31), and there is some evidence that lactose increases zinc absorption (32). Amine and Hegsted (24), for example, reported an increased iron retention when iron-deficient rats were fed a 60°/o lactose diet. The iron retention values notified by Amine and Hegsted were lower that those found in our study, but the tendency of lactose to increase iron absorption is evident in both their experiments, and ours.

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RESUMEN

EFECTO DE ALGUNOS CARBOHIDRATOS EN LA ABSORCION DE HIERRO

Se llevaron a cabo dos experimentos, con el propósito de examinar el efecto de varios carbohidratos (fructosa, lactosa, almidón de maíz, almidón de papa y almidón de trigo) en la utilización del hierro, por ratas deficientes en este elemento.

Las ratas recibieron una sola comida marcada con ^{59}Fe que contenía el carbohidrato a ensayar a un nivel de 60°/o. En el resto del experimento las ratas fueron alimentadas *ad libitum* con una dieta que contenía glucosa como fuente de carbohidratos al nivel de 60°/o. En ambos ensayos las ratas no recibieron alimento durante

la noche anterior, y la dosis se ofreció como una comida, en la mañana.

Para determinar el hierro 59, las ratas se contaron en un "contador de cuerpo entero" entre dos y cuatro horas después de la dosis, y cada día durante los 10 días siguientes. Se determinaron el porcentaje de retención y el porcentaje de absorción, así como los valores de hemoglobina.

En el primer experimento, el reemplazo de glucosa por fructosa al nivel de 60°/o aumentó significativamente la retención y la absorción del hierro. Con la lactosa también se notó un aumento de estos parámetros, pero dicho incremento no fue estadísticamente significativo.

La administración de ^{59}Fe como un quelato ^{59}Fe-fructosa no tuvo efecto significativo en la retención y absorción del hierro, comparada con el efecto resultante de dosificar con el ^{59}Fe absorbido a la fructosa. Este hallazgo no descarta la posibilidad de que la quelación sea el mecanismo responsable del efecto promotor de la fructosa en la absorción del hierro. El complejo puede haberse formado en el estómago, dando como resultado una absorción similar para la comida marcada con ^{59}Fe, y la marcada con el complejo ^{59}Fe-fructosa.

En el segundo experimento, la administración de una comida marcada con ^{59}Fe que contenía cualquiera de los almidones al nivel de 60°/o, se tradujo en una reducción en la retención y en la absorción de ^{59}Fe. El efecto, sin embargo, fue estadísticamente significativo sólo para el almidón de maíz cocinado, el almidón de trigo crudo, y el almidón de trigo cocinado. El efecto de la cocción en reducir el porcentaje de retención y el porcentaje de absorción fue estadísticamente significativo para el almidón de maíz.

El efecto inhibidor de los almidones en la absorción de hierro, es importante en la nutrición humana. En los países en vías de desarrollo, las dietas por lo general son ricas en cereales y pobres en productos animales. Los efectos inhibitorios de los cereales han sido atribuidos tradicionalmente a la presencia de fitatos y fibra, pero los datos aquí presentados sugieren que una alta ingestión de almidones puede contribuir notoriamente a inducir este efecto inhibitorio.

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