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# Influence of Dietary Carbohydrate on Copper, Iron, and Zinc Status of the Rat (39106)

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Carbohydrates make up the major portion of normal diets and vary in form from simple compounds such as glucose and fructose to complex molecules of starch and cellulose. The role of dietary carbohydrate in trace mineral absorption and utilization has only recently begun to receive attention, with major emphasis on iron metabolism. Several investigators have reported that certain monosaccharides, notably fructose, form chelates with iron and that these complexes may be readily absorbed through the intestinal mucosa (1–3). Similar chelate complexes involving other trace minerals may also occur in the diet. Amine and Hegstad (4) fed several dietary carbohydrates to iron-deficient rats and found decreasing iron retention in animals receiving diets containing lactose plus starch, sucrose, glucose, and starch. This observation and others dealing mainly with anemia in humans and animals consuming large quantities of starch (5, 6) indicate that dietary carbohydrate influences absorption of iron. Studies discussed in this report were conducted to investigate the effects of dietary carbohydrate on distribution of copper, iron, and zinc in tissues of rats.

*Materials and methods.* Animals used in these studies were weanling Sprague–Dawley male rats obtained from Charles River Breeding Laboratories.

In the first experiment animals received diets containing (in percent): casein, 14.3; corn oil, 5.0; cellulose, 1.5; mineral mix, 4.0; vitamin mix, 2.2 (ICN Pharmaceuticals, Inc., Cleveland, Ohio, Salt Mix P.H. and Vitamin Diet Fortification Mixture); l-methionine, 0.2; l-arginine, 0.1; and carbohydrate to equal 100%. The four carbohydrates fed were corn starch, sucrose, glucose, and fructose. In the second experiment diets were similar except that the salt mix used was William and Briggs-Modified (General Biochemicals, Chagrin Falls, Ohio), and it was added at 3.5% of the diet. The salt mix used in the first experiment provided in excess of four times the rat's requirement for iron and just met its need for copper, while that used in the second experiment supplied adequate amounts of both of these minerals, but an excess of neither. Copper and iron contents of the diets obtained by chemical analysis are given in Table I.

For the two experiments, groups of 15 animals were housed individually in stainless steel cages and were fed diets containing one of the carbohydrate sources. Food and deionized water were supplied *ad libitum* throughout the 42-day experimental period. Food consumption and weight gain were determined at regular intervals.

The animals were exsanguinated under ether anesthesia. Hemoglobin concentration (7), packed cell volume using heparinized microtubes, and red blood cell counts in a hemocytometer were determined on fresh blood; and serum was obtained from clotted blood by refrigerated centrifugation. Copper was determined in samples of serum wet-ashed with nitric, sulfuric, and perchloric acids using bis-(1-piperdylthiocarbonyl) disulfide as the color reagent (8). Ceruloplasmin (Cp) was determined on serum samples by the method of Sunderman and Nomoto (9). Serum iron and total iron binding capacity were determined by the method of Goodwin *et al.* (10) using Ferrozine (11) as the color reagent.

Livers, hearts, and spleens were removed for separate analyses, and the spleens and portions of the livers were wet-ashed in nitric, sulfuric, and perchloric acids. The carcasses and remaining organs were washed to remove adhering blood and intestinal contents, and analyzed as one sample after disintegration in hot nitric acid and separation of fat. Aliquots of carcass samples were then wet-ashed with sulfuric and perchloric acids. These wet-ashed samples were used for deter-

TABLE I. COPPER AND IRON CONTENT OF DIETS[a]

| Mineral | Dietary carbohydrate | | | |
|---|---|---|---|---|
| | Starch | Sucrose | Glucose | Fructose |
| Experiment 1 | | | | |
| Copper (mg/kg) | 5.15 | 6.33 | 5.06 | 5.40 |
| Iron (mg/kg) | 191 | 198 | 187 | 191 |
| Experiment 2 | | | | |
| Copper (mg/kg) | 7.10 | 7.32 | 7.46 | 7.68 |
| Iron (mg/kg) | 41.0 | 37.5 | 37.1 | 40.9 |

[a] Values were determined by chemical analysis.

mination of iron with orthophenanthroline as the color reagent, of copper as described above, and of zinc by atomic absorption spectroscopy.

The data were subjected to analysis of variance (12), and Duncan's multiple range test (13) was applied where appropriate.

*Results and discussion.* The animals quickly adapted to the experimental diets with no adverse effects, but those receiving fructose and glucose diets had a lower feed efficiency and were smaller than those receiving the other diets in both experiments (Table II). This phenomenon may have been due to a reduced appetite caused by rapid absorption of dietary monosaccharide.

There was marked liver enlargement in the animals receiving the fructose diet. These animals along with those on the sucrose diet had elevated levels of liver lipid compared to animals receiving starch and glucose diets (unpublished data), and this accounted for some of the liver enlargement. Also, fructose is a gluconeogenic sugar (14), and there may have been elevated deposition of glycogen in livers of the fructose-fed animals.

Gross hematological parameters of the animals in these experiments were not altered by dietary treatments and were essentially the same in both studies. Hemoglobin concentration averaged 15.5 g per 100 ml of blood and packed cell volume was 52% of the whole blood.

Serum copper was markedly affected by dietary carbohydrate in the first experiment (Table III). Starch-fed animals had the highest level and glucose-fed animals the lowest with sucrose- and fructose-fed animals having intermediate levels. This pattern of response was repeated in the second experiment although the differences were not as great. Serum ceruloplasmin, a copper-containing protein in serum that functions in mobilization and utilization of storage iron (15), closely paralleled serum copper content in both experiments. Even though the level of

TABLE II. FEED EFFICIENCY, FINAL WEIGHT, AND LIVER WEIGHT AS PERCENT BODY WEIGHT OF RATS FED DIFFERENT CARBOHYDRATES

| Dietary carbohydrate | Feed efficiency[a,b] | Final weight (g) | Liver weight (as % body weight) |
|---|---|---|---|
| Experiment 1 | | | |
| Starch | 0.349[a] | 368[a] | 4.24[c] |
| Sucrose | 0.359[a] | 345[ab] | 4.63[b] |
| Glucose | 0.336[b] | 328[b] | 4.07[c] |
| Fructose | 0.327[b] | 313[b] | 5.23[a] |
| Experiment 2 | | | |
| Starch | 0.352[ab] | 389[a] | 4.18[c] |
| Sucrose | 0.356[a] | 381[ab] | 4.54[b] |
| Glucose | 0.347[ab] | 364[b] | 4.05[c] |
| Fructose | 0.340[b] | 336[c] | 5.21[a] |

[a] Feed efficiency is expressed as g gain/g feed intake.

[b] Values in a column followed by the same superscript are not different at the 5% level of probability according to Duncan's multiple range test.

TABLE III. SERUM COPPER, CERULOPLASMIN, IRON, AND TOTAL IRON BINDING CAPACITY OF RATS FED DIFFERENT CARBOHYDRATES[a]

| Dietary carbohydrate | Copper (μg/100 ml) | Cp (mg/100 ml) | Iron (μg/100 ml) | (TIBC) μg/100 ml |
|---|---|---|---|---|
| Experiment 1 | | | | |
| Starch | 113[a] | 57.3[a] | 330 | 688[bc] |
| Sucrose | 73[b] | 30.4[b] | 318 | 744[ab] |
| Glucose | 43[c] | 15.3[c] | 329 | 647[c] |
| Fructose | 67[bc] | 28.4[bc] | 297 | 798[a] |
| Experiment 2 | | | | |
| Starch | 121[a] | 63.3[ab] | 308 | 668 |
| Sucrose | 115[ab] | 61.3[ab] | 348 | 706 |
| Glucose | 105[b] | 54.8[b] | 334 | 696 |
| Fructose | 121[a] | 65.1[a] | 335 | 712 |

[a] Values in a column followed by the same superscript are not different at the 5% level of probability according to Duncan's multiple range test.

TABLE IV. CARCASS AND LIVER COPPER IRON, AND ZINC AND SPLEEN IRON CONTENT OF RATS FED DIFFERENT CARBOHYDRATES[a]

| Dietary carbohydrate | Copper | | | | Iron | | | | | | Zinc | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Liver | | Carcass | | Liver | | Carcass | | Spleen | | Liver | | Carcass | |
| | (μg) | (μg/g) | (μg) | (μg/g) | (mg) | (μg/g) | (mg) | (μg/g) | (μg) | (μg/g) | (μg) | (μg/g) | (mg) | (μg/g) |
| Experiment 1 | | | | | | | | | | | | | | |
| Starch | $93^{a}$ | $5.94^{a}$ | $470^{a}$ | $1.40^{a}$ | 1.46 | $94^{b}$ | 4.36 | 13.3 | $227^{a}$ | 325 | $385^{a}$ | $24.6^{a}$ | 7.55 | $22.4^{b}$ |
| Sucrose | $83^{ab}$ | $5.21^{b}$ | $327^{b}$ | $1.05^{b}$ | 1.63 | $101^{b}$ | 4.21 | 13.6 | $200^{ab}$ | 272 | $330^{b}$ | $20.6^{c}$ | 6.84 | $22.0^{b}$ |
| Glucose | $63^{c}$ | $4.73^{b}$ | $279^{c}$ | $0.94^{b}$ | 1.98 | $147^{a}$ | 4.02 | 13.6 | $171^{b}$ | 277 | $304^{b}$ | $22.9^{b}$ | 6.55 | $22.1^{b}$ |
| Fructose | $81^{b}$ | $4.95^{b}$ | $288^{bc}$ | $1.04^{b}$ | 1.72 | $106^{b}$ | 4.01 | 14.5 | $173^{b}$ | 263 | $329^{b}$ | $20.2^{c}$ | 7.15 | $26.0^{a}$ |
| Experiment 2 | | | | | | | | | | | | | | |
| Starch | $70^{a}$ | 4.28 | 413 | 1.18 | $0.84^{a}$ | $52^{a}$ | $4.06^{a}$ | 11.5 | $106^{a}$ | 141 | 223 | 13.7 | 11.77 | 33.6 |
| Sucrose | $71^{a}$ | 4.14 | 387 | 1.11 | $0.80^{a}$ | $46^{ab}$ | $3.84^{a}$ | 11.0 | $95^{ab}$ | 119 | 211 | 12.3 | 12.59 | 36.1 |
| Glucose | $60^{b}$ | 4.02 | 379 | 1.13 | $0.62^{b}$ | $42^{b}$ | $3.73^{ab}$ | 11.1 | $82^{b}$ | 109 | 193 | 13.0 | 11.84 | 34.9 |
| Fructose | $70^{a}$ | 4.06 | 381 | 1.26 | $0.75^{a}$ | $43^{b}$ | $3.47^{b}$ | 11.5 | $84^{b}$ | 115 | 213 | 12.2 | 11.45 | 37.8 |

[a] Values in a column followed by the same superscript are not different at the 5% level of probability according to Duncan's multiple range test.

this protein was low in the glucoxe-fed animals of the first experiment, adequate hemoglobin levels were observed. Serum iron level was not affected by dietary carbohydrate, but level of iron in serum of the animals from the first experiment was no greater than that of the second experiment even though the diets used contained an excess of iron. Serum total iron binding capacity was greater in animals receiving fructose and sucrose diets in both experiments with the difference being significant in the first experiment.

In the first experiment both copper content and concentration in liver and carcass were highest in starch-fed animals and lowest in the glucose-fed group, with sucrose- and fructose-fed animals being intermediate (Table IV). It should be noted here that animals receiving sucrose and fructose diets exhibited hepatomegaly (Table II); therefore concentrations of minerals in the liver were diluted to some extent. In the second experiment concentration of copper in these tissues was not affected significantly, but total copper in the liver of glucose-fed animals was lower than the three other groups.

In the first experiment where there was an abundance of dietary iron, the concentration of iron in livers of the animals was significantly affected by dietary carbohydrate. Animals receiving the glucose diet had the highest concentration of iron and those on the starch diet had the lowest. However, in the second experiment where the level of iron in the diet more closely matched the requirement of the rat, liver iron concentration was highest in animals receiving the starch diet and lowest in those receiving the glucose diet.

Iron content of the carcass was not affected by dietary carbohydrate in the first experiment, but total carcass iron was significantly lower in the fructose-fed animals in the second experiment. This appeared to be due to reduced size of the animals. Total iron in spleens of animals from both experiments was affected by dietary carbohydrate in a similar manner, with the highest level in the animals receiving starch and the lowest level in the animals receiving glucose and fructose diets.

In both experiments starch fed-animals accumulated more zinc in their livers than the other animals, with the accumulation being significant in the first experiment. Carcass zinc concentration was highest in fructose-fed animals in both experiments, with this accumulation being significantly higher in the first experiment.

Sourkes *et al.* (16) have shown an inverse relationship in hepatic copper and iron concentrations in rats fed diets deficient in copper or iron for 8 weeks or longer. This is supported to some extent by this investigation although the animals were fed for only 6 weeks. From *in vitro* studies using sacs of everted small intestine from rats, Schwarz and Kirchgessner (17) concluded that absorption of copper was reduced by high con-

centrations of glucose. The inverse relationship between copper and iron status of starch- and glucose-fed animals of the first experiment of this investigation may indicate that reduced absorption of copper observed in animals fed glucose was exaggerated by antagonism between copper and excessive iron at the site of absorption.

*Summary.* Rats were fed diets containing starch, sucrose, glucose, or fructose as the carbohydrate source, and the influence of these carbohydrates on copper, iron, and zinc status was determined. It was found that copper absorption was reduced in animals receiving glucose. This reduction was exaggerated when a high level of iron was present in the diet, indicating a possible antagonism between iron and copper at the site of absorption. Iron and zinc status of the animals also appeared to be influenced to some extent by dietary carbohydrate.

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