The Effect of Phytic Acid on the Levels of Blood Glucose and Some Enzymes of Carbohydrate and Lipid Metabolism

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ABSTRACT

In this study, six groups of rats were fed as follows: Groups 1 and 2 were fed formulated diets supplemented with zinc or without zinc respectively. Groups 3 and 4 were fed formulated diets supplemented with zinc plus phytic acid extracted from sweet potato (Ipomea batatas) or commercial phytic acid respectively. Groups 5 and 6 were fed formulated diets supplemented with phytic acid extract from sweet potato or commercial phytic acid respectively. The animals were fed for three weeks and then sacrificed. The activities of key enzymes of carbohydrate and lipid metabolism as well as transaminases in the liver were determined. Blood glucose level was also assessed. Phytic acid extract consumption from sweet potato and commercial phytic acid plus zinc supplement lowered blood glucose levels. There was no significant change in the activity of 6-phosphogluconate dehydrogenase among the groups. Similarly, phytic acid supplementation showed no significant decrease in the activity of pyruvate kinase compared to the group fed formulated diets. There was a significant increase in the activity of glucose-6-phosphate dehydrogenase in the groups fed phytic extract from sweet potato compared to the other groups. There was a lowering of blood glucose levels which is desirable for diabetics who consume sweet potato diets. The changes in some of the hepatic metabolic enzymes are geared towards compensating for the decreased glycolytic responses.

El Efecto del Ácido Fítico sobre los Niveles de la Glucosa en Sangre y sobre Algunas Enzimas del Metabolismo de Carbohidratos y Lípidos

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RESUMEN

En este estudio, se alimentaron seis grupos de ratas de la forma que a continuación se describe. Los grupos 1 y 2 fueron alimentados con dietas formuladas con o sin suplemento de zinc respectivamente. Los grupos 3 y 4 fueron alimentados con dietas formuladas con suplemento de zinc más ácido fítico extraído del boniato (Ipomea batatas) o el ácido fítico comercial respectivamente. Los grupos 5 y 6 fueron alimentados con dietas formuladas con suplemento de extracto de ácido fítico del boniato o ácido fítico comercial respectivamente. Los animales fueron alimentados durante tres semanas y luego sacrificados. Se determinó la actividad de las enzimas claves del metabolismo de carbohidratos y lípidos, así como las transaminasas en el hígado. Asimismo se evaluó el nivel de glucosa en sangre. El consumo de extracto de ácido fítico del boniato y el ácido fítico comercial más el suplemento de zinc, disminuyeron los niveles de glucosa en sangre. No hubo cambios significativos en la actividad de la 6-fosfogluconato deshidrogenasa entre los grupos. De modo similar, la suplementación con ácido fítico no mostró una disminución significativa de la actividad de la piruvato quinasa en comparación con el grupo alimentado con dietas formuladas. Sin embargo, hubo un aumento significativo en la actividad de la glucosa-6-fosfato deshidrogenasa en los grupos alimentados con extracto fítico de boniato en comparación con el otros grupos. No hubo alteración significativa de las actividades de la enzima malática y la ATP-citratosa en este estudio. Hay una disminución de los niveles de glucosa en sangre,
INTRODUCTION
Phytic acid is a major phosphorus compound in plant seeds and is also found in significant quantities in roots and tubers (1). Phytic acid binds to essential minerals, thus rendering them unavailable for intestinal uptake and unable to participate in essential metabolic processes in the body. The ratio of phytic acid to minerals present in foods may serve as an indication of the availability of the minerals in question. For example, a high phytic acid to zinc molar ratio (> 15:1) indicates low mineral availability from that food. Preliminary study in our laboratory shows that sweet potato has high phytic acid to zinc molar ratio even after cooking. Sweet potato is consumed on a wide scale in the tropics and in the subtropical regions (2).

The physiological effects of phytic acid are applicable to human and animal nutrition and metabolism, as they have been reported to regulate the process of digestion by binding to some digestion products which may in turn delay the onset of diabetes and hyperlipidaemia (3, 4). Carbohydrate foods are digested and absorbed at different rates. Some liberate their products of digestion rapidly in the upper part of the gastrointestinal tract, while others release their breakdown products slowly as they travel through the length of the small intestine (5). This phenomenon is therefore thought to affect the activity of enzymes of carbohydrate and lipid metabolism. The presence of phytic acid and other antinutrients in the diet may result in carbohydrates being slowly digested. When this occurs, some may remain unabsorbed in the colon, and then become fermented by bacterial microflora to short chain fatty acids. These short chain fatty acids are important for maintaining colonic health and for carbohydrate and lipid metabolism (4).

Studies by Hallberg (6) showed that phytic acid rather than dietary fibre is mostly responsible for the reduced availability of minerals in man. Evidence has been presented showing that phytic acid causes a decrease in calcium and zinc balance in rats and humans (7). Phytic acid has also been reported to interfere with iron absorption in man and forms complexes of varying composition with proteins (8, 4). However, the effect of phytic acid on hepatic carbohydrate and lipid metabolism is not known. The objective of this study was to investigate the effect of consumption of phytic acid extract from sweet potato on carbohydrate and lipid metabolism in the liver.

MATERIALS AND METHODS
Fresh mature sweet potato (Ipomea batatas) tubers were harvested from a farm in the parish of Manchester, Jamaica. Tuber samples were washed with distilled water to avoid introducing minerals from external sources, peeled, sliced, oven dried at 65°C to constant weight and ground to a fine powder.

The experimental animals were 36 Wistar rats (18 males and 18 females) aged four weeks. They were assigned by weight into six groups of six rats each, with mean body weights 236.4g. Diets were prepared according to standard methods of diet preparations [Purified Rodent diet was obtained from PMI Feeds Inc Lab Diet #5001, vitamin mix was from Tuco Products Co, Orangeville, Ontario, Canada, and mineral mix was prepared according to the American Institute of Nutrition guidelines (AIN-76A), (9, 10)]. Commercial phytic acid was purchased from Sigma-Aldrich, St Louis, Mo, USA.

Phytic acid was extracted from sweet potato by the method of Samotus and Schwimmer (11). A known amount of sweet potato was blended with a know volume of 10% trichloroacetic acid in a Waring Blender for five minutes. The slurry was filtered by suction in a sintered glass funnel. The residue was then washed successively with 5% trichloroacetic acid. Filtrates were combined, neutralized with 5M NaOH and freeze-dried. The dried extract was used for the preparation of phytate extract supplemented diets.

The groups were fed diets as follows: Group 1 was fed formulated diet supplemented with zinc; Group 2 was fed formulated diet without zinc supplementation; Group 3 was fed formulated diet plus zinc supplement, along with phytic acid extract from sweet potato; Group 4 was fed formulated diet plus zinc supplement, along with commercial phytic acid; Group 5 was fed formulated diet supplemented with phytic acid extract from sweet potato; Group 6 was fed formulated diet supplemented with commercial phytic acid. Note: Groups 1 and 2 were control groups. Rats were housed in stainless steel cages in a room kept on a 12-hour light-dark cycle and were allowed access to their respective diets and water ad libitum. The cages were cleaned daily. Prior to the start of the experiment, all the animals were fed with standard rat chow for one week to allow for acclimatization to the new diet. The rats were then fed their respective diets for three weeks. Changes in body weight and total food intakes were recorded weekly. At the end of the experiment, the rats were sacrificed by a blow to the head. Approval for the study was obtained after the presentation of the protocol to the board of the Department of Basic Medical Sciences.

Blood samples were obtained in oxalate/fluoride containers for glucose determination as described by Teller (12). The activities of liver transaminases were determined according to the method of Reitman and Frankel (13) as...
reported by Bergmeyer and Erlich (14). The activities of the enzymes of lipid and carbohydrate metabolism were measured using the method of Storey and Bailey (15).

Statistical analyses
Results were expressed as mean ± SEM. Analysis of variance (ANOVA) was used to test for differences among the groups. Post hoc analysis was carried out using the Duncan’s Multiple Range test to test for significant difference among the means (p < 0.05) (16). All statistical analyses were done using the statistical programme SPSS version 10.1.

RESULTS
Table 1 shows body weight changes and food intake of the six groups of rats fed control diets as well as phytic acid supplemented diets. Final body weight was significantly reduced in rats fed phytate extract without zinc (Group 5) compared to the groups fed control diets (Groups 1, 2) or commercial phytate plus zinc (Group 4) even though daily food intakes among the groups were not significantly different.

Table 1: Body weight changes and food intake of rats fed control diets as well as phytic acid supplemented diets

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Initial body weight</th>
<th>Final body weight</th>
<th>Daily food intake/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulated diet – Zn (1)</td>
<td>236.8 ± 16.5</td>
<td>256.3 ± 23.9</td>
<td>12.1 ± 1.1</td>
</tr>
<tr>
<td>Formulated diet + Zn (2)</td>
<td>236.5 ± 14.5</td>
<td>260.9 ± 19.3</td>
<td>11.9 ± 0.8</td>
</tr>
<tr>
<td>Formulated diet + Zn + sweet potato phytate extract (3)</td>
<td>236.3 ± 14.9</td>
<td>225.6 ± 9.3’</td>
<td>11.2 ± 1.9</td>
</tr>
<tr>
<td>Formulated diet + Zn + commercial phytate (4)</td>
<td>236.5 ± 21.2</td>
<td>250.0 ± 21.1</td>
<td>11.4 ± 0.8</td>
</tr>
<tr>
<td>Formulated diet – Zn + sweet potato phytate extract (5)</td>
<td>236.0 ± 15.7</td>
<td>202.5 ± 8.9’</td>
<td>10.1 ± 0.4</td>
</tr>
<tr>
<td>Formulated diet – Zn+ Commercial phytate (6)</td>
<td>236.7 ± 16.5</td>
<td>241.4 ± 17.1’</td>
<td>13.4 ± 0.8</td>
</tr>
</tbody>
</table>

Means ± SEM, n = 6 in each group
Final body weight: Vertical’ denotes significant differences (p < 0.05) from groups 1, 2 and 4 (Duncan’s Multiple Range Test).

The figure shows blood glucose levels at the end of the experiment for rats fed phytic acid extract or other diets. There were no significant changes among the groups. However, the groups fed phytic acid extract (Groups 3, 5) or commercial phytic acid (Groups 4, 6) displayed lower blood glucose levels than the controls (Groups 1, 2).

Table 2 shows activities of some enzymes of carbohydrate and lipid metabolism in the liver of rats fed phytic acid supplemented diets and other diets. There was no significant difference in the activity of ATP citrate lyase among the groups. Malic acid activity was significantly increased in the group fed phytic acid extract without zinc (Group 5) compared to the group fed commercial phytic acid plus zinc (Group 4). The activity of 6-phosphogluconate dehydrogenase did not vary significantly among the groups. However, the activity of NADP⁺ isocitrate dehydrogenase was significantly increased in the group fed formulated diet plus zinc (Group 2) compared to the group fed the same diet but without zinc (Group 1). The activity of NADP⁺ isocitrate dehydrogenase was not significantly altered in the groups fed phytic acid extract or commercial phytic acid without zinc (Groups 5 and 6) compared to the group fed formulated diet without zinc (Group 1). However, the activity of this enzyme in Groups 5 and 6 was significantly reduced when compared to the group fed formulated diet plus zinc (Group 2). Pyruvate kinase activity was significantly reduced in the group fed phytic acid extract without zinc (Group 5) compared to the control (Group 1). The activity of glucose-6-phosphate dehydrogenase was significantly increased in the group fed phytic acid extract without zinc (Group 5) compared to the other groups. Similarly, the activity of this enzyme in the group fed phytic acid extract plus zinc (Group 3) was significantly higher than the groups fed commercial phytic acid plus zinc or formulated diet without zinc (Groups 4 and 1).
DISCUSSION

Although no significant difference was observed in the blood glucose levels between the groups (Fig.), the results suggest that animals fed phytic acid supplemented diets had a tendency towards lower blood glucose levels than groups without phytic acid supplementation. Of the four phytic acid fed groups (3–6), blood glucose levels were lowest in the two groups that had zinc supplementation (3 and 4). This suggests that adequate supplementation of the diet with zinc is necessary in order for phytic acid to be truly effective in lowering blood glucose.

One of the regulatory reactions of glycolysis is catalyzed by pyruvate kinase while key reactions of pentose phosphate pathway are catalyzed by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (15, 17). Data from this study did not show any significant changes in the activity of 6-phosphogluconate dehydrogenase among the groups. However, the significant increase in the activity of glucose-6-phosphate dehydrogenase in the group fed phytic extract from sweet potato is an indication of specific activation of this enzyme by the metabolic products resulting from the acute consumption of this supplement. This would suggest a non-involvement of the hepatic glycolytic pathway in the hypoglycaemic property observed in rats fed phytic acid supplements.

Malic enzyme generates NADPH for reductive biosynthesis and ATP-citrate lyase produces acetyl-CoA for the synthesis of steroids and fatty acids (18). The non-significant change in the activities of these enzymes in this study among the groups shows that there is no heightened demand for NADPH by the liver except for the group fed phytic acid extract compared to the commercial phytic acid plus zinc fed group. The observed significant increases in the activities of glucose-6-phosphate dehydrogenase and malic enzymes in the group fed phytic extract from sweet potato may be geared towards biosynthetic reactions to compensate for the decreased glycolytic responses (19, 20).

The lowering of blood glucose levels observed in this study is desirable for diabetics who consume sweet potato diets.

REFERENCES

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