Concomitant Repression by Gluten of HMP Shunt Dehydrogenases, Citrate Cleavage Enzyme and Malic Enzyme in Rat Liver and Adipose Tissue

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ABSTRACT On the basis of previous results which indicate that a gluten diet represses hexose monophosphate shunt dehydrogenases (HMPD) in the rat liver, the activities of glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), citrate cleavage enzyme (CCE) and malic enzyme (ME), as well as plasma insulin levels, were examined in the liver and epididymal fat pad of male rats fed diets containing as their nitrogenous component, either casein, gluten, or gluten supplemented with Lys and Thr after protein deprivation. The gluten diet repressed the activities of HMPD, CCE and ME. The intensity and the nature of the repression was different in liver and in adipose tissue. In the liver the repression of HMPD was greater than that of CCE and ME and in no case was the enzyme repression prevented by addition of Lys and Thr to the gluten diet. In the adipose tissue the repression of CCE and ME was greater than that of HMPD and in all cases the repression was prevented by addition of Lys and Thr. The enzyme responses were independent of any direct effect of the diets on insulin secretion and, as might be inferred from the constant levels of liver glycogen, independent of any effect on other hormones involved in the coordination of various phases of lipid metabolism and in its integration with carbohydrate metabolism. These results are consistent with the view that in the liver the levels of lipogenic enzymes are controlled by different regulatory mechanisms than in the adipose tissue. J. Nutr. 106: 335-341, 1976.

INDEXING KEY WORDS gluten • liver • adipose tissue • HMP shunt dehydrogenases • citrate cleavage enzyme • malic enzyme

It has been known for many years (1) that the enzyme profile of the liver cell can be drastically changed by a great variety of dietary, hormonal, and pharmacologic stimuli (2). Of the NADP-linked liver enzymes, the hexose monophosphate shunt dehydrogenases (HMPD) and malic enzyme (ME) are among those which exhibit the largest variation from one physiologic circumstance to another (2).

The activity of liver glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) are regulated by dietary stimuli since they are elevated in fasting and refeeding (2-8), intermittent starvation and refeeding (6, 9-11), after feeding diets high in glucose (12) or in fructose (12-15). They are also lowered by feeding diets high in fats (16) or in refeeding, after a period of starvation, with diets low in protein (17). The above modifications are accompanied by analogous changes in the various enzymes of lipogenesis (8, 18-20).
In our previous studies of the effect of dietary protein on the response of HMPD we have shown that in rats fed a gluten diet, the HMPD activity did not reach the level found in the rats fed the casein diet. This effect was independent of both the amino acid composition of the gluten and of its essential fatty acids content (21-25).

The above results have led to the hypothesis that this response of HMPD to the gluten diet is independent of the nutritive value of the gluten, but is dependent on the presence in the gluten of unknown repressive factors or of factors different from those already known capable of repressing HMPD activity.

Since the activity of the HMPD is connected with the formation of NADPH which is a storage form of "reductive energy" necessary for the synthesis of fatty acids, steroids, reduced glutathione and acts as a reductive cofactor for a number of hydroxylases, feeding a diet containing gluten as its only protein source might impair the reduction of NADP and therefore affect the rate of several metabolic pathways.

The hypothesis of the possible effects of the dietary gluten on lipogenesis and on steroidogenesis is supported by the fact that modifications of the HMPD activities induced by means of hormones or diet (8, 16-20) were usually accompanied by parallel changes of the activity of lipogenic enzymes.

Therefore, before performing the metabolic experiments of lipogenesis and steroidogenesis, we conducted enzymological tests to ascertain if the gluten diet: 1) repressed not only the activities of HMPD but also the levels of some enzymes of the extra-mitochondrial pathway of fatty acid synthesis such as citrate cleavage enzyme (CCE) and ME in the liver; and 2) had an equal effect on the HMPD, CCE and ME in the adipose tissue.

The second question was raised because the possibility of a different dietary regulation of lipogenic enzymes in the liver and in the adipose tissue has been suggested (15, 17, 26).

Finally, since insulin increases the extra-mitochondrial synthesis of fatty acids and exerts a stimulating effect on the activities of G6PD, 6PGD, ME and CCE (20), plasma insulin levels were also determined in order to ascertain whether the modification of the activities of the four enzymes under examination depends upon the β-cytotropic action of the diet.

**MATERIALS AND METHODS**

Adult male WAG rats (240 to 340 g) were caged from weaning at constant temperature (23°) and had free access to a stock diet. At the beginning of the experiment, the animals were housed individually and fed a protein-free diet for 6 days; subsequently some were fed for 4 days and others for 6 days a balanced diet containing, as its exclusive nitrogen source, casein or wheat gluten with or without 1% L-lysine-HCL and 0.40% L-threonine. In order to equalize the essential fatty acid content of the casein- and gluten diet, the former was supplemented at the expense of starch (w/w) with 1.2% corn oil and 0.019% methyl-linolenate. The composition of the diets has been described in a preceding paper (23). At the end of re-feeding the rats were anaesthetized with sodium pentobarbital and the abdomen was opened in order to extract the blood from the vena cava and to remove the liver and the epididymal pads. Both were homogenized in 0.154 M KCl and the homogenates were centrifuged at 35,000 × g for 1 hour at 2°.

G6PD (EC 1.1.1.49), 6PGD (EC 1.1.1.44), ME (EC 1.1.1.40) and CCE (EC 4.1.3.8) assays were conducted at 25° on the supernatant with the methods of Bottomley (Method II) (27), Ochoa (28) and Cot tam (29) respectively. Liver glycogen and total liver protein were also assayed by methods previously described (23). Plasma insulin was determined using rat insulin as standard. Body, liver and adipose tissue parameters were related to body weight at the end of protein deprivation.

One enzyme unit was defined as the amount of enzyme that reduced or oxidized 1 μmole of NADP or NADH under the experimental conditions. The statistical analysis was performed using the Student's t-test.
TABLE 1

Effect of the dietary protein component on some body, liver and adipose tissue parameters

<table>
<thead>
<tr>
<th>Days of feeding and nature of the protein component</th>
<th>4 day</th>
<th>6 day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein (5)*</td>
<td>Gluten Lys-HCl 1% Thr 0.4% (5)</td>
</tr>
<tr>
<td>Parameter</td>
<td>(5)*</td>
<td>(5)</td>
</tr>
<tr>
<td>Original body wt, g</td>
<td>277 ± 8.1*</td>
<td>295 ± 6.9</td>
</tr>
<tr>
<td>Body wt at end of protein deprivation, g</td>
<td>257 ± 8.5</td>
<td>288 ± 8.9</td>
</tr>
<tr>
<td>Body wt at time of killing, g</td>
<td>275 ± 8.4</td>
<td>283 ± 10.3</td>
</tr>
<tr>
<td>Cumulative post deprivation body wt gain, g/100 g body wt</td>
<td>6.70±1.7</td>
<td>4.80±0.94</td>
</tr>
<tr>
<td>Liver size, g/100 g body wt</td>
<td>3.61±0.12</td>
<td>3.47±0.09</td>
</tr>
<tr>
<td>Liver protein, mg/100 g body wt</td>
<td>679 ± 25.9 AB</td>
<td>622 ± 18.3 A</td>
</tr>
<tr>
<td>Liver glycogen, mg/100 g body wt</td>
<td>277 ± 55.5</td>
<td>307 ± 38.3</td>
</tr>
<tr>
<td>Epipidymal fat pad wt, g/100 g body wt</td>
<td>1.21±0.14</td>
<td>1.04±0.13</td>
</tr>
</tbody>
</table>

* In parenthesis is given the number of rats.  Mean ±st.  Means on the same day not sharing a common superscript letter are significantly different P < 0.001. If different letters are not both capitals the statistical significance is at P < 0.05.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tissue</th>
<th>Casein (5)</th>
<th>Gluten Lys-HCl 1% (5)</th>
<th>Casein (8)</th>
<th>Gluten Lys-HCl 1% (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>G6PD³</td>
<td>Liver</td>
<td>34.5 ± 4.8 A B</td>
<td>9.4 ± 1.6 B</td>
<td>34.7 ±5.0 A</td>
<td>14.7 ± 3.3 B</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>0.85±0.08</td>
<td>0.61±0.09</td>
<td>0.83±0.08 A</td>
<td>0.43±0.03 B</td>
</tr>
<tr>
<td>6PGD³</td>
<td>Liver</td>
<td>23.5 ±1.2</td>
<td>20.2 ±0.84</td>
<td>34.5 ± 4.3 A</td>
<td>20.5 ± 1.9 B</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>0.457±0.06</td>
<td>0.555±0.09</td>
<td>0.81±0.13 A</td>
<td>0.413±0.04 B</td>
</tr>
<tr>
<td>ME³</td>
<td>Liver</td>
<td>19.5 ±2.5</td>
<td>12.5 ±0.83</td>
<td>20.2 ± 1.9 A</td>
<td>12.1 ± 1.6 B</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>0.693±0.16</td>
<td>0.689±0.14</td>
<td>1.08 ± 0.19 a</td>
<td>0.345±0.04 B</td>
</tr>
<tr>
<td>CCE³</td>
<td>Liver</td>
<td>—</td>
<td>—</td>
<td>14.9 ± 1.16 A</td>
<td>9.04 ± 1.04 B</td>
</tr>
<tr>
<td></td>
<td>Adipose</td>
<td>—</td>
<td>—</td>
<td>0.327±0.05 A</td>
<td>0.092±0.01 B</td>
</tr>
</tbody>
</table>

1 The number of rats is given in parenthesis.  ³ Enzyme activity expressed in units where one unit is defined as that amount of enzyme which reduces or oxidizes 1 µmole of NADP or NADH under the experimental conditions. ³ Units/100 g body wt, mean ± se.  ³ Means on the same day with different capital letters are significantly different P < 0.001. ³ If different letters are not both capital the statistical significance is at P < 0.05. ³ This value is the mean ± se of four rats.
TABLE 3

Plasma insulin levels of rats fed diets containing gluten or casein as their protein component

<table>
<thead>
<tr>
<th>Days of feeding and nature of protein component</th>
<th>4 day</th>
<th>6 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (8)</td>
<td>90.5±19.1</td>
<td>90.5±19.1</td>
</tr>
<tr>
<td>Gluten Lys-HCl 1% Thr 0.4% (10)</td>
<td>86.7±13.5</td>
<td>101.4±17.3</td>
</tr>
<tr>
<td>Casein (12)</td>
<td>133.9±19.9</td>
<td>128.1±19.1</td>
</tr>
<tr>
<td>Gluten Lys-HCl 1% Thr 0.4% (10)</td>
<td>108.9±14.8</td>
<td>108.9±14.8</td>
</tr>
</tbody>
</table>

Insulin μU/ml

In parenthesis is given the number of rats. * Mean ±SE.

analysis was performed by an analysis of variance. The significance level of the difference between the means was examined using methods for samples of equal or unequal size, as appropriate (30).

RESULTS

The effects of the three diets (casein, gluten and gluten supplemented with Lys and Thr) upon: a) body, liver and adipose tissue weight; b) liver protein and glycogen content; c) HMPD, CCE and ME activities of the liver and adipose tissue, are presented in tables 1 and 2. In table 3 the levels of plasma insulin are shown. As has previously been found (21-24), rats fed the gluten diet for 6 days had lower body weight gain, smaller liver and less liver protein than rats fed the casein diet. These effects were overcome by adding a sufficient amount of Lys and Thr to the gluten. The weight of the epididymal pads, however, did not change with the different dietary treatments. As has been shown before, feeding the gluten diet for 6 days produced a significant decrease in G6PD and 6PGD activities in the liver compared to feeding the casein diet. This decrease could not be prevented by increasing the nutritive value of the gluten with Lys and Thr (table 2) in contrast to body weight, liver weight and liver protein results. The gluten diet also repressed the HMPD of the adipose tissue, but this repression, unlike that observed in the liver, did not occur when Lys and Thr were added to the gluten diet. This observation has not been reported previously. The gluten diet also repressed the activity of CCE and ME both in the liver and in the adipose tissue and in the liver this repression, like that of the HMPD, could not be prevented by addition of Lys and Thr to the gluten, whereas it could be prevented in the adipose tissue. The response for all enzymes was greater on the sixth day of the experiment than on the fourth day and was quite different in the adipocytes and the hepatocytes. In the adipocytes, by the sixth day, the depression of CCE and ME (70%) was greater than the depression of the HMPD (48%). In the hepatocytes, however, on the fourth day, when ME activity still had not undergone any modification, the activity of the G6PD had decreased by 44% and, on the sixth day, the G6PD activity had decreased by 58% while that of CCE and ME had decreased only 40%. All the above results appeared independent of the plasma insulin levels which did not show any significant difference among the rats fed the three different diets.

DISCUSSION

The effect of the diet on the activity of some lipogenic enzymes is largely dependent on the nature of the dietary protein. The gluten diet was less effective than the casein diet in increasing G6PD, 6PGD, CCE and ME. This different response was independent of any direct influence of the gluten diets on the secretion of insulin or on any of the other hormones which play a role in the coordination of various phases of lipid metabolism and in its integration with carbohydrate metabolism. This conclusion is based on the normality of both the plasma insulin and liver glycogen levels.

A number of experimental results have shown: a) the activity of the CCE and of NADP-dependent enzymes, i.e. HMPD
and ME, is related to the intensity of the fatty acid synthesis in the liver and in the adipose tissue (2, 11, 31); b) under conditions favoring lipogenesis in adipose tissue, when pentose pathway activity is high, only about 60% to 60% of the required reducing equivalents are generated by this pathway (11, 32, 33); c) the depression of the activity of ME (provoked by the gluten) should consequently also depress the source of reducing power represented by the coupling of the malic dehydrogenase and NADP-malic dehydrogenase (8, 16, 31, 33). This would therefore catalyze the reaction oxalacetate → malate → pyruvate and, in the process, oxidize 1 mole of NADH and reduce 1 mole of NADP effecting a transhydrogenation from NADH to NADP. This mechanism could account for the adaptability of malic enzyme in conditions promoting rapid lipogenesis (11).

Although these arguments support the hypothesis that the gluten diet may repress lipogenesis under particular conditions, the lipogenic response of the adipose tissue of the rat and of the liver of the chick may be independent of concomitant modifications of ME, G6PD, and 6PGD (11). This fact still does not explain the nature of the relationship between fluctuations in the rate of lipogenesis and concurrent changes in the activities of the NADP-linked dehydrogenases (2).

In the present work, the addition of adequate amounts of Lys and Thr to the gluten diet prevented the depression of HMPD, CCE and ME in the adipose tissue, but not in the liver. This is another example of the different response of enzymes of liver and adipose tissue to the same stimulus (15, 17, 26) and is consistent with the view that the regulatory mechanisms for these enzymes are different in the liver than in the adipose tissue. Such a difference is not surprising if we consider that adipocytes and hepatocytes, although occupying a central role in lipid metabolism, differ both morphologically and functionally. The adipocytes are cells of mesenchymal derivation belonging to the reticulo-endothelial system and function in all the metabolic processes characteristic of any kind of active tissue. Under nervous and hormonal control these cells are active in lipogenesis, and the condensation, storage and hydrolysis of triglycerides. The hepatocytes are cells of entodermal derivation, differentiated in order to effect a greater variety of metabolic functions. In lipid metabolism they demonstrate a versatility and specialization far greater than that of the adipocytes, functioning in the synthesis and removal of phospholipids and lipoproteins, the degradation of fatty acids, ketogenesis, etc. In the adipocytes, the system synthesizing fatty acids in normal lipogenesis depends principally upon ME for NADPH, while the pentose shunt (which in the adipose tissue is less sensitive to insulin) represents the supplementary source of NADPH acting in conditions of hyperlipogenesis. In the hepatocytes, however, the pentose pathway represents the major supply of NADPH, and the ME represents the supplementary source of NADPH in conditions of enhanced lipogenesis.

Independent of the different responses to the same dietary stimulus by the same enzymes in the liver and in the adipose tissue, the prevention of the gluten-caused depression of the liver of the chick may be independent of concomitant modifications of ME, G6PD, and 6PGD (11). This fact still does not explain the nature of the relationship between fluctuations in the rate of lipogenesis and concurrent changes in the activities of the NADP-linked dehydrogenases (2).

In support of this hypothesis, it has been found that adipose tissue of the rat differs markedly from liver in the response of fatty acid synthesis to a lack of dietary protein (17). Orotic acid enhances activities of the key lipogenic enzymes in adipose tissue, but produces a marked depression in the activities of the hepatic lipogenic enzymes (26), and dietary fructose depresses tissue lipogenesis from fructose and glucose but increases fatty acid synthesis from fructose in the liver (15). In addition, lysine and threonine are two essential amino acids clearly connected to lipid metabolism: the ε-amino nitrogen atom of lysine is the site of attachment of the biotin prosthetic group of several carboxylases such as acetyl-CoA carboxylase; threonine deficiency increases fatty acid synthesis in the liver and in the adipose tissue.

LITERATURE CITED


