THE EFFECT OF A HIGH-PROTEIN DIET ON PLASMA GLUCOSE CONCENTRATION, INSULIN SENSITIVITY AND PLASMA INSULIN IN RATS

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SUMMARY
The administration of a high-protein diet to rats, from the end of lactation until they reached a weight of 150 g., produced significant changes in the concentrations of insulin in the plasma and pancreas. Compared with controls, the animals receiving this diet had higher plasma glucose and insulin concentrations and a higher hormone content in the pancreas.

The levels of hepatic glycogen were similar to those in the control rats, but the muscle glycogen was significantly lower. Adipose tissue in vitro was less sensitive to the action of insulin on glucose uptake.

INTRODUCTION
Rats fed on a high-fat diet (Blázquez & López Quijada, 1968) showed diabetic features: plasma insulin decreased, plasma glucose was higher, and the sensitivity of the tissues to insulin decreased. With a high-carbohydrate diet (Blázquez & López Quijada, 1969), obesity, hyperinsulinism and decreased sensitivity of the tissues to insulin were demonstrated. On the other hand, the different composition of the food alters the enzymic processes which constitute a basic part of the metabolism (Vaugham, Hannon & Vaughan, 1960; Freeland & Harper, 1957; Mokrasch, Davidson & McGilvery, 1956; Rosen, Roberts & Nichol, 1959). Animals fed on diets rich in fat, protein and carbohydrate have enzyme patterns different from animals fed on a control diet.

In the present study, an investigation was made of the effect of a high-protein diet on: (1) plasma insulin compared with insulin extractable from the pancreas; (2) the sensitivity to insulin of adipose tissue; (3) plasma glucose concentration and liver and muscle glycogen.

METHODS
Groups of 25 male rats of the Wistar strain, weighing approximately 60 g., were separated immediately after lactation. Group A, designated as the control group, was fed on a standard diet (protein, 21.4%; carbohydrates, 49.5%; fat, 3.8%). Group B received a high-protein diet (Nutritional Biochemical Corp., Ohio, USA) comprised of vitamin-free casein, 64%; sucrose, 22%; vegetable oil, 8%. Minerals and vitamins were given to both groups in suitable amounts. The rats were allowed...
to eat *ad libitum* until they weighed 150 g. All rats were killed by decapitation. Blood was collected from neck veins into heparinized tubes; plasma was separated immediately and stored at −10°. The composition of the high-fat and high COH diets were: high-fat diet (vegetable oil, 45%; sucrose, 29%; vitamin-free casein, 18%) and high-carbohydrate diet (vegetable oil, 8%; sucrose, 68%; vitamin-free casein, 18%).

**Determination of glucose and glycogen.** Glucose was measured in 0·02 ml. plasma by a glucose oxidase method (Sols & de la Fuente, 1957). Glycogen in liver and diaphragm was measured by the anthrone reaction after alkaline digestion and precipitation by ethanol (Carrol, Longley & Roe, 1956).

**Measurement of plasma and pancreatic insulin.** The radioimmunoassay originally described by Hales & Randle (1963) was used. The antisera, precipitating sera and 131I-labelled insulin were supplied by the Radiochemical Centre, Amersham, England. The antiserum (against pig insulin, obtained in the guinea-pig) was pre-precipitated with antisera to guinea-pig serum proteins (obtained in the rabbit); the 131I-labelled insulin was bovine. The standard curve was obtained with crystalline ox insulin (Eli Lilly and Co.) and rat insulin (Novo). Therefore, the present results are expressed as ox-insulin ‘equivalents’, and are considered only of comparative value for samples assayed in the same run. All samples in one experiment were determined against the same standard curve. The values obtained are likely to underestimate the true insulin levels in rat plasma and the differences encountered (Hales & Kennedy, 1964). The validity of the procedure was established by obtaining a good correlation of the dilution curves of rat insulin (Novo) and the bovine insulin, when the amounts of unlabelled insulin were not higher than 50 μu./ml.

Pancreatic insulin was extracted and partially purified by the procedure of Coore & Randle (1964). The extract was diluted with 0·04 m-phosphate buffer, pH 7·4, to the appropriate concentrations.

**Sensitivity to insulin** in vitro. Rat epididymal fat pads were used. Approximately 100 mg. were removed from the distal part of the epididymis; one fat pad was incubated with insulin (20 μu./ml.) and the other without insulin. The incubations were carried out in Krebs–Ringer bicarbonate buffer with glucose (3 mg./ml.) in a Dubnoff agitator bath at 37° in an atmosphere of O₂ and CO₂ (95%:5%) for 4 hr. After incubation, the concentration of glucose in the medium was measured according to Nelson (1944). The results are expressed as mg. glucose uptake/100 mg. tissue. The rats used were not fasted before they were killed.

**RESULTS**

**Caloric studies**

Table 1 shows that diets rich in carbohydrate, fat or protein produced more energy than the control diet. However, the rats on a diet with lower caloric content (control group) ate more, thus equilibrating their energy balance. The control diet produced 326 kcal./100 g., the rats ate 13·5 g./day and their calorie intake per day was 44 kcal. The high-protein diet produced 427 kcal./100 g., the rats ate 10·4 g./day and their calorie intake was 44·4 kcal. The same phenomenon also occurred with the high-carbohydrate and high-fat diets.
The diets rich in carbohydrate, fat and protein were better used by the animals than the control diet, since the transformation ratio (the amount of food necessary to produce a weight increase of 1 g.) was smaller.

Table 1. Calorie content of diets and calorie intake of rats fed on different diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Calorie content of diets (kcal./100 g.)</th>
<th>Increase in body weight (g./day)</th>
<th>Food consumption (g./day)</th>
<th>Caloric intake/rat/24 hr.</th>
<th>Transformation ratio (Food consumed:weight increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>326</td>
<td>2-0</td>
<td>13-5</td>
<td>44-0</td>
<td>6-75</td>
</tr>
<tr>
<td>High-carbohydrate diet</td>
<td>427</td>
<td>3-6</td>
<td>10-5</td>
<td>44-8</td>
<td>2-91</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>611</td>
<td>2-0</td>
<td>6-3</td>
<td>38-5</td>
<td>3-17</td>
</tr>
<tr>
<td>High-protein diet</td>
<td>427</td>
<td>2-7</td>
<td>10-4</td>
<td>44-4</td>
<td>3-85</td>
</tr>
</tbody>
</table>

Twenty-three rats per group.

Fig. 1. Concentration of glucose in plasma. Unshaded bars, controls; shaded bars, rats on high-protein diet. The standard deviation of the mean is indicated on each bar. Number of experiments in parentheses.

Table 2. Glycogen concentrations of liver and diaphragm of control rats and of rats fed on a high-protein diet. Means ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>Liver (mg./100 mg.)</th>
<th>Diaphragm (mg./100 mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed ad libitum</td>
<td>Fasted for 17–20 hr.</td>
</tr>
<tr>
<td>Group A (control)</td>
<td>3-75 ± 0-43 (12)</td>
<td>0-08 ± 0-02 (10)</td>
</tr>
<tr>
<td>Group B (high-protein diet)</td>
<td>3-00 ± 0-35 (11)</td>
<td>0-09 ± 0-02 (11)</td>
</tr>
<tr>
<td>P</td>
<td>&gt; 0-05</td>
<td>&gt; 0-05</td>
</tr>
</tbody>
</table>

Number of experiments in parentheses.
Plasma glucose

Fig. 1 shows that the plasma glucose concentration increased significantly (P < 0.01) in rats fed on the high-protein diet. However, after 17–20 hr. starvation the concentrations were similar in both groups (P < 0.05).

Glycogen content

Table 2 shows the glycogen content of liver and diaphragm. The values for liver glycogen were similar in both groups, but the concentration of glycogen in the diaphragm in the high-protein diet group was decreased (P < 0.05).

Table 3. Relationship between plasma insulin and insulin extractable from pancreas of controls and rats on high-protein diet. Means ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>Plasma insulin (µU./ml)</th>
<th>Insulin in pancreas (µg./100 mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Fasted 17-20 hr.</td>
</tr>
<tr>
<td>Group A (control)</td>
<td>40.1 ± 11.0 (12)</td>
<td>18.4 ± 5.0 (11)</td>
</tr>
<tr>
<td>Group B (high-protein diet)</td>
<td>43.0 ± 11.0 (11)</td>
<td>29.0 ± 7.6 (10)</td>
</tr>
<tr>
<td>P</td>
<td>&gt; 0.05</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Insulin values are given as equivalents of bovine insulin (potency 25 international units/mg.). Number of experiments in parentheses.

Table 4. Effect in vitro of insulin on glucose uptake of adipose tissue of groups A and B incubated for 4 hr. Means ± s.E.

<table>
<thead>
<tr>
<th></th>
<th>No. of rats</th>
<th>Glucose uptake (mg. glucose/100 mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (control)</td>
<td>13</td>
<td>0.175 ± 0.020</td>
</tr>
<tr>
<td>Insulin</td>
<td>13</td>
<td>0.455 ± 0.030</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Group B (high-protein diet)</td>
<td>10</td>
<td>0.100 ± 0.020</td>
</tr>
<tr>
<td>Insulin</td>
<td>10</td>
<td>0.202 ± 0.030</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Insulin in blood and pancreas

The plasma insulin levels in groups A and B were compared with the values for insulin extracted from their pancreases. Table 3 shows that when the animals were killed after feeding, both groups had the same concentrations of insulin. After starvation for 17–20 hr., the differences in plasma and pancreatic insulin between the two groups were highly significant in that, in comparison with 18.4 µU. insulin/ml. plasma and 2.53 µg. insulin/100 mg. pancreas in the control group, 29 µU./ml. and 3.46 µg./100 mg. were found in group B.

Sensitivity to insulin in vitro

Table 4 shows that the adipose tissue of the high-protein group of rats was less sensitive to insulin than the same tissue in the control group.
**Discussion**

The ingestion of diets different from the usual ones alters the metabolic and hormonal mechanisms in the normal animal. Thus the activities of enzymes involved in glycolysis and gluconeogenesis change when a high-fat, high-carbohydrate or high-protein diet is given (Vaugham et al. 1960; Freeland & Harper, 1957; Mokrasch et al. 1956; Rosen et al. 1959; Niemeyer, Clark-Turri, Garcia & Vergara, 1962). Diets rich in fat, carbohydrate or protein produce significant changes in insulin metabolism (Blázquez & López Quijada, 1968; Blázquez & López Quijada, 1969): the sensitivity of peripheral tissues to insulin lessens and insulin levels in the plasma and pancreas are modified. Moreover, other metabolic parameters, such as liver and muscle glycogen and plasma glucose, alter considerably. The secretion rate of other hormones also changes when dietary changes are introduced. For example, the intravenous injection of amino acids or the ingestion of high-protein diets produce a greater secretion of growth hormone (Knopf, Conn, Fajans, Floyd, Guntsche & Rull, 1965; Knopf, Conn, Floyd, Fajans, Rull, Guntsche & Thiffault, 1966). However, it may be assumed that these changes are due to a greater caloric value of the diet, and not specifically to the greater content of carbohydrates, fat or protein. Although the calorie content of the experimental diets was greater than that of the control diet, the calorie intake was similar in all groups (Table 1). This shows that the animal regulates the consumption of calories.

In the rats fed on the control diet, the concentration of insulin in plasma was correlated with the plasma glucose level. Thus, when the animals ate *ad libitum*, plasma glucose and insulin increased, but decreased to basal levels after a 17–20 hr. fast. However, this correlation did not obtain when the animals ate a diet different from normal. With a high-fat diet, the plasma insulin remained low, in spite of hyperglycaemia in the animals fed *ad libitum*, and hypoglycaemia after a 17–20 hr. fast. A high-carbohydrate diet also modified this correlation. The same modification was seen with high-protein diet. Rats fed *ad libitum* had a higher concentration of plasma glucose than the controls, but plasma insulin remained at the same level in both groups; after 17–20 hr. of fasting the glucose levels decreased in both control and experimental groups, but insulin concentrations remained noticeably high in the experimental groups.

The increase in plasma glucose in rats on a high-protein diet, despite the low carbohydrate content of the diet and the smaller amount of food ingested compared with the controls, suggests that gluconeogenic function is activated in the former. This assumption is supported by the fact that hepatic glycogen concentrations were similar in both groups, in spite of the smaller proportion of carbohydrate in the high-protein diet. The lower glycogen content of the diaphragm of rats on this diet may be explained by the observation of a lack of gluconeogenic mechanism in muscle (Scrutton & Utter, 1968). Vaughan et al. (1960) found that in animals on a high-protein diet hexokinase activity decreases in the liver and others (Freeland & Harper, 1957; Mokrasch et al. 1956; Rosen et al. 1959; Pitot & Peraino, 1964; Freeland & Avery, 1964) have reported that gluconeogenesis is increased by other enzymes. On the other hand, it is known that the administration of gluconeogenic amino acids increases glycaemia and that gluconeogenesis occurs immediately in livers perfused with these
amino acids (Exton & Park, 1967; Floyd, Fajans, Conn, Knopf & Rull, 1966a). It was also shown that in incubated liver slices the formation of glucose from these amino acids is important (Krebs, Nutton & Hems, 1966).

The increase in plasma glucose is facilitated by decreased utilization of hexose in peripheral tissues. The decreased sensitivity to insulin can be related to enzymic changes or to other factors which impede the action of the hormone. A high-protein diet, or the intravenous injection of amino acids, stimulates the secretion of growth hormone. Injection of this hormone or of hypophysial extracts produces a decrease in the glucose uptake of the diaphragm (Park, Brown, Cornblath, Daughaday & Kralh, 1952; Cotes, Reid & Young, 1949). Growth hormone also has a lipolytic effect on adipose tissue (Greenbaum & McLean, 1953) and liberates fatty acids, which are antagonistic to insulin (Randle, Garland, Hales & Newsholme, 1963).

The changes in the insulin levels in peripheral blood are interpreted as a reflexion of the secretory activity of the pancreas. It was demonstrated that certain amino acids augment the release of insulin (Floyd, Fajans, Conn, Knopf & Rull, 1966b). According to these authors, this is an important physiological phenomenon because the ingestion of protein and certain amino acids increases insulin secretion, and the hyperinsulinaemia could facilitate intracellular utilization of amino acids for protein synthesis. In our experiments with rats we found that a high-protein diet significantly modified the insulin levels in the pancreas and plasma. When rats were killed after a 17–20 hr. fast, plasma and pancreatic insulin concentrations were noticeably higher than those in control rats, which suggests that hormone synthesis and secretion were increased.

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REFERENCES


High-protein diet on insulin metabolism


