Effect of a High-Protein Diet on Insulin and Glucagon Secretion in Ventromedial Hypothalamic (VMH) Lesioned Rats

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Summary The effects of a high-protein diet on insulin and glucagon secretion in ventromedial hypothalamic (VMH) lesioned and sham-operated (sham) rats were studied in vivo as well as in perfusate from isolated pancreas. Two weeks after VMH destruction or sham operation, the rats were given either a balanced diet (protein 27%, carbohydrate 61%, fat 12%) or a high-protein diet (protein 55%, carbohydrate 30%, fat 15%) for the following 2 weeks. The calorie intake and body weight changes after the commencement of the diets were almost the same in the groups of VMH lesioned rats, but these were much greater than those in the two sham-operated groups. Fasting blood glucose, plasma insulin, and plasma glucagon concentrations were also similar between the two VMH groups, but in the sham-operated rats fasting blood glucose and plasma insulin concentrations of those rats on high-protein diet were significantly increased when compared to those on balanced diet. In the isolated, perfused pancreas, an arginine-induced excess insulin and glucagon secretion was not significantly different between the VMH lesioned rats. An arginine-induced rise in insulin concentration in the sham-operated rats on high-protein diet was significantly higher than for rats on balanced diet. We therefore suggest that hyperinsulinemia already produced in the VMH lesioned rats may not be influenced by the change in the composition of the dietary protein and carbohydrate.

Key Words high-protein diet, ventromedial hypothalamic lesions, food intake, blood glucose, insulin, glucagon, isolated perfused rat pancreas
In a previous study (1), we demonstrated that plasma insulin concentration and arginine-induced insulin secretion in rats fed on a high-protein diet were significantly higher than in rats fed on a balanced diet.

It is well known that destruction of the ventromedial hypothalamic (VMH) nuclei causes marked obesity associated with hyperinsulinemia in rats (2-4). Frohman et al. (5) reported that in VMH lesioned rats fed on a high-fat diet, plasma insulin levels were lower when compared to those in rats fed on a low-fat diet. On the contrary, Inoue et al. (6) demonstrated that plasma insulin levels in VMH lesioned rats were slightly increased with a high-fat diet. In addition, the latter authors showed that plasma glucagon levels did not differ between those two dietary groups of animals, and that insulin secretion from the isolated, perfused pancreatic islets of the VMH lesioned rats was significantly increased with a high-fat diet.

The present study was undertaken to investigate the effects of a high-protein diet on plasma insulin and glucagon levels and secretion of these hormones from the isolated, perfused pancreas of VMH lesioned rats.

MATERIALS AND METHODS

Wistar-strain female rats weighing 190–200 g each were used. Under sodium pentobarbital anesthesia, the rats were placed in a stereotaxic apparatus. Electrolytic lesions of the bilateral ventromedial hypothalamic nuclei were produced as previously described (2). After the operation, the animals were housed individually in an air-conditioned room and maintained on a balanced diet (345 kcal/100 g; protein 27% kcal, carbohydrate 61% kcal, fat 12% kcal) for 2 weeks. The rats were then randomly divided into two groups, one of which continued on the same balanced diet ad libitum for another 2 weeks, the other starting with a high-protein diet (360 kcal/100 g; protein 55% kcal, carbohydrate 30% kcal, fat 15% kcal) ad libitum for the same period. The composition of the diets was described previously (1). As controls, the sham-operated rats were used and divided into two groups as described above.

At the end of the feeding period, the rats were fasted overnight and were then anesthetized with sodium pentobarbital intraperitoneally. Peripheral blood was withdrawn from the jugular vein into heparinized tubes containing 1,000 U Trasylol for the assays of glucose, insulin, and glucagon. The rat pancreas was isolated and perfused by the procedure previously reported (2). All perfusions were accomplished with Krebs-Ringer bicarbonate buffer solution containing 0.25% bovine serum albumin and 4.6% dextran (mean mol. wt. 70,000). The medium was gassed with 95% O₂ and 5% CO₂ and maintained at pH 7.4 at 37°C. After an equilibration period with 4.4 mM glucose, L-arginine hydrochloride was added into the perfusate for 15 min to provide a final concentration of 19 mM. Each 1-min sample of effluent from the portal vein was collected in chilled tubes containing 1,000 U Trasylol, frozen immediately, and stored at -20°C until assayed. Blood glucose was measured by the glucose oxidase method (7). Insulin was determined by poly-

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ethylene glycol radioimmunoassay (8) and glucagon was determined by radioimmunoassay using the talcum adsorption technique (9) using antiserum 30K. Statistical analysis was performed by the Student’s "t" test.

RESULTS

Calorie intake and body weight change (Fig. 1)

The balanced diet used contained 345 kcal/100 g, and the VMH lesioned rats consumed an average 31 ± 3 (± SE) g/day or 110 ± 11 kcal/day. The high-protein diet comprised 360 kcal/100 g, and the VMH lesioned rats consumed a mean 26 ± 11 g/day or 94 ± 5 kcal/day. There was no significant difference between their calorie intakes. The food intake of the sham-operated rats on high-protein diet (20 ± 1.5 g/day or 72 ± 5 kcal/day) did not differ from that (22 ± 1.6 g/day or 77 ± 6 kcal/day) of the sham-operated rats on balanced diet. The body weight was increased markedly both in the VMH lesioned rats fed on balanced diet and high-protein diet (360 ± 8 vs. 370 ± 6 g, respectively), while body weight gain was less marked in the sham-operated rats fed on both diets (255 ± 8 vs. 246 ± 5 g, respectively) as shown in Fig. 1.

Fasting blood glucose, plasma insulin, and plasma glucagon levels (Table 1)

Fasting blood glucose levels in the VMH lesioned rats fed on both high-protein diet and balanced diet were 115 ± 7 mg/dl and 120 ± 5 mg/dl, respectively. In contrast, fasting blood glucose levels of the sham-operated rats on high-protein diet were 124 ± 6 mg/dl, significantly higher than those (100 ± 5 mg/dl) of the rats on balanced diet. Fasting plasma insulin levels in the VMH lesioned rats were

![Graph showing changes in body weight of VMH and sham groups fed balanced and high-protein diet.](image-url)

Fig. 1. Changes of body weight in the VMH lesioned and sham-operated rats fed on a balanced diet (---) and a high-protein diet (—-).

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Table 1. Fasting blood glucose, plasma insulin and glucagon levels in the VMH lesioned and sham-operated rats fed on a balanced diet and a high-protein diet.

| Rat                | Blood glucose (mg/dl) | Plasma insulin (ng/ml) | Plasma glucagon (pg/ml) |
|--------------------|-----------------------|------------------------|-------------------------|
| VMH lesioned       |                       |                        |                         |
| Balanced diet (6)  | 120 ± 5\(^a\)         | 2.8 ± 0.53\(^b\)      | 141 ± 18                |
| High-protein diet (6) | 115 ± 7\(^a\)   | 2.1 ± 0.29\(^b\)      | 152 ± 25                |
| Sham operated      |                       |                        |                         |
| Balanced diet (6)  | 100 ± 5               | 0.43 ± 0.06            | 127 ± 8                 |
| High-protein diet (6) | 124 ± 6\(^a\)   | 0.94 ± 0.12\(^a\)      | 124 ± 9                 |

Values are shown as means ± SE, with the numbers of animals given in parentheses. Values not superscripts are not significantly different, \( p \geq 0.05 \). \(^a\) Different from the sham-operated animals fed on a balanced diet, \( p < 0.01 \). \(^b\) Different from the sham-operated animals fed on a balanced diet or fed on a high-protein diet, \( p < 0.001 \).

significantly higher than those in the sham-operated rats, but there was no difference between the VMH lesioned rats fed on either a high-protein diet or a balanced diet (2.1 ± 0.29 vs. 2.8 ± 0.53 ng/ml, respectively). Fasting plasma insulin levels in the sham-operated rats on high-protein diet were significantly higher than those in the sham-operated rats on balanced diet. Fasting plasma glucagon levels in all the four groups of animals were not significantly different from each other.

Pancreas perfusion study (Fig. 2)

A 19 mM arginine and 4.4 mM glucose infusion evoked biphasic insulin and glucagon responses in all the four groups (Fig. 2). In the VMH lesioned rats on high-protein diet, there were first and second peaks of insulin secretion which reached 22.9 ± 5.6 and 22.3 ± 6.0 ng/ml, respectively. These figures were lower than the corresponding figures of 26.1 ± 8.7 and 33.9 ± 8.6 ng/ml obtained in the VMH lesioned rats on balanced diet, but the difference was not significant. The increased insulin secretion in the VMH lesioned rats was maintained at a significantly higher level than in the sham-operated rats. In the sham-operated rats on high-protein diet, the peak values were 5.6 ± 1.1 and 6.1 ± 0.88 ng/ml, which were significantly higher than the corresponding values of 3.0 ± 0.68 and 4.2 ± 1.1 ng/ml in the rats on balanced diet. On the other hand, the response of glucagon secretion to an arginine and glucose infusion did not show any significant difference between the two groups of VMH lesioned and sham-operated animals.

DISCUSSION

The present study has demonstrated that a high (55\%) protein diet does not affect an already increased secretion of insulin in VMH lesioned rats under
Fig. 2. Immunoreactive insulin (IRI) and glucagon (IRG) concentrations before and after an infusion of 19 mM arginine solution in the presence of 4.4 mM glucose in the isolated, perfused pancreas of VMH lesioned and sham-operated rats fed on a balanced diet (---) and a high-protein diet (—). Values are means ± SE.

conditions of either in vivo or in perfused pancreas. The high-protein diet, however, significantly the elevated fasting blood glucose level and plasma insulin level and also enhanced arginine-induced insulin secretion from the perfused pancreas in the sham-operated rats, as we previously reported in normal rats (1). The reason for the different effects of the high-protein diet on insulin release in the VMH lesioned and sham-operated rats remains unclear. Inoue et al. (6) reported that secretion of insulin from perfused pancreatic islets in the VMH lesioned rats fed on a high-fat diet was significantly higher than that from rats fed on a low-fat diet, and they suggested that increased insulin secretion might have resulted from the different degrees of obesity observed in the two dietary groups. It is possible that altered carbohydrate and fat intake might affect blood glucose and plasma insulin levels. However, the fat contents of the diets are almost the same between the two groups employed in the present study and it is not likely that a low-carbohydrate diet itself contributes to enhanced insulin secretion and elevated blood glucose levels (10). In the present experiment, there was no difference in the animals’ food intake and body weight between the VMH lesioned rats fed on either a high-protein diet or a
balanced diet. It was suggested, therefore, that an already stimulated insulin release in the VMH lesioned rats is not affected by the changes in protein content of the diet.

It has been demonstrated that in VMH lesioned rats maintained on restricted food intake, excess insulin secretion is not lowered to normal control levels (11, 12). From these results, Martin et al. (11) have proposed that the VMH lesions enhance the responsiveness of the pancreatic islets to insulin secretory stimulants without any changes in food intake.

Since hyperinsulinemia produced after induction of VMH lesions has been shown to be associated with hyperactivity of the vagal nerve system (13–16), it seems possible that increased vagal nerve activity stimulates secretion of insulin in VMH lesioned rats.

In the present experiment, the high-protein diet failed to influence either plasma glucagon or glucagon secretion in the isolated perfused pancreas of VMH lesioned rats. The results reported on glucagon secretion of VMH lesioned rats in vivo and in vitro have been contradictory and inconclusive (4, 6, 17). Rohner-Jeanrenaud and Jeanrenaud (17) recently reported that both the basal and arginine-induced glucagon secretion from the perfused pancreas of VMH lesioned rats in the absence of glucose are significantly higher than in controls. Our results are not in accordance with their findings, but the difference of animal ages, feeding periods after VMH destruction, and of glucose and arginine concentrations in the perfusate must be considered as contributing to the different results.

The present study demonstrated that the change in the composition of the diet fails to influence elevated insulin secretion established in VMH lesioned rats. Further studies on the effects of changes in the composition of the food in diets are required to clarify the dietary factors regulating insulin and glucagon secretion in VMH lesioned rats.

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