PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 393-397 (1976)

Kidney Response to Cold Stress and High Protein Intake[1, 2] (39404)

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Substantial increases in kidney size have been observed in animals exposed to cold (1, 2) or fed diets containing high levels of protein (3-5). In fact, this effect of cold and diet appears to be additive as evidenced by the markedly greater kidney enlargement seen in cold-exposed rats receiving a high protein diet as compared to those fed a high carbohydrate diet (6). Whether achieved by diet or environmental temperature, the size increase is the result of actual tissue growth. Biochemically, such growth is evidenced by a normal tissue protein content (5), while histologically, it is evidenced by hyperplasia and hypertrophy of the glomeruli, tubules, and interstitial cells (7, 8). Presumably, the cold-induced or diet-induced enlargement of the kidney represents a compensatory response to an added functional load. In the case of high protein diets, and probably in the case of cold exposure where the caloric intake can be increased twofold (9), this added functional load may be caused by an increased metabolic turnover of nitrogen in the body. Indeed, when animals are fed high protein diets, without cold exposure, increases in the activities of renal enzymes mediating the catabolism of amino acids, e.g., glutamic dehydrogenase (4), and amino nitrogen, e.g., arginase (3), have been reported. Such animals also exhibit an increase in the rate of renal gluconeogenesis from amino acid precursors (10). In contrast, the activities of renal glutamic-pyruvic and glutamic-oxalacetic transaminase are essentially normal in rats fed high protein diets (11). With cold exposure alone, slight increases in the activities of renal xanthine oxidase (12) and arginase (13) have been reported, as have increases in DNA content (1) and gluconeogenic capacity (9). As in the case of high protein diets, cold exposure alone does not affect renal glutamic-oxalacetic transaminase activity (1). These observations indicate that high protein intake or cold stress produce adaptive changes in several synthetic and degradative metabolic pathways of the kidney. Concomitant effects of cold stress and high protein intake on the activity of specific gluconeogenic and urea cycle enzymes associated with such pathways remain to be determined. Accordingly, the present study was directed at this problem.

Methods. Forty male Holtzman rats weighing from 280-300 g were used in this study. They were individually housed at 25° and fed a control, high carbohydrate diet for 10 days prior to experimentation. The diet contained, by weight, the following components: casein, 20%; sucrose, 73%; corn oil, 3%; mineral mix, 4%; and a complete vitamin supplement. Following this dietary adjustment period, the rats were divided into two treatment groups of 20 animals each. One group remained at 25° and the second group was placed in a cold room held at 5°. Ten animals in each group were continued on the control, high carbohydrate diet while the remaining animals were given a high protein diet. The latter contained by weight: casein, 85%; sucrose, 8%; plus the remaining ingredients at the levels described in the control diet. Food as well as water were available *ad libitum*. Food intake and change in body weight were recorded daily. Both the feeding and cold exposure periods were of 10 days' duration.

Thereafter, the animals were sacrificed,

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

[2] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institutes of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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the kidneys were removed, chilled in ice-cold saline, and weighed. One kidney from each animal was dried in a vacuum oven at 65° for 24 hr. The dried material was analyzed for total lipids with continuous (8 hr) ethyl ether extraction, and for total nitrogen by the micro-Kjeldahl procedure (14). The second kidney was used for preparation of tissue homogenates and for enzyme assays according to the following methods: glucose-6-phosphatase (GPase), the method of Cori and Cori (15); fructose-1,6-diphosphatase (FDPase), the method of Racker (16); phosphoenolpyruvate carboxykinase (PEPase), the method of Nordlie and Lardy (17) and Shrago and Lardy (18); glutamic-oxalacetic transaminase (GOT), the method of La Due *et al.* (19); glutamic-pyruvic transaminase (GPT), the method of Wroblewski and La Due (20); tryrosine alpha ketoglutaric transaminase (TGT), the method of Diamondstone (21); glutamic dehydrogenase (GDH), the method of Wergedal and Harper (4); arginase and arginine synthetase, the method of Brown and Cohen (22) and ornithine transcarbamylase, the method of Schimke (23). Since there were substantial differences in the final body weights and the size of the kidneys among the treatment groups, enzymatic activity was expressed as micromoles of product per minute per organ per 100 g body weight, as this method best expresses the total metabolic requirement of the kidney (5). All data were statistically evaluated with analyses of variance and significant F ratios were further evaluated with the Tukey multiple range test. Differences between means at the 0.05 level of probability were considered significant.

Results. As indicated in Table I, the cold-exposed animals lost body weight, the loss being significantly more pronounced in the group fed the high protein diet than in the group fed the control diet. In addition, the animals fed the high protein diet at 20° gained less weight than the corresponding control group. Cold-exposed animals consumed more food than the corresponding animals at 20°. However, food intake of the high protein group was lower than that of the control group at both 5 and 20°.

TABLE I. EFFECT OF COLD STRESS AND HIGH PROTEIN DIET ON SEVERAL KIDNEY COMPONENTS AND ENZYME ACTIVITIES TEMPERATURE AND DIET.

	20°		5°	
	Control	Protein	Control	Protein
Av change in BW/day, g	3.5 ± 0.28[a]	2.1 ± 0.19[c]	−3.3 ± 0.20[d]	−5.6 ± 0.3[cd]
Av food intake/day, g	17.6 ± 1.6	13.1 ± 1.9[c]	25.5 ± 1.3[d]	19.5 ± 1.7[cd]
Kidneys/pair, g	2.04 ± 0.02	2.46 ± 0.08[c]	2.23 ± 0.13[d]	2.94 ± 0.03[cd]
Moisture, %	77.71 ± 0.14	77.34 ± 0.12	77.81 ± 0.10	77.90 ± 0.15
Nitrogen, % wet wt	2.58 ± 0.10	2.52 ± 0.09	2.60 ± 0.08	2.53 ± 0.12
Lipids, % wet wt	2.93 ± 0.11	2.85 ± 0.12	2.89 ± 0.14	2.97 ± 0.22
Enzyme activity[b]				
Glucose-6-phosphatase	9.39 ± 0.69	19.26 ± 1.02[c]	9.72 ± 0.89	30.16 ± 1.83[cd]
Fructose-1,6-diphosphatase	2.47 ± 0.29	4.47 ± 0.36[c]	2.50 ± 0.18	7.50 ± 0.49[cd]
Phosphoenolpyruvate carboxykinase	1.72 ± 0.09	2.92 ± 0.30[c]	1.78 ± 0.12	4.51 ± 0.28[cd]
Glutamic-oxalacetic transaminase	18.77 ± 1.29	22.29 ± 1.43	19.89 ± 1.83	37.85 ± 2.19[cd]
Glutamic-pyruvic transaminase	0.49 ± 0.09	0.52 ± 0.03	0.48 ± 0.04	2.51 ± 0.12[cd]
Tyrosine-α-ketoglutaric transaminase	0.92 ± 0.21	0.88 ± 0.24	0.91 ± 0.19	0.96 ± 0.17
Glutamic dehydrogenase	15.16 ± 0.96	27.59 ± 1.38[c]	14.54 ± 1.13	38.89 ± 1.28[cd]
Arginine synthetase	0.14 ± 0.02	0.27 ± 0.03[c]	0.12 ± 0.03	0.31 ± 0.05[c]
Ornithine transcarbamylase	0.28 ± 0.05	0.65 ± 0.04[c]	0.25 ± 0.04	0.73 ± 0.08[c]
Arginase	9.43 ± 1.13	19.74 ± 1.83[c]	11.29 ± 0.93	20.75 ± 1.90[c]

[a] Mean ± SE from 10 rats.
[b] Micromoles/min/100 g BW ($P \leq 0.05$).
[c] Indicates significant ($P \leq 0.05$) difference from controls, same temperature.
[d] Indicates significant ($P \leq 0.05$) difference from 20°, same diet.

Compared to the controls, high protein intake caused an enlargement of the kidneys, the increase being significantly more pronounced at 5° than at 20°. Neither diet nor cold stress had any effect on the moisture, lipid, or nitrogen content of the kidneys.

At both environmental temperatures, the activities of GPase, FDPase, PEPase, GDH, and of the three urea cycle enzymes were enhanced by the high protein diet. Furthermore, the activities of GPase, FDPase, PEPase, and GDH were significantly higher when the high protein diet was fed at 5° as compared to 20°. In addition, the high protein diet stimulated the activity of GOT and GPT only when fed at 5°. Neither cold nor high protein intake affected the activity of TGT. Cold had no effect on enzymatic activity in animals fed the control diet.

Discussion. Initial stages of cold exposure are associated with a stimulation of shivering thermogenesis which may be largely dependent on glucose as an energy source. During this period, a marked enhancement of hepatic gluconeogenesis takes place, apparently in response to an increased energy demand of the cold environment (9). The present data demonstrate that, in addition to the liver, the kidney is an effective gluconeogenic organ in the cold environment when dietary protein is the major source of energy. In many respects, gluconeogenic responses of the kidney and the liver to the high protein intake are similar. The activities of the urea cycle enzymes increase in both organs (24, 25) as do the activities of GPase, FDPase, and GDH (4, 25). In contrast to the liver, renal GOT, GPT, and TGT do not respond to high protein diets when fed in the warm environment (25). However, as the present data indicate, the activity of GOT and GPT is stimulated in the cold environment. These findings suggest that aspartic acid, alanine, and tyrosine are not significant precursors of renal glucose in the warm environment. This suggestion is supported by a report showing that alanine does not stimulate the rate of gluconeogenesis in perfused kidney (26). The present data, however, also suggest that aspartic acid and alanine have significant roles in the renal synthesis of glucose in animals subjected to cold stress and when limited to low carbohydrate intake. These findings would further imply that the enhanced activities of GOT and GPT observed under cold stress represent the maximal rate of gluconeogenic processes since the two enzymes were stimulated by dietary and environmental conditions which impose an apparent, maximal demand for glucose. In general, it appears that enhanced gluconeogenesis in cold-exposed animals fed high protein diets is substrate-induced, as a result of increased intake and catabolism of dietary protein, and not induced by cold per se.

Other factors which influence or control renal gluconeogenic capacity remain to be clarified. It would appear likely that increases in adrenocortical and thyroid activity observed during the initial stages of adaptation to cold stress (27) are responsible, at least in part, for enhancing the activities of renal gluconeogenic enzymes. Specific enzymes which respond to such hormonal stimuli include GPase (28), PEPase (29), GPT and GOT (30), and FDPase (31). A recent report indicating an increase in the renal GOT only when high protein intake was combined with the administration of hydrocortisone (25) gives credence to such a conclusion.

The present data demonstrate, as have the data of many others (32–34), that cold-exposed animals increase their food intake to compensate for heat loss to the environment. However, animals fed the high protein diet ate less than those fed the control diet; consequently, the loss in body weight was more severe. Apparently, cold stress failed to overcome the hypophagia associated with the intake of high protein diets. This observation confirms and extends the conclusion of Beaton (34) who reported that rats exposed to cold for 8 days and fed a 40% casein diet ate less than rats receiving a diet containing 5 or 20% casein.

Metabolic events related to adaptation to high protein diets are not thoroughly understood; however, prominent among these are depression of food intake and body weight gain, hypertrophy of the kidney and the liver, alterations in the activity of several enzyme systems participating in the catabo-

lism of amino acids, and changes in the profile of plasma-free amino acids (5). In general, such metabolic events seem to be related to the animal's capacity to dispose of a large influx of amino acids resulting from ingestion of excess protein. If the influx exceeds the oxidative capacity for specific amino acids, the tissues may be presented with an imbalanced amino acid mixture which would adversely affect the food intake and the synthesis of new proteins. In this respect, previous data from several laboratories show that cold-exposed rats readily consume diets having a moderately imbalanced pattern of amino acids (35–37). However, when the imbalance was made more severe food intake was depressed to the level observed in a warm environment (38). Such data also show that cold-exposed animals oxidize the excess amino acids more rapidly than normothermic rats at 20° (39). The foregoing observations suggest that rats maintained on a high protein diet do not have the capacity to degrade excess amino acids at a rate that would have allowed an increase in food intake commensurate with the elevated energy demands of a cold environment.

Summary. Compared to rats fed a high sugar diet, consumption of a high protein diet caused an enlargement of the kidneys, the increase being more pronounced at 5° than at 20°. High protein intake enhanced activities of renal glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase, glutamic dehydrogenase, and of the urea cycle enzymes. However, the activities of the gluconeogenic enzymes were higher at 5° than at 20°. In contrast, enzymatic activity was not changed in cold-exposed animals fed the high sugar diet. Since cold exposure stimulates food intake, it appears that enhanced renal gluconeogenesis observed in this study is substrate-induced rather than induced by cold per se.

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Received March 29, 1976. P.S.E.B.M. 1976, Vol. 152.