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Depressed Response of Plasma Iron and Zinc to Endotoxin and LEM in STZ-Diabetic Rat[1,2] (41585)

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Abstract. Laboratory and epidemiological evidence indicate that the enhanced flux of iron and zinc from the plasma to the storage compartments, such as liver, serves as a protective host response to combat infection. Studies were performed to determine the status of this nonspecific immune response in the diabetic animal, since it is commonly held that the diabetic has an increased incidence and susceptibility to infection. Normal rats and rats previously rendered diabetic by streptozotocin (STZ) were injected with either saline or *Escherichia coli* endotoxin, and plasma levels of zinc, iron, and copper were monitored 8 hr thereafter. Diabetic rats reduced their plasma zinc and iron levels by 35 and 25%, respectively, in response to endotoxin injection whereas control rats had a 70% decrease in zinc and a 46% depression in iron. Insulin administration to the diabetic rats restored the ability to decrease plasma zinc and iron to the same degree as control rats. Plasma copper did not change in any group. Further investigation suggested that the defect in trace metal response occurred after the secretion of leukocytic endogenous mediator (LEM) in the inflammatory response pathway. It is concluded that STZ-diabetic rats have a diminished ability to decrease plasma zinc and iron in response to endotoxin, and that this defect is due to an ineffective response of target tissues to the effects of leukocytic endogenous mediator. Furthermore, it is postulated that the hyperinsulinemia associated with the stress of infection functions to lower plasma zinc and, possibly, iron, thereby allowing the host to better combat infection.

In addition to activating cellular immune systems, the response of higher animals to infection and inflammation is characterized by marked alterations in carbohydrate, lipid, nitrogen, and trace metal metabolism (1, 2). The host-mediated reduction in plasma levels of iron and zinc has received increased attention during recent years. Epidemiological and laboratory studies have provided evidence that the enhanced flux of iron from plasma to the liver represents a defense mechanism, viz., "nutritional immunity," whereby the host attempts to withold this growth-limiting nutrient from the invading parasite (3). Although the basis for depressed plasma zinc content remains unknown, it has been proposed that this process is necessary for activation of the quiescent population of phagocytic cells (4). Thus, the impaired ability of the host to reduce plasma iron and zinc levels in response to microbial invasion would be expected to increase the probability of morbidity.

It is a common, although controversial, clinical axiom that poorly controlled diabetics have enhanced susceptibility to and severity of infections. Indeed depressed T-cell function has been observed in diabetic humans (5), as well as in chemically induced (6, 7) and congenitally (6, 8) diabetic rodents. Likewise, nonlymphocytic cells of bone marrow origin from diabetics exhibit decreased chemotaxis (9, 10), adherence (11), phagocytic capability (7, 12–14), and bactericidal activity (14, 15). We recently found altered tissue levels and metabolism of trace metals in streptozotocin-diabetic rats (16, 17). Here we report that diabetic rats have decreased ability to reduce plasma iron and zinc levels following the administration of either endotoxin or leukocytic endogenous mediator (LEM). These results suggest compromised nutri-

[1] Abbreviations: streptozotocin, STZ; leukocytic endogenous mediator, LEM.

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tional immunity in the insulin-deficient diabetic animal.

Materials and Methods. Male Sprague–Dawley rats were purchased from Charles River Breeding Laboratories and maintained on a 12 hr light–dark schedule (0400–1600 hr). To induce the insulin-deficient diabetic state, freshly prepared streptozotocin (STZ) was administered intraperitoneally (ip) on two consecutive days at a dose of 50 mg/kg body weight. Control and diabetic animals were given free access to chow (Ralston Purina Rodent Chow 5012) and deionized water, except on the day of sacrifice when food was removed at 0600 hr.

Two weeks after STZ treatment, diabetic and age-matched control rats were injected at 0800–0900 hr with one of the following: (a) 0.4–0.6 ml saline (0.9% w/v); (b) 0.4–0.6 ml saline containing *Escherichia coli* (serotype 0111:B4) endotoxin at a dose of 0.2 mg/kg body weight; (c) 0.95 ml crude rabbit leukocyte endogenous mediator (LEM); and, (d) 0.95 ml heat-inactivated LEM. Endotoxin was purchased from Sigma Chemical Co. LEM (Batch No. 53) was a gift from Dr. P. Z. Sobocinski and Dr. D. M. Bunner, U.S.A Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. Inactivated LEM was prepared by heating the native material at 95°C for 30 min and subsequently removing denatured material by centrifugation at 12,000*g* for 15 min. Native LEM was centrifuged similarly. Animals were killed by decapitation 8 hr after injection. Blood was collected in heparinized tubes and centrifuged at 1086*g* for 10 min to prepare plasma.

Zinc, copper, and iron levels in plasma diluted 1:5 with deionized water were measured by atomic absorption spectrometry (Perkin Elmer Model 560). Plasma glucose was determined by glucose oxidase-/peroxidase-coupled enzyme assay (Sigma Chemical Co. Diagnostic Kit No. 510). Data were statistically analyzed by analysis of variance and Duncan's multiple range test was used to determine means significantly different at $\alpha < 0.05$.

Results. Animals receiving streptozotocin exhibited characteristic changes associated with the insulin-deficient condition, viz., increased food and water consumption and urine output, decreased growth rate, and marked hyperglycemia. Although the plasma glucose level of controls was not altered by exposure to either endotoxin or LEM, both materials caused a slight (approx 15%), but significant ($\alpha < 0.05$), increase in the degree of hyperglycemia in diabetic rats. This exaggerated hyperglycemia in endotoxin-treated diabetic rats has been previously reported (18).

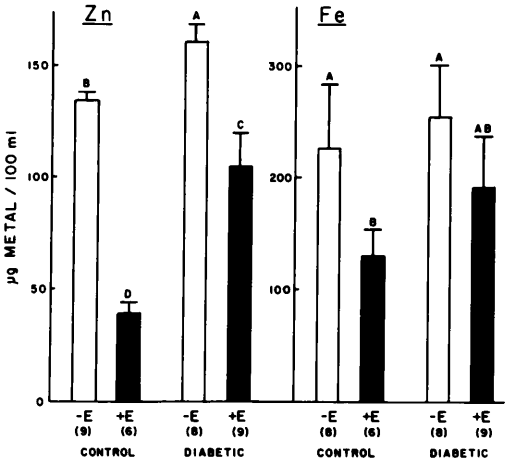

FIG. 1. Plasma zinc and iron levels in control and STZ-diabetic rats administered endotoxin. Age-matched control and 2-week STZ-diabetic rats received either saline (−E) or endotoxin (+E; 0.2 mg/kg body wt) and were sacrificed 8 hr later. The numbers in parentheses are the number of animals in each group in this and subsequent figure. Data represent mean ± SEM. Common letters above error bars indicate no significant difference at $\alpha < 0.05$.

Plasma zinc was approximately 20% higher in diabetic animals than controls (Fig. 1), whereas iron (Fig. 1) and copper levels of the two groups were not significantly different. Injection of control rats with endotoxin depressed plasma zinc and iron by 70 and 46%, respectively (Fig. 1). In contrast, when diabetic rats were exposed to endotoxin, the plasma zinc and iron levels were reduced by only 35 and 25%, respectively (Fig. 1). Daily subcutaneous injections of insulin (2 units NPH, Eli Lilly and Co.) from Day 7 through 13 of the study restored the ability of the diabetic host to reduce plasma zinc and iron to the same degree as control rats following challenge with endotoxin. When diabetic rats not exposed to endotoxin were treated with insulin, the concentration of plasma zinc was reduced to control levels (16). Plasma copper levels in control, diabetic, and insulin-treated

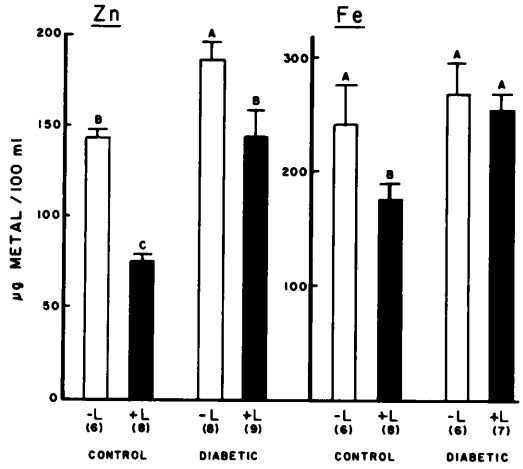

FIG. 2. Plasma zinc and iron levels in control and STZ-diabetic rats treated with either native or heat-treated LEM. Control and diabetic rats were injected with either crude (+L) or heat-inactivated (−L) LEM intraperitoneally. Animals were sacrificed 8 hr later and plasma content of zinc and iron measured. Data represent mean ± SEM. Common letters above error bars indicate no significant difference at $\alpha < 0.05$.

diabetic rats remained unchanged at 8 hr after endotoxin administration.

Crude LEM was administered to test whether the reduced ability of chemically diabetic rats to depress plasma levels of zinc and iron following endotoxin injection was due to decreased secretion of endogenous mediators by the phagocytic cells. LEM injection depressed plasma zinc and iron levels of control animals by 48 and 29%, respectively, compared to the group receiving heat-inactivated material (Fig. 2). When diabetic rats were injected with LEM, plasma iron content did not change and the level of plasma zinc, although significantly decreased, was only restored to the amount normally present in control animals (Fig. 2). Finally, the copper content of LEM-treated control and diabetic rats increased from 98 to 116 ($\alpha < 0.05$) and 97 to 108 ($\alpha < 0.05$), respectively.

Discussion. The introduction of microbes, endotoxin, and other foreign materials into reptiles, birds, and mammals initially activates the nonspecific cellular immune response. Once stimulated, these cells release a family of hormone-like substances (collectively referred to as LEM) that mediate elevation of the circulating levels of pituitary, pancreatic, and adrenal hormones (1, 2). This altered endocrine profile appears to be directly responsible for the diverse metabolic changes that occur during episodes of infection and inflammation.

Data presented above show that STZ-diabetic rats have a diminished ability to reduce plasma concentrations of iron and zinc in response to endotoxin treatment. Since the chemotactic responsiveness (9, 10) and intracellular metabolism (14, 15, 19) of phagocytic cells from poorly controlled diabetics are impaired, it was possible that the diabetic animal was unable to produce sufficient quantities of LEM. However, LEM-treated diabetic rats also failed to depress plasma iron and only reduced zinc to the normal level after injection with crude rabbit LEM. These data suggest that the neuroendocrine system of the STZ-diabetic rat does not respond properly to LEM and/or there is some abnormality in the hormone-mediated transfer of plasma zinc and iron to the liver.

The classic endocrine response to acute stress includes an elevation of plasma glucagon and glucocorticoid levels and a reduction of the plasma insulin content. The hormonal status of animals with an infection or inflammation is unique compared to other types of stress, because it is characterized by hyper-, rather than hypo-, insulinemia (20, 21). The role(s) of the additional complement of insulin in biochemical adaptation to infection remains unknown. We propose that endotoxin and LEM-mediated elevation of plasma insulin content may be necessary to depress plasma zinc and, possibly, iron. Insulin-deficient diabetic rats are hyperzincemic [Figs. 1 and 2 (16)], whereas the hyperinsulinemic *ob/ob* mouse has been reported to have significantly lower plasma zinc levels than lean littermates (22). Moreover, insulin treatment of STZ-diabetic rats reduces their plasma zinc content to that of control animals (16) and restores their ability to respond to endotoxin and LEM in a normal manner. Further support for a role for insulin in zinc metabolism is provided by the recent observations of Hurley and associates (23). These investigators have found circadian variation in the plasma zinc concentration in normal rats and that this phenomenon is related to food intake.

Plasma zinc levels were constant during the light period when animals consumed little diet. A sharp decline (25%) in the plasma content of this metal was correlated with the initiation of feeding during the dark period. Nutrient absorption is correlated with the secretion of additional amounts of insulin. Further studies are required to determine if the influence of insulin on zinc metabolism is direct or indirect.

Finally, considering the proposed rationales for host-mediated reduction of plasma iron (3) and zinc (4) during periods of infection and inflammation, our data suggest that altered trace-metal metabolism in the STZ-diabetic rat contributes to impaired immunocompetance.

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1. Beisel WR. Metabolic response to infection. Annu Rev Med **26**:9–20, 1975.
2. Powanda MC. Changes in body balance of nitrogen and other key nutrients: description and underlying mechanisms. Amer J Clin Nutr **30**:1254–1268, 1977.
3. Weinberg ED. Iron and infection. Microbiol Rev **42**:45–66, 1978.
4. Chvapil M. The role of zinc in the function of some inflammatory cells. In: Brewer GJ, Prasad AS, eds. Zinc Metabolism: Current Aspects in Health and Disease. New York, AR Liss, pp103–122, 1977.
5. MacCuish AC, Urbanick SJ, Campbell CJ, Duncan LJP, Irvine WJ. Phytohemogglutinin transformation and circulating lymphocyte subpopulations in insulin-dependent diabetic patients. Diabetes **23**:708–712, 1974.
6. Mahmoud AAF, Rodman HM, Mandel MA, Warren KS. Induced and spontaneous diabetes mellitus and suppression of cell-mediated immunologic responses: granuloma formation, delayed dermal reactivity, and allograft rejection. J Clin Invest **57**:362–367, 1976.
7. Saiki O, Negorw S, Tsuyuguchi I, Yamamura A. Depressed immunological defense mechanisms in mice with experimentally induced diabetes. Infect Immun **28**:127–131, 1980.
8. Mandel MD. Immune competence and diabetes mellitus. II. Experimental mouse studies J Surg Res **26**:199–205, 1979.
9. Mowat AG, Baum J. Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. N Engl J Med **284**:621–627, 1971.
10. Hill HR, Sauls HS, Dettloff JL, Quie PG. Impaired leukocyte responsiveness in patients with juvenile diabetes mellitus. Clin Immunol Immunopathol **2**:395–403, 1974.
11. Bagdade JD, Stewart M, Walters E. Impaired granulocyte adherence. A reversible defect in host defense in patients with poorly controlled diabetes. Diabetes **27**:677–681, 1978.
12. Bagdade JD, Root RK, Bulger RJ. Impaired leukocyte function in patients with poorly controlled diabetes. Diabetes **23**:9–15, 1974.
13. Bybee JD, Rogers DE. The phagocytic activity of polymorphonuclear leukocytes obtained from patients with diabetes mellitus. J Lab Clin Med **64**:1–13, 1964.
14. Nolan CM, Beaty HN, Bagdade JD. Further characterization of the impaired bactericidal function of granulocytes in patients with poorly controlled diabetes. Diabetes **27**:889–894, 1978.
15. Tan JS, Anderson JL, Watanakunakorn C, Phair JP. Neutrophil function in diabetes mellitus. J Lab Clin Med **85**:26–33, 1975.
16. Failla ML, Kiser RA. Altered tissue content and cytosol distribution of trace metals in experimental diabetes. J Nutr **111**:1900–1909, 1981.
17. Failla ML, Kiser RA. Hepatic and renal metabolism of copper and zinc in the diabetic rat. Amer J Physiol **244**, E115–E121, 1983.
18. Kelleher DL, Bagby GJ, Spitzer JJ. Glucose metabolism following endotoxin administration in diabetic rats. Horm Metab Res **12**:595–601, 1980.
19. Klebhanoff SJ. Antimicrobial mechanisms in leukocytes. Semin Hematol **12**:117–142, 1975.
20. George DT, Abeles FB, Mapes CA, Sobocinski PZ, Zenser TV, Powanda MC. Effect of leukocyte endogenous mediators on endocrine pancreas secretory responses. Amer J Physiol **233**:E240–E245, 1977.
21. Rayfield EJ, Curnow RT, Reinhard D, Kochicheril NM. Effect of acute endotoxemia on glucoregulation in normal and diabetic subjects. J Clin Endocrinol Metab **45**:513–521, 1977.
22. Begin-Heick N, Dalpe'-Scott M, Rowe J, Heick HMC. Zinc supplementation improves the insulin secretory response in the ob/ob mouse. Fed Proc **41**:1088, 1982.
23. Hurley LS, Gordon P, Keen CL, Merkhofer I. Circadian variation in rat plasma zinc and rapid effect of dietary zinc deficiency. Proc Soc Exp Biol Med **170**:48–52, 1982.

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