Behavioral Manipulation of the Diabetic Phenotype in *ob/ob* Mice

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SUMMARY

The genetically obese mouse (C57BL/6J *ob/ob*) is a commonly used model of non-insulin-dependent diabetes mellitus. However, our studies demonstrate that, while the animal is significantly hyperinsulinemic, it in fact does not show consistent hyperglycemia in the resting state. During stress, both obese animals and their lean littermates become hyperglycemic, but the magnitude of the hyperglycemia is exaggerated in the obese mice. Obese animals also show an exaggerated plasma glucose increase in response to epinephrine injection. This increase in plasma glucose is accompanied by a decrease in plasma insulin in response to both stress and epinephrine. Our findings suggest that environmental stimuli influence the expression of diabetes in the C57BL/6J obese mouse and therefore must be considered in studies of this animal model of diabetes. DIABETES *33*:616–618, July 1984.

The genetically obese mouse (C57BL/6J *ob/ob*) is characterized by a syndrome of obesity, hyperinsulinemia, insulin resistance, hyperglycemia, and glucose intolerance.[1] For this reason it has been used as a model of non-insulin-dependent diabetes in humans.[2] However, different laboratories report varying glucose levels in mice of the same age. For example, 12–16-wk-old fed animals' plasma glucose has been reported to be 500 mg/dl[3] and 359 mg/dl,[4] and blood glucose 200 mg/dl[5] and 150 mg/dl.[5] While this variability could result from substrain genetic variation, it is possible that different experimental environments may have affected the results obtained.

This study was first presented in abstract form at the Society for Psychophysiological Research 23rd Annual Meeting in Monterey, California, in September 1983.

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Received for publication 31 October 1983.

One major methodologic variable, seldom considered in experimental endocrinology, is environmental stress. There are few studies concerning the interaction of stress and behavior with the development or expression of diabetes in animals. Although Cannon[6] described stress-induced hyperglycemia in normal animals a half century ago, the effects of stress on spontaneously diabetic animals have not been studied. It has been postulated that stress can precipitate diabetes in susceptible individuals,[7,8] but the degree to which stress contributes to the expression of the diabetic phenotype is not known. We now report that the *ob/ob* mouse is not consistently hyperglycemic in the resting state, but that exaggerated hyperglycemia in response to stress or exogenous epinephrine is a characteristic of this strain.

MATERIALS AND METHODS

Two experiments were performed to study the effects of environmental stress and adrenergic responsivity on plasma glucose in this model of diabetes. Obese (C57BL/6J *ob/ob*) mice and their lean (C57BL/6J ob/?) littermates between the ages of 6 and 20 wk, obtained from the Jackson Laboratory (Bar Harbor, Maine) at 1 mo of age, were used in both experiments. All animals were housed in group cages, with four to six animals per cage. They were provided with water and Purina laboratory chow ad libitum. No animals except mice were present in the area in which these mice were kept. The mice were accustomed to repeated handling before initiating the experimental procedures. In the first experiment, six obese and nine lean animals were immobilized in wire mesh cages for 60 min. After 30 min of immobilization, they were given a 5-min period of moderate shaking (mechanical shaker, 200 strokes/min, 6-cm excursion). No animals appeared to suffer any behavioral effects of shaking. After 60 min, blood samples were drawn via retro-orbital sinus puncture. Plasma was analyzed for glucose, insulin, and corticosterone. Eight obese and 12 lean control animals remained undisturbed in their home cages until blood samples were drawn. Glucose was analyzed with a Beckman Glucose Analyzer. Insulin was determined via double-antibody radioimmunoassay with a commercially available kit

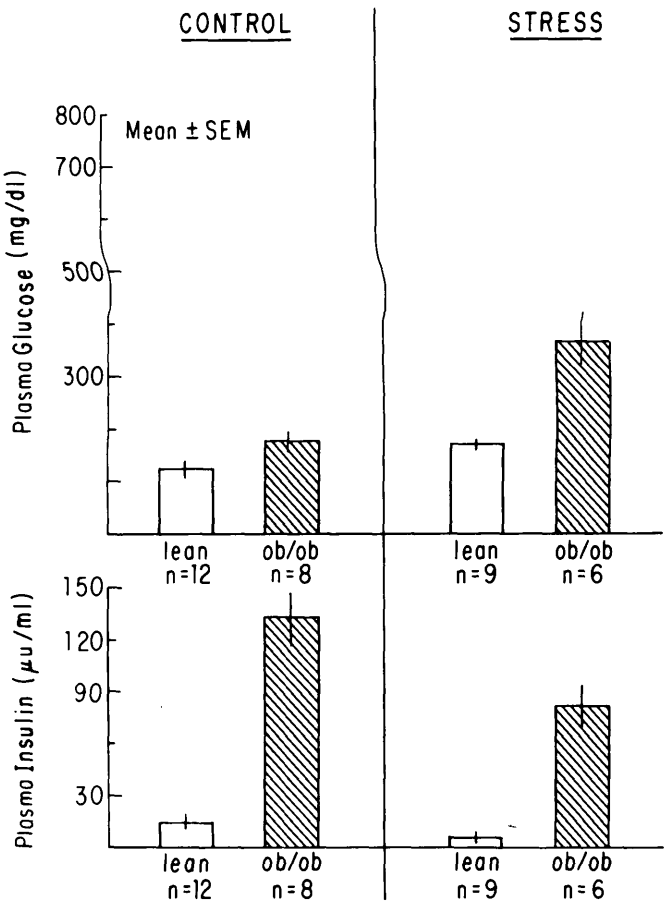

FIGURE 1. The effects of stress on plasma glucose and insulin in C57BL/6J *ob/ob* mice and their lean littermates (mean ± SEM).

(Cambridge Medical Diagnostics). Corticosterone was measured via radioimmunoassay using ^{3}H-corticosterone from New England Nuclear (Boston, Massachusetts) and antisera from Radio Assay Systems Laboratories (Carson, California), after extraction from plasma with ethyl acetate.

A similar design was used to investigate the effects of epinephrine on plasma glucose and insulin. Epinephrine bitartrate (3 μg/10 g body wt) was injected subcutaneously into six obese and ten lean mice. Six obese and nine lean controls were injected with an identical volume of saline. Retro-orbital blood samples were drawn for analysis 1 h after the injection. Stress and epinephrine effects were analyzed with a 2 × 2 factorial ANOVA for each dependent variable. Student's *t* tests were used to assess the effects of stress on corticosterone in obese and lean animals separately.

RESULTS

As is shown in Figures 1 and 2, resting plasma glucose values for lean and obese mice were not consistently different. Mean plasma glucose for 12 obese animals was 166 ± 16 mg/dl (mean ± SEM) while mean plasma glucose for 18 lean animals was 137 ± 8 mg/dl. As can be seen in Figure 1, immobilization and shaking produced an increase in plasma glucose in both lean and obese animals ($P < 0.01$). However, this effect was significantly greater in the obese animals than in their lean littermates ($P < 0.01$). Similarly, while plasma insulin decreased significantly in all stressed animals ($P < 0.01$), the decrease was greater in the obese animals ($P < 0.02$).

In agreement with previous reports,[4] basal plasma corticosterone was significantly ($P < 0.01$) higher in obese mice (114 ± 19 μg/ml) than in their lean littermates (41 ± 7 μg/ml). Plasma corticosterone was significantly increased during stress in both groups of animals (obese, 189 ± 18 μg/ml, $P < 0.05$; lean, 160 ± 8 μg/ml, $P < 0.001$), but stress did not affect obese and lean animals differentially.

The effects of epinephrine (Figure 2) were analogous to those of stress. Epinephrine produced a significant increase in plasma glucose in all animals ($P < 0.001$), with obese mice showing a greater response than their lean littermates ($P < 0.001$). This increase in plasma glucose was not accompanied by a compensatory increase in plasma insulin in lean animals, while in obese mice a significant ($P < 0.001$) decrease in plasma insulin occurred.

DISCUSSION

While obese animals were clearly hyperinsulinemic at baseline, resting plasma glucose values were not consistently elevated in obese as compared with lean mice. This is noteworthy in light of the reports of significantly elevated glucose levels in this strain. We have demonstrated that the experimental environment alone could account for these el-

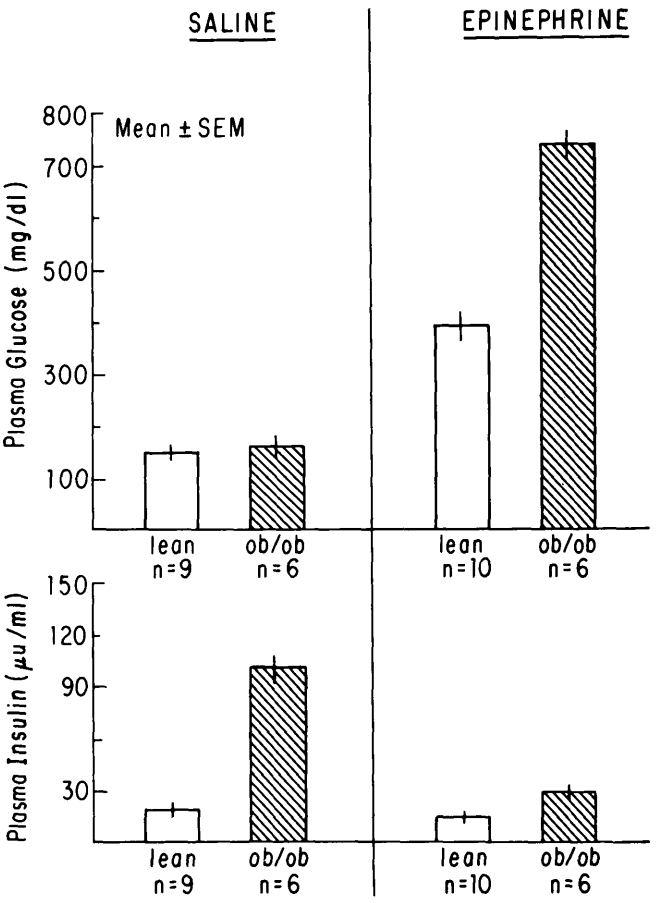

FIGURE 2. The effects of epinephrine (3 μg/10 g, injected s.c.) on plasma glucose and insulin in C57BL/6J *ob/ob* mice and their lean littermates (mean ± SEM).

evated values. This suggests that environmental stimulation may be partially responsible for the expression of the diabetic phenotype in this animal model of diabetes. One mechanism by which the environment can produce hyperglycemia is by activation of the sympathoadrenal axis. Such activation can produce, among other effects, the inhibition of glucose-stimulated insulin secretion.[8,9] Our finding that both stress and epinephrine increase plasma glucose and decrease plasma insulin in obese more than in lean animals suggests altered adrenergic sensitivity of the pancreatic islets and possibly of other sites as well. Because stress did not differentially affect corticosterone in the obese mice, our data do not support a primary role for it in the exaggerated glycemic response of these animals to stress. However, it is possible that chronic elevation of circulating corticosterone in obese mice[1] contributes to the altered adrenergic responsivity[10] or to other mechanisms involved in stress-induced hyperglycemia. Fujimoto et al.[11] reported that the KK mouse, also a diabetic model, has a similar hypersensitivity to epinephrine, as well as a greater insulin secretory response to isoproterenol. They argued that heightened β-adrenergic sensitivity could be responsible for both the hyperinsulinemia and insulin resistance of the KK strain. Further studies are required to elucidate the mechanism of the increased susceptibility to stress in the *ob/ob* strain.

Details of the experimental environment and animal handling are not traditionally included in metabolic studies. Our data indicate that environmental conditions must be considered for the results of such studies to be properly interpreted. In addition, the findings of these animal studies are important in light of our recent demonstration that progressive relaxation training improves glucose tolerance in non-insulin-dependent diabetic humans.[12] The animal model that we have described might prove useful for investigating pharmacologic and behavioral factors of potential therapeutic benefit for the non-insulin-dependent diabetic patient.

ACKNOWLEDGMENTS

This study was supported in part by NIMH grant no. MH-K01-00303 and NIH grant no. R01 AM-29989. Data were analyzed using the Clinfo Data Analysis System of the Clinical Research Unit of Duke University Medical Center (C.G.R.C. RR-30).

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