*Pharmacology Biochemistry & Behavior*, Vol 26, pp 491–495 Copyright © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

# Exaggerated Peripheral Responses to Catecholamines Contributes to Stress-Induced Hyperglycemia in the ob/ob Mouse[1]

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KUHN, C M , C COCHRANE, M N FEINGLOS AND R S SURWIT *Exaggerated peripheral responses to catecholamines contributes to stress-induced hyperglycemia in the ob/ob mouse* PHARMACOL BIOCHEM BEHAV **26**(3) 491–495, 1987 —The present study investigated the contribution of altered sympathetic reactivity to the stress-induced hyperglycemia observed in the c57BL/6J (ob/ob) mouse, an animal model of type II diabetes Blood glucose and insulin responses to sympathetic agonist and antagonist administration were evaluated in ob/ob mice and their nondiabetic, lean (ob/?) littermates In addition, the ability of nutritional status to modify these responses was determined These studies demonstrated that epinephrine administration to ob/ob mice caused an exaggerated increase in blood glucose and decrease in plasma insulin in ob/ob mice relative to lean littermates The dose response curve for epinephrine-induced increases in blood glucose were shifted to the left, and the duration of the blood glucose and plasma insulin responses was longer Differences between ob/ob mice and their nondiabetic littermates were greater when animals were tested in the fasted state In addition, administration of the alpha adrenergic antagonist phentolamine caused a larger increase in plasma insulin in ob/ob mice than was observed in lean littermates These results suggest that altered peripheral responses to sympathetic stimuli contribute to stress-induced hyperglycemia in ob/ob mice, and raise the possibility that altered sympathetic function is an etiologic factor in development of diabetes in these animals

Diabetes Stress Sympathetic nervous system

NON-INSULIN dependent (type II) diabetes in humans is characterized by obesity, hyperinsulinemia, insulin resistance, hyperglycemia and glucose intolerance A number of animal models of this disease exist, including the genetically obese mouse (c57BL/6J, ob/ob), which shows a physiologic profile like that described above [4]. Although the ob/ob mouse is clearly diabetic, there is a wide variation in reported values for plasma glucose, which range from mildly elevated (150 mg/dl) to extremely hyperglycemic (500 mg/dl) [8, 11, 12]. Recent studies from this laboratory have shown that basal glucose in the fed ob/ob mouse is not consistently elevated in comparison to littermates, but that plasma glucose is markedly hyperreactive to environmental stress [19,20]. We showed further that the blood glucose response to a single dose of epinephrine is significantly greater in ob/ob mice [19] This finding suggests that altered postsynaptic responsivity to sympathetic agonists contributes to exaggerated glycemic responses in ob/ob mice, an hypothesis which is supported by similar findings of enhanced glycemic responses to adrenergic agonists in a similar mouse model of non-insulin dependent diabetes [10] Furthermore, sympathetic activity, as assessed by norepinephrine turnover, is normal in most peripheral organs including heart, spleen and pancreas of adult ob/ob mice, further supporting a postsynaptic site as the mediator of supersensitivity [13]

The goal of the present study was to investigate further the contribution of altered peripheral sympathetic function to the exaggerated glycemic response to ob/ob mice to stress To investigate this question, plasma glucose and insulin responses to a sympathetic agonist (epinephrine) and a sympathetic antagonist (phentolamine) were determined in ob/ob mice and their lean (ob/?) littermates. The results of this study support our previous hypothesis that exaggerated peripheral responses to sympathetic stimuli contribute to the exaggerated glycemic responses of ob/ob mice to stress.

## METHOD

Obese (c57BL/6J, ob/ob) and their lean littermates (c57BL/6J, ob/?) were obtained from the Jackson Laboratory (Bar Harbor, ME) at 1 month of age Animals were used for

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TABLE 1
PLASMA GLUCOSE AND INSULIN IN OBESE AND LEAN MICE

| | Glucsoe (mg/dl) | Insulin ($\mu$U/ml) |
|---|---|---|
| Lean | | |
| Fed | 153 ± 7 | 40 ± 5 |
| Fasted | 91 ± 4* | 16 ± 3 |
| Obese | | |
| Fed | 184 ± 8 | 544 ± 39 |
| Fasted | 185 ± 8 | 223 ± 32* |

Mice were fed ad lib, or were fasted for 18 hours, and blood was collected by retroorbital sinus puncture, centrifuged and plasma assayed for glucose and insulin N=10–15 in each group *Indicates $p<0.05$ or better relative to fed control

experiments between 2 and 3 months of age (20–30 g body weight for leans, 40–50 g body weight for ob/ob) Animals were housed in group cages with four to six animals per cage in an animal room with a 12 hour light-12 hour dark day-night cycle They were provided with water and Purina laboratory chow ad lib, except in the fasting experiment, in which food was removed for a 24 hour period before the experiment. The mice were accustomed to handling and blood collection before the experiments by daily handling and two basal blood collections during a 1 month habituation period

Animals were stressed for 1 hour as described previously by immobilization combined with shaking [19], or were injected subcutaneously with the indicated dose ($\mu$g salt/10 g body weight) of epinephrine bitartrate or phentolamine mesylate. Epinephrine was used as a model adrenergic agonist which has been shown to stimulate both alpha and beta receptors in peripheral organs of mice at the doses used [10] Phentolamine was used as an alpha adrenergic antagonist which is known to elevate insulin secretion in normal and diabetic mice [10], presumably by blockade of alpha receptors in the pancreas Controls in the immobilization experiment were not handled, while controls in the epinephrine experiment were injected subcutaneously with an equal volume of saline

After one hour of stress, or at the indicated time after drug injection, blood (300 $\mu$l) was collected by retroorbital sinus puncture into heparinized pipettes. Plasma was separated by centrifugation at 1,000 × g for 15 minutes, and frozen for later analysis

Plasma glucose was analyzed with a Beckman Glucose analyzer Insulin was determined with a double-antibody radioimmunoassay using a commercially available kit (Cambridge Medical Diagnostics).

Results were analyzed by 3-way ANOVA and post-hoc unpaired Student's $t$-tests to determine the significance of single points.

## RESULTS

### *Basal Glucose and Response to Stress*

Table 1 shows basal plasma glucose in ob/ob and lean (+/?) littermates in the fed or fasted state. As reported previously [6], basal plasma glucose values in fed ob/ob mice were not different from their lean littermates. However, a significant difference between lean and obese mice was observed in basal blood plasma glucose in the fasted state ($p<0.05$) Ob/ob animals were markedly hyperinsulinemic in the fed or fasted state ($p<0.05$ for an effect of nutritional status) In addition, the plasma glucose and plasma insulin responses to immobilization stress were exaggerated in fed ob/ob mice in comparison to lean (ob/?) controls (Fig 1) Both the increase in glucose and the decrease in insulin were significantly greater in obese than in lean mice ($p<0.01$ for an effect of stress, $p<0.01$ for an effect of strain and $p<0.05$ for the interaction of strain with stress response for glucose, $p<0.05$ for the interaction of strain with stress response for glucose, $p<0.05$ for the interaction of strain and stress effects for insulin).

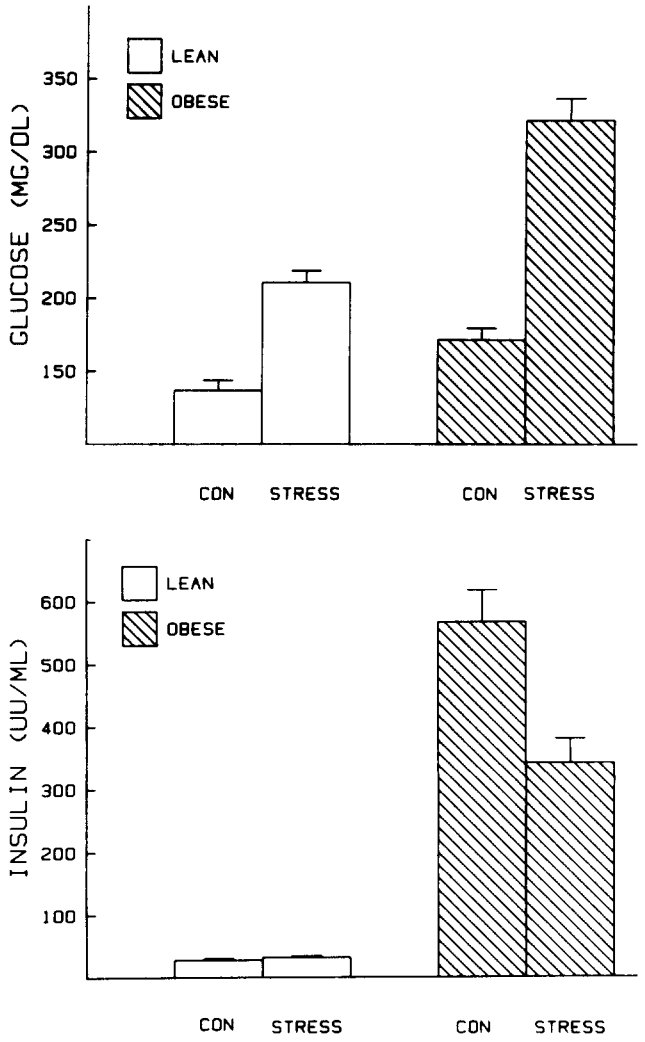


FIG 1 Effect of immobilization stress on plasma glucose and insulin in ob/ob and lean mice Mice were bled after 60 minutes of immobilization and plasma glucose and insulin determined as described in the Method section Results are expressed as mean±SEM (mg/dl) glucose or ($\mu$ units/ml) insulin N=9–12 in each group *Indicates statistically different from nonstressed control, $p<0.05$ or better

### *Response to Epinephrine*

The appearance of significant differences between blood

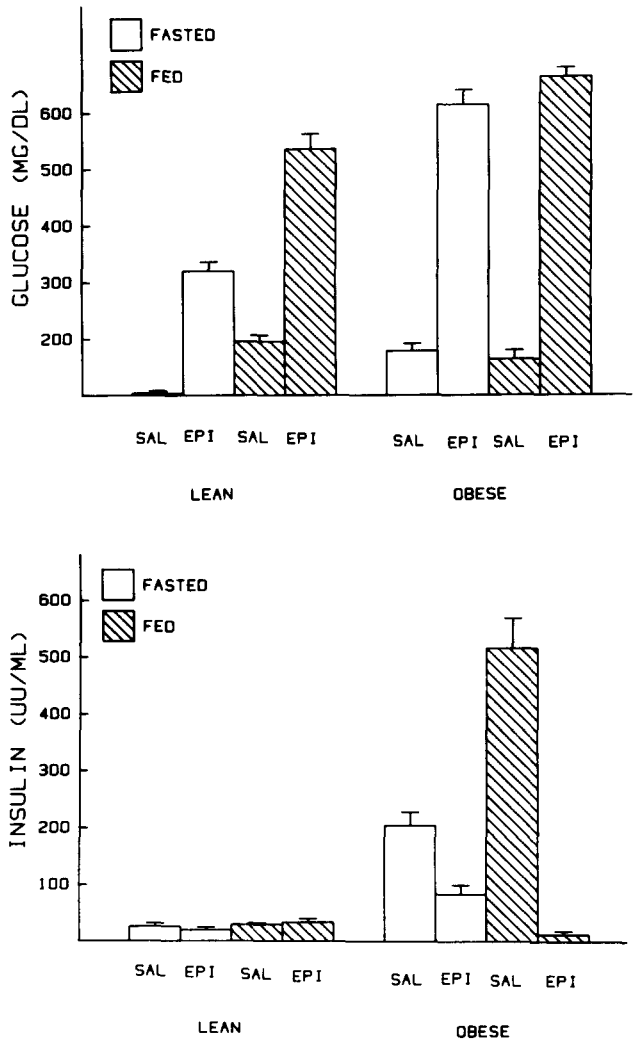


FIG 2 Plasma glucose and insulin response to epinephrine in fed and fasted mice Plasma glucose was determined 30 minutes after epinephrine (3 $\mu$g/10 g b wt ) Controls were injected at the same time with saline Results are expressed as mean±SEM glucose (mg/dl) or insulin ($\mu$ units/ml) N=8–10 in each group *Indicates statistically different from fasted control, $p<0$ 05 or better

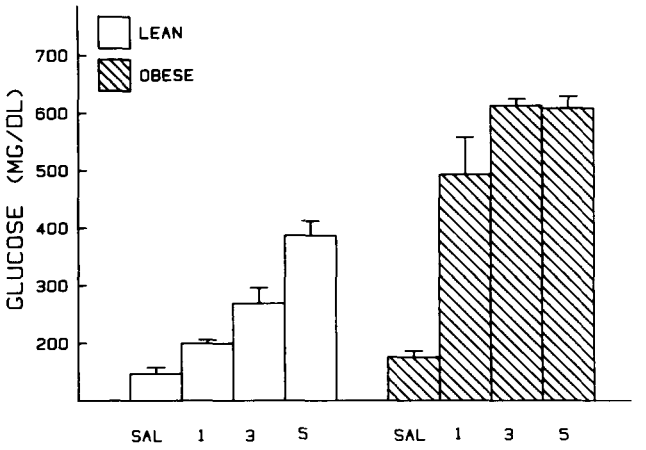


FIG 3 Effect of increasing doses of epinephrine in plasma glucose in ob/ob and lean mice Mice were bled 60 minutes after epinephrine administration Saline-injected controls are shown as sal Dose is expressed as $\mu$g salt/10 g b wt Results are expressed as mean±SEM glucose (mg/dl) N=5–8 in each group

glucose of lean and ob mice only in fasted animals suggested that further characterization of sympathetic responsivity in these animals required careful consideration of nutritional status. Therefore, we repeated our original study of glucose and insulin responses to epinephrine under both fed and fasted conditions The results of this experiment are shown in Fig. 2. The increase in blood glucose after epinephrine in lean animals was considerably greater in the fed state ($p<0$ 01 for an effect of treatment, $p<0.01$ for a strain effect, and $p<0$ 01 for an interaction of strain with response to epinephrine). The glucose response to epinephrine in obese mice did not change with nutritional status. Therefore, the differences between the obese and lean mice were greater in the fasted state ($p<0.01$ for an interaction of strain with nutritional status). Interestingly, plasma insulin levels in lean animals were not affected by epinephrine at this dose, while insulin was markedly suppressed in both fed and fasted obese mice ($p<0.01$ for an interaction of strain with response to epinephrine).

The plasma glucose response of fasted obese and lean mice to varying doses of epinephrine is shown in Fig. 3. While epinephrine caused a dose related increase in plasma glucose in lean animals in the dose range from 1–5 $\mu$g/10 g body weight (10 $\mu$g/10 g proved toxic), plasma glucose responses were maximal at the lowest dose of epinephrine tested in obese mice ($p<0.01$ for an effect of dose, $p<0.01$ for a difference between obese and lean mice, and $p<0.01$ for an interaction of strain with epinephrine dose)

To determine if the apparent differences in the dose-response relationship for plasma glucose elevation by epinephrine in obese and lean mice resulted from a shift in the time course of epinephrine action in the obese animals, the time course of the glucose and insulin response to a submaximal dose of epinephrine was tested. The results of this experiment are shown in Figs. 4 and 5. Epinephrine administration caused a marked rise in plasma glucose in both obese and lean mice, although the increase was significantly greater in obese animals ($p<0$ 01 for an effect of time, $p<0.01$ for an effect of strain, and $p<0$ 01 for an interaction of strain with time). The effects of epinephrine on plasma insulin were striking (Fig. 5). Epinephrine caused an extremely rapid and marked suppression of plasma insulin in obese mice, but caused no change in plasma insulin of lean mice ($p<0$ 01 for an effect of time, $p<0.01$ for a difference between strains and $p<0.01$ for an interaction of time with strain).

*Response to Phentolamine*

In normal animals, sympathetic stimuli inhibit insulin secretion tonically via an alpha adrenergic mechanism [16]. The results of the previous experiment suggested that postsynaptic responses to exogenous challenge were markedly abnormal in obese mice However, this experiment did not evaluate the response of the pancreas to changes in endogenous neural activity. To determine if manipulation of endogenous sympathetic activity evoked similarly marked

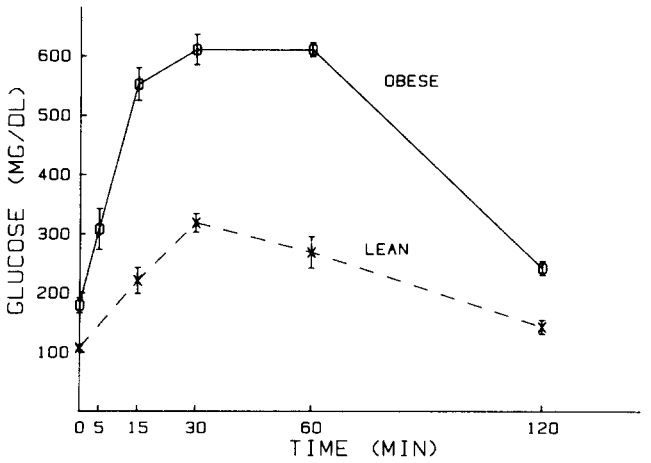


FIG 4 Time course of plasma glucose response to epinephrine (3 μg/10 g b.wt ) Animals were injected with epinephrine at t=0, and different groups of animals were bled at the indicated time after injection As no significant effect of saline injection on basal glucose was observed, control glucose values have been pooled and shown at t=0 Results are expressed as mean±SEM glucose (mg/dl) Dotted line indicates ob/ob mice, and solid line represents data from lean controls N=8–12 in each group

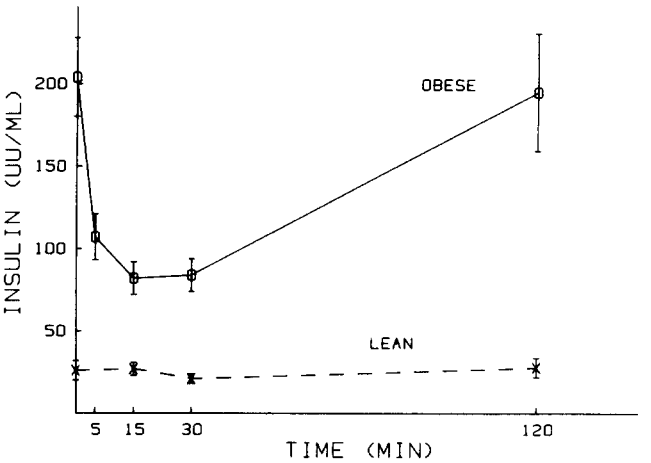


FIG 5 Time course of plasma insulin response to epinephrine Animals were treated as described in legend to Fig 5 Results are expressed as mean±SEM insulin (μ units/ml) Dotted line represents ob/ob mice, and solid line represents lean controls N=8–12 in each group

changes in plasma insulin, insulin responses to adrenergic blockade with phentolamine were evaluated. In this experiment, both fed and fasted animals were tested, as tonic sympathetic activity to the pancreas is affected significantly by nutritional status [14] The results of this experiment are shown in Fig. 6. In obese animals, basal insulin levels were markedly higher, and were increased more (in terms of absolute increases in insulin) than in leans ($p<0.01$ for an effect of drug, $p<0$ 01 for an effect of strain, and $p<0$ 05 for an interaction of strain with response to phentolamine). Interestingly, nutritional status did not have a dramatic effect on the insulin response to adrenergic blockade. Blood glucose was decreased slightly but significantly in fed mice (303±21 to 252±11 in obese mice, from 177±17 to 134±9 mg/dl in fed lean mice, $p<0.01$ for an effect of treatment) but did not change significantly in fasted animals.

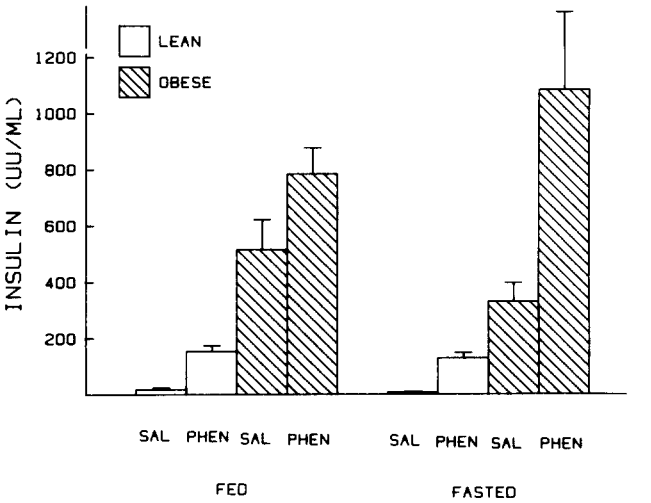


FIG 6 Plasma insulin response to phentolamine Animals were bled 60 minutes after injection with saline or phentolamine (200 μg/10 g b wt ) Results are expressed as mean±SEM insulin (μ units/ml) *Indicates statistically different from fed control N=8–10 in each group

## DISCUSSION

The results of this study confirm and extend our previous reports of exaggerated glycemic responses of ob/ob mice to both behavioral and pharmacologic manipulations. The present findings demonstrate clearly that blood glucose responses to the adrenergic agonist epinephrine are markedly enhanced in ob/ob mice in comparison to their lean littermates. The increased glycemic responses of ob/ob mice to stress or to epinephrine are accompanied by marked suppression of insulin secretion in diabetic but not in control mice. These results corroborate a previous report of altered blood glucose and insulin responses to sympathetic stimuli in another mouse model of non-insulin-dependent diabetes, the KK mouse [10]

The findings of this study suggest that altered peripheral responses to sympathetic stimuli contribute to the enhanced glycemic responses of ob/ob mice to behavioral stimuli The pattern of increased glucose and suppressed insulin parallels the changes elicited by stress Furthermore, reports that cold-stressed-induced increases in norepinephrine turnover in fat, pancreas, liver and heart in ob/ob mice [13] suggest that increased nerve impulse activity does not contribute to the observed effects. Finally, we have demonstrated previously that glucocorticoid secretion, the other likely mediator of stress effects, is not differentially affected by stress in obese mice [19] However, recent reports that endogenous opioids enhance glycemic responses to epinephrine in dogs [9], in combination with the number of studies demonstrating abnormalities in endogenous opioid function in ob/ob mice

[15,17], suggest that stress-induced changes in endogenous opioid secretion could also contribute to this phenomenon.

An even more intriguing possibility raised by these findings is that altered sympathetic function is an etiologic factor in the development of diabetes in this mouse strain. Our findings suggest that altered peripheral responsivity to adrenergic agonists in peripheral organs involved in glucose output or glucose uptake might contribute significantly to the hyperglycemia observed in the ob/ob mouse. However, such an hypothesis must be tested cautiously, as the exaggerated glycemic response observed in this study might result in part from other factors which alter the cellular response to catecholamines. A similarly enhanced response might be observed following changes in levels of other glucoregulatory hormones.

The relative contribution of altered insulin secretion and peripheral glucose utilization to the hyperglycemia caused by epinephrine administration is not clear. Phentolamine administration to these animals elicited a large change in insulin secretion that was associated with a suppression of blood glucose. This finding suggests that decreases in insulin associated with stress or epinephrine administration contribute to the observed changes in blood glucose. However, impaired peripheral utilization of glucose, which is markedly abnormal in these animals, could also contribute to this effect [4].

The specific receptor defect mediating the exaggerated glucose response to epinephrine is not clear. One simple possibility is that pancreatic or peripheral adrenergic receptors are supersensitive as a consequence of diabetic neuropathy, as has been reported in db/db mice [5]. However, an alternative is suggested by recent biochemical studies of adenylate cyclase function in ob/ob mice. Studies in normal mice suggest that epinephrine-induced increases in glucose reflect a combination of alpha-2 receptor mediated inhibition of insulin secretion, and beta receptor mediated changes fat and glucose metabolism [16,18]. Several laboratories have shown that inhibitory influences on adenylate cyclase are less effective in ob/ob mice because of a change in the interaction of the guanine nucleotide regulatory protein with the catalytic subunit of adenylate cyclase [1, 2, 7]. This effect could result in a failure of normal inhibitory influences to offset beta adrenergic stimulation of cyclase This hypothesis is supported by the reported ability of the beta antagonist propranolol to block epinephrine-induced hyperglycemia in the absence of changes in insulin [10]. Although this conclusion is highly speculative, it represents an avenue for further investigation into this problem.

In summary, the findings of the present study suggest that the enhanced glycemic responses of ob/ob mice to behavioral stimuli result in part from exaggerated peripheral responses to changes in sympathetic stimuli The increased glucose and insulin responses to epinephrine observed in the present study support a role for altered receptor and/or post-receptor responses in this effect. However, sympathetic stimulation of the pancreas or peripheral tissues could also contribute to this phenomenon An additional finding of the present study is that plasma insulin, like glucose, is extremely sensitive to behavioral and pharmacologic manipulations in ob/ob mice. This lability of insulin secretion might provide a significant mechanism for glucoregulation, despite the relative insulin resistance that has been reported in these animals

## REFERENCES


1 Begin-Heick, N Adenylate cyclase in lean and obese (ob/ob) epididymal white adipocytes *Can J Biochem* **58:** 1033–1038, 1980

2 Begin-Heick, N The interaction of guanine nucleotides with the adenylate cyclase complex in white adipocyte membranes of lean and obese (ob/ob) mice *Can J Biochem* **62:** 816–820, 1984

3 Beloff-Chain, A , N Freund and K A Rookledge Blood glucose and serum insulin levels in lean and genetically obese mice *Horm Metab Res* **1:** 374–378, 1975

4 Bray, G A. and D A York Hypothalamic and genetic obesity in experimental animals an autonomic and endocrine hypothesis *Physiol Rev* **59:** 719–809, 1979

5 Brown, M R., P J Dyck, G E McClearn, A A Sima, H C Powell and D Porte Central and peripheral nervous system complications *Diabetes* **31:** Suppl 1, 65–70, 1982

6 Danowski, T S Emotional stress as a cause of diabetes *Diabetes* **12:** 183–184, 1963

7 Dehaye, J P , J Winand and J Christophe Adrenergic mechanisms in the hyperglycemia and hyperinsulinemia of diabetic kk mice *Diabetologia* **15:** 45–51, 1978

8 Dubuc, P U , P J Cahn, S Ristimaki and P L Willis Starvation and age effects on glycoregulation and hormone levels of C57BL 16j ob/ob mice *Horm Metab Res* **14:** 532–535, 1982

9 Eutayeb, K M A , B M Arano and H L A Lickey Beta-endorphin modulation of the glucoregulatory effects of repeated epinephrine infusion in normal dogs *Diabetes* **34:** 1293–1300, 1985

10 Fujimoto, K , T Sakaguchi and M Ui Adrenergic mechanisms in the hyperglycemia and hyperinsulinemia of diabetic kk mice *Diabetologia* **20:** 568–572, 1981

11 Garthwaite, L , D R Martinson, L F Tseng, T C Hagen and J Menahan A longitudinal hormonal profile of the genetically obese mouse *Endocrinology* **107:** 671–676, 1980

12 Grundleger, M L , V Y Godbole and S W Thenen Age dependent development of insulin resistance of soleus muscle in genetically obese (ob/ob) mice *Am J Physiol* **239:** E363–E371, 1980

13 Knehans, A W and D R Romsos Norepinephrine turnover in obese (ob/ob) mice effects of age, fasting and acute cold *Am J Physiol* **244:** E567–E574, 1983

14 Landsberg, L and J B Young Fasting, feeding and regulation of the sympathetic nervous system *N Engl J Med* **298:** 1295–1301, 1978

15 Margules, D L , B Moisset, M J Lewis, H Shibuya and C B Pert Beta-endorphin is associated with overeating in genetically obese (ob/ob) mice and rats (fa/fa) *Science* **202:** 988–989, 1978

16 Nakadate, T , T Nakaki, T Muraki and R Kato Regulation of plasma insulin levels by alpha-2 adrenergic receptors *Eur J Pharmacol* **65:** 421–424, 1980

17 Rossier, J , J Rogers, T Shibasaki, R Guillemin and F E Bloom Opioid peptides and alpha melanocyte-stimulating hormone in genetically obese (ob/ob) mice during development *Proc Natl Acad Sci USA* **76:** 2077–2080, 1979

18 Shikama, H and M Ui Adrenergic receptor and epinephrine-induced hyperglycemia and glucose tolerance *Am J Physiol* **229:** 962–966, 1975

19 Surwit, R S , M N Feinglos, E G Livingston, C M Kuhn and J A McCubbin Behavioral manipulation of the diabetic phenotype in ob/ob mice *Diabetes* **33:** 616–618, 1984

20 Surwit, R S , J A McCubbin, C M Kuhn, D McGee, D Gerstenfeld and M N Feinglos Alprazolam reduces stress hyperglycemia in ob/ob mice *Psychosom Med* **48:** 278–282, 1986