



PAPER

Increased responsiveness to the hyperglycemic, hyperglucagonemic and hyperinsulinemic effects of circulating norepinephrine in *ob/ob* mice

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OBJECTIVE: Several studies have implicated increased sympathetic tone as a contributing factor to the hyperglycemia and hyperglucagonemia of *ob/ob* mice. However, the responsiveness of plasma glucose, insulin and glucagon to circulating norepinephrine (NE) in *ob/ob* vs normal lean mice has never been described. Therefore, the present study investigated the effect of a 15 min intravenous NE infusion (1 pmol/min/g) on plasma glucose, insulin and glucagon in anesthetized lean, *ob/ob*, *ob/ob*-concurrent yohimbine (α_2 antagonist) treated, and *ob/ob*-chronically sympatholytic dopamine agonist treated (for 14 days prior to infusion) mice. In an effort to gain insight into a possible relation between norepinephrine, hyperglucagonemia and hyperinsulinemia in *ob/ob* mice, this study also examined the isolated islet responses to NE and glucagon in lean, *ob/ob* and *ob/ob*-sympatholytic dopamine agonist treated mice.

RESULTS: Basal humoral values of glucose, insulin and glucagon were all elevated in *ob/ob* vs lean mice (by 63, 1900 and 63%, respectively, $P < 0.01$). However, NE infusion further increased levels of glucose, insulin and glucagon in *ob/ob* (by 80, 90 and 60%, respectively, $P < 0.05$) but not in lean mice (between group difference for all parameters $P < 0.05$). Acute concurrent yohimbine treatment as well as chronic prior sympatholytic dopamine agonist treatment (bromocriptine plus SKF38393) simultaneously strongly abrogated or abolished all these humoral hypersensitivity responses to intravenous NE in *ob/ob* mice ($P < 0.05$). Clamping the plasma glucose level in untreated *ob/ob* mice at a high level (30 mM) established by NE infusion did not significantly alter the plasma insulin level, suggesting that some other influence of NE was responsible for this insulin effect. Direct NE administration at 1 μ M to islets from lean and *ob/ob* mice inhibited 15 mM glucose-stimulated insulin secretion in both groups, but at 0.1 μ M it was inhibitory only in islets from *ob/ob* mice. However, glucagon (10 nM) increased 15 mM glucose-stimulated insulin secretion in *ob/ob* (by 170%, $P < 0.05$) but not lean mice (between group difference $P < 0.05$).

CONCLUSION: These findings suggest that hypersensitivity to circulating NE may potentiate hyperglycemia and hyperglucagonemia in *ob/ob* mice, and the subsequent hyperglucagonemia coupled with increased islet β -cell insulin secretory responsiveness to glucagon in *ob/ob* mice may support hyperinsulinemia, thus explaining the increased plasma insulin level response to intravenous NE in these animals. These findings further support a role for increased peripheral noradrenergic activities in the development and maintenance of the hyperglycemic, hyperglucagonemic and hyperinsulinemic state, characteristic of type 2 diabetes.

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Introduction

The leptin deficient, obese C57BL/6J mouse (*ob/ob*) is characterized by numerous neuroendocrine and metabolic abnormalities including a decreased thermogenic activity of brown adipose tissue (BAT).^{1,2} The decreased thermogenic

capacity of BAT is associated with decreased sympathetic nervous system (SNS) activation of the tissue.² This decreased SNS activation is due in part to the absence of leptin, which is a potent stimulus for SNS stimulation of BAT.^{3,4} Furthermore, this influence of leptin is mediated centrally within the ventromedial hypothalamus (VMH), wherein it activates SNS fibers innervating BAT in the periphery.⁵ A second contributing factor to the decreased SNS stimulation of BAT in *ob/ob* mice is the increased VMH levels of and responsiveness to norepinephrine (NE) in these animals.^{6–9} NE activity within the VMH inhibits glutamate-

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evoked neuronal activation therein,⁸ which is a strong stimulus for SNS activation of BAT.^{10,11}

Despite these VMH influences to reduce SNS stimulation of BAT thermogenesis in *ob/ob* mice, data from several sources indicate that increases in other arms of the SNS may contribute to the increases in plasma glucose and glucagon observed in these animals and other animal models of the obese insulin resistant state. First, increased VMH NE activity, which is a common feature among a wide variety of obese insulin resistant animal models including the *ob/ob* mouse,^{6-9,12-15} is known to stimulate hepatic glucose output (HGO), adipose lipolysis and glucagon secretion via activation of the SNS.¹⁶⁻¹⁹ Such VMH NE activities may indirectly increase plasma insulin levels via increases in plasma glucagon, islet β -cell responsiveness to glucagon, and/or plasma glucose and free fatty acid (FFA) levels, as well as via desensitization of islet responsiveness to NE and/or increased responsiveness to acetylcholine.²⁰⁻²⁵ Chronic NE infusion into the VMH of normal rodents produces glucose intolerance, hyperglucagonemia and hyperinsulinemia as well as leptin resistance while increasing circulating norepinephrine levels.²⁵⁻²⁷ Secondly, increased corticotropin-releasing hormone stimulation of the dorsomedial hypothalamus (DMH) observed in *ob/ob* mice²⁸ can potentiate SNS stimulation of HGO.²⁹ Thirdly, chemical sympathectomy reduces elevated plasma glucose and insulin levels in *ob/ob* but not lean mice.³⁰ Fourthly, hyperglycemic responses to adrenergic agonists are exaggerated in *ob/ob* vs lean mice,³¹ and importantly the urinary excretion rates for NE in *ob/ob* mice are equal to or greater than that observed in lean mice.³²

Therefore, increased liver and pancreatic tissue responsiveness to peripheral NE may contribute to the increased HGO, hyperinglucagonemia and hyperinsulinemia observed in *ob/ob* mice and other obese-diabetic species. However, the relative influence of plasma NE on plasma glucose, insulin and glucagon levels in hyperglycemic *ob/ob* vs normal mice has never been investigated. Therefore, the present study was undertaken in an effort to ascertain an involvement of peripheral norepinephrine in the development of this metabolic syndrome. Specifically, the present study investigated (a) the plasma glucose, insulin and glucagon responses to intravenous NE infusion in *ob/ob* and normal mice, (b) the effect of acute (yohimbine) or chronic (sympatholytic dopamine agonist treatment) noradrenergic blockade on the above humoral responses to intravenous NE in *ob/ob* mice, and (c) the insulin secretory responses to NE and glucagon in isolated islets of lean, *ob/ob* and *ob/ob*-sympatholytic dopamine agonist treated mice.

Methods

Experimental design

Female C57BL/6J obese (*ob/ob*) mice (body weight: 40–45 g, 6–7 weeks of age) and their lean littermates (+/?) were purchased from Jackson Laboratory (Bar Harbor, Maine) and

housed on 12 h daily photoperiods (light onset 07:00). After a 7 day adaptation to the animal care facility, *ob/ob* mice were randomly divided into two groups and injected (i.p.) daily for 14 days with either vehicle (0.005% aqueous ethanol) or bromocriptine (BC; 13 mg/kg/day) plus SKF38393 (SKF; 10 mg/kg/day) at 1 h after light onset. Bromocriptine is a potent sympatholytic agent by virtue of its D₂ agonist, α_1 antagonist and α_2 agonist properties.³³⁻³⁵ SKF38393 is a D₁ agonist that enhances central nervous system responses to D₂ agonists such as bromocriptine.³⁶ Beyond being sympatholytic, chronic BC/SKF treatment also reduces tissue responsiveness to NE (via the hypothalamus) and abrogates the metabolic syndrome in *ob/ob* mice.^{8,9,13,28,37,38} Lean mice were also similarly vehicle treated. Approximately 24–28 h after the termination of the 14 day treatment, mice were utilized (at 07:00–11:00 h) for either (a) *in vivo* plasma glucose, insulin and glucagon responses to intravenous norepinephrine (NE) infusion, or (b) *in vitro* insulin release studies. The experimental protocol was reviewed and approved by the animal care committee at Ergo Science Corp.

The *in vivo* plasma glucose, insulin and glucagon responses to NE

Mice were anesthetized with sodium pentobarbital (60 mg/kg body weight) and a catheter was inserted into the right jugular vein for drug delivery and blood sample collection. Following a 60 min recovery period from surgery, an initial blood sample was collected to measure basal blood glucose, plasma insulin and glucagon levels. Then, NE was infused intravenously (1 pmol/min/g) through the catheter for 15 min with or without yohimbine, a potent noradrenergic α_2 antagonist (1 pmol/min/g; 5 min prior to and throughout the 15 min NE infusion). Subsequently, a second blood sample was collected to measure these aforementioned parameters. The NE dose was based on studies of urinary NE excretion in female *ob/ob* mice.³² The increase in blood volume is proportionate to the weight gain in *ob/ob* vs lean mice. As such, the volume distribution of drug delivery based on body weight is similar between the two groups.

The *in vitro* insulin secretory response to NE and glucagon

Pancreatic islets were isolated from mice by collagenase digestion and Ficoll gradient centrifugation, as previously described.³⁹ Islets (10 islets per incubation well) were pre-incubated at 37°C with Krebs–Ringer–HEPES buffer (pH 7.4) without glucose for 20 min. Islets were then transferred to another well with 4 ml of this incubation buffer containing glucose (15 mM) with or without NE (0.1 μ M) or glucagon (10 nM; time 0) and incubated for 60 min at 37°C in a metabolic shaker.⁴⁰ A 20 μ l aliquot of the incubation buffer was sampled at time 0 and 60 min to measure insulin concentration. A sample of 20 islets was collected before incubation and stored at –80°C until subsequent measurements of

islet DNA and insulin content were performed. All analyses were performed in duplicate. Insulin secretion data were computed both as percentage of baseline islet insulin content and as fmol insulin/ng DNA/h. for comparative analyses.

Analytical procedures

Blood glucose level was measured by a glucose monitor (Accu-Check Advantage, Boehringer, Indianapolis, IN). Commercially available RIA kits (Linco Research, Inc., St Charles, MO) were utilized to measure plasma insulin and glucagon, as well as the insulin content in the incubation buffer of the static islet incubation.¹¹ Plasma FFA and triglycerides (TG) levels were measured via enzymatic assays from commercially available kits as previously described²³ (Wako Chemicals, Richmond VA and Sigma Chemical Co., St Louis, MO, respectively). The DNA content of the islet tissue was determined using a fluorometric method using a DyNA Quant 200 Fluorometer (Hoefer Pharmacia Biotech).²³ Insulin content in isolated islets was measured by RIA following acid-ethanol extraction.²³ Data are presented as mean \pm s.e.m. and ANOVA followed by the Student *t*-test as appropriate was applied for the statistical analyses of parameter differences between treatment groups.

Results

Effect of intravenous NE on plasma glucose, insulin and glucagon levels

Basal (time 0) values for glucose, insulin, and glucagon were all elevated in *ob/ob* vs lean mice (by 63, 1900 and 63%, respectively; $P < 0.01$). In lean mice, a 15 min i.v. infusion of NE (1 pmol/min/g) induced no significant change in either blood glucose, plasma insulin or glucagon levels. In vehicle-treated obese mice, however, this NE infusion triggered a 1.8-fold increase of blood glucose ($P < 0.01$), and 1.9-fold increase in plasma glucagon level ($P < 0.01$). The plasma insulin level of *ob/ob* mice was not reduced, but rather increased 1.6-fold after NE infusion ($P < 0.01$). Consequently, glucose, insulin and glucagon responses to intravenous NE infusion were greater in *ob/ob* than lean mice ($P < 0.01$). Similar infusion of saline into *ob/ob* mice did not alter basal blood levels of glucose (7.6 ± 0.9 vs 10.2 ± 1.6 after infusion; $P = 0.1$). When yohimbine (1 pmol/min/g, i.v.) was infused 5 min prior to the NE infusion and maintained simultaneously with the NE infusion for 15 min, the stimulatory NE effects on insulin and glucagon release were completely blocked ($P < 0.05$, Figure 1). Although glucose levels were still significantly increased following yohimbine plus NE infusion (36%, $P < 0.05$), the magnitude of the increase was less than for NE alone (90%) ($P < 0.05$). In an effort to determine whether or not the NE-induced increase in plasma insulin of *ob/ob* mice was at all attributable to the concurrent rise in blood glucose level, we tested the effects of a hyperglycemic clamp condition on plasma insulin level in

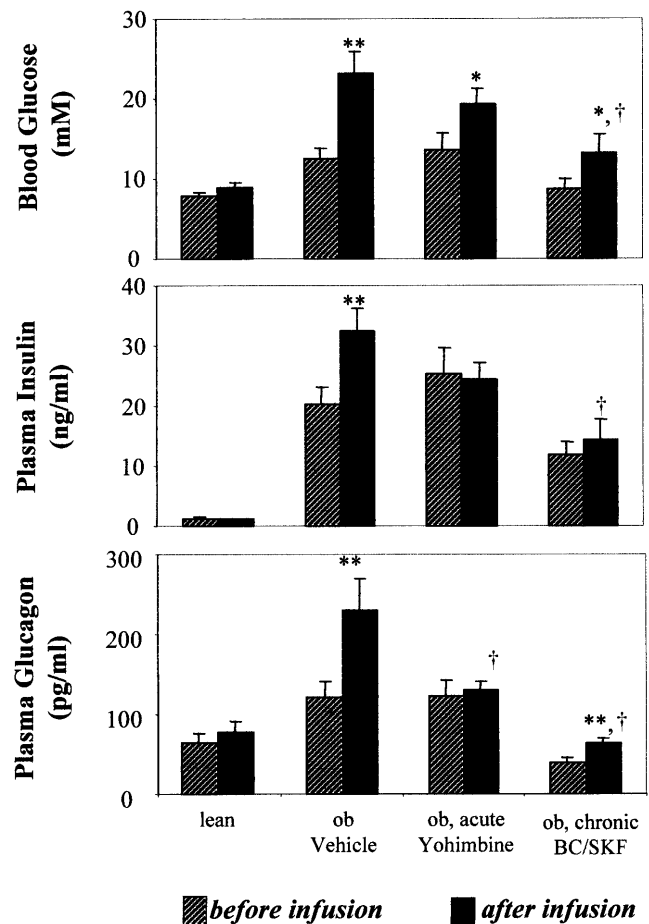


Figure 1 Effect of intravenous NE infusion on blood glucose, plasma insulin and plasma glucagon levels in lean, *ob/ob*, *ob/ob*-concurrent yohimbine infused, or *ob/ob* sympatholytic BC/SKF treated (for 14 days prior to NE infusion) mice. Values are means \pm s.e.m. of five animals per group. * $P < 0.05$; ** $P < 0.01$ relative to pre-NE infusion values; † $P < 0.01$ compared to respective values in NE infused *ob/ob* (*ob*) control mice. See Results section for description of comparative differences in responsiveness to NE among treatment groups.

an additional similarly designed experiment wherein the blood glucose level was maintained at 30 mM for 15 min. Glucose was infused at a variable rate over a 15 min period to achieve a constant blood glucose level of 30 ± 1.5 mM during the 10–15 min period of infusion in each of the lean control, *ob/ob* control, and *ob/ob*-BC/SKF treated groups. Under this hyperglycemic condition, plasma insulin level was significantly increased in lean mice (from 2.6 ± 0.4 to 4.4 ± 0.8 ng/ml; $P < 0.05$) but not in *ob/ob* mice (from 45.8 ± 20.1 to 68.9 ± 23.6 ng/ml; $n = 6$ animals/group).

Influence of BC/SKF on metabolic factors and humoral responses to NE infusion

As in previous studies,^{23,37,38} following 14 days of treatment, BC/SKF markedly reduced plasma glucose (42%, $P < 0.01$),

insulin (40%, $P < 0.01$), glucagon (59%, $P < 0.01$), FFA (34%, $P < 0.01$) and TG (43%, $P < 0.01$) levels as well as body weight (6.9 g, $P < 0.01$) relative to vehicle-treated *ob/ob* controls (Table 1). It has been demonstrated previously that, although BC/SKF treatment attenuates hyperphagia of *ob/ob* mice, such influences on feeding do not account for the observed impact on the above-described humoral factors with the exception of plasma TG level.^{37,38} Such a relation between BC/SKF effects on feeding and metabolism was also observed in this study as well respecting pair-fed and BC/SKF-treated animals (data not shown).

Furthermore, BC/SKF treatment blunted the intravenous NE-induced increase of blood glucose and plasma glucagon (from 80% to 50%, $P < 0.01$ and from 90% to 50%, $P < 0.05$, respectively), and abolished the abnormal NE induced increase of plasma insulin level ($P < 0.05$; Figure 1). Pair feeding control *ob/ob* mice to match the food consumption of BC/SKF treated mice did not prevent the glucose or insulin response to NE infusion (glucose, 22 ± 2 to 30 ± 3 mM; insulin, 12 ± 5 to 25 ± 7 ng/ml; $P < 0.05$). Thus, chronic systemic treatment with the sympatholytic dopamine agonists, BC/SKF and acute pretreatment with yohimbine similarly block the stimulatory effect of intravenous NE administration on plasma glucose, insulin and glucagon levels in *ob/ob* mice. However, chronic treatment with BC/SKF also reduced basal levels of glucose, insulin and glucagon in *ob/ob* mice. Consequently, the absolute values of plasma glucose, insulin and glucagon following NE infusion in *ob/ob* mice were also reduced by BC/SKF treatment (by 50, 50 and 75%, respectively; $P < 0.05$).

Direct NE and glucagon effect on islet insulin secretion

Direct regulation of insulin release by glucose, glucagon and NE was also examined *in vitro*, utilizing a static islet incubation method. The object of this experiment was to examine possible differences in the islet insulin secretory responses to NE and glucagon between lean, *ob/ob* and *ob/ob*-BC/SKF-treated mice. Therefore, differences in change from respective baseline islet insulin secretion following NE or glucagon administration were compared among these groups. We have previously reported the influence of BC/SKF on basal and

glucose-stimulated insulin secretion from isolated islets.²³ Insulin secretion data expressed either as percentage of baseline islet insulin content or as fmol insulin/ng DNA/h produced the same results and are summarized as follows.

Glucagon (10 nM) did not induce a significant increase in the insulin release response to 15 mM glucose from islets of lean mice. However, glucagon did significantly increase (by 2.7-fold, $P < 0.05$) this islet response from islets of *ob/ob* mice, which was greater than that (1.3-fold) of lean mice ($P < 0.05$). This hypersensitivity to glucagon among *ob/ob* mice was abolished by BC/SKF treatment ($P < 0.05$ vs *ob/ob* control), which resulted in an insulin secretory response to glucagon similar to that observed in islets from lean mice (1.4-fold increase; NS).

The direct effect of NE on insulin release was also tested *in vitro*. The addition of 0.1 μ M NE to the incubation medium did not affect the islet insulin secretory response to 15 mM glucose in islets from lean mice. However, such glucose-induced insulin release from obese mice (both BC/SKF- and vehicle-treated) was inhibited by 0.1 μ M NE by 60–70 and 70–90%, respectively ($P < 0.05$, compared with that in 15 mM glucose alone). At a concentration of 1 μ M, NE in the islet incubation buffer significantly inhibited this glucose-induced-insulin release in all three groups of mice (data not shown).

Discussion

The present study indicates that relative to lean euglycemic mice, obese hyperglycemic *ob/ob* mice exhibit a hypersensitivity to the stimulatory effects of plasma NE on plasma glucose and glucagon levels. Furthermore, they demonstrate that increases in plasma NE in *ob/ob* but not lean mice stimulate a rise in plasma insulin level. This unique hypersensitivity response to NE may be attenuated or abolished by acute noradrenergic α_2 antagonist or chronic sympatholytic dopamine agonist treatments. Moreover, these data suggest that the stimulatory effect of NE on plasma insulin level is mediated, at least in part, indirectly via stimulation of glucagon secretion. The present findings respecting the increased plasma glucose response to NE in *ob/ob* vs lean mice are in good agreement with previous studies wherein (a) hyperglycemic responses to adrenergic agonists were exaggerated and (b) chemical sympathectomy reduced elevated plasma glucose and insulin levels in *ob/ob* but not lean mice.^{30,31} In all, the results of this study implicate a role for peripheral noradrenergic activities in the hyperglycemic, hyperglucagonemic and hyperinsulinemic state of *ob/ob* mice. It should be noted that, although hypothalamic NE activities increase peripheral SNS functions, including a rise in circulating NE levels,^{18,19,27} the exogenous NE infusion in this study may be expected to exert its effects primarily on peripheral tissues inasmuch as monoamines poorly traverse the blood–brain barrier.⁴¹ Increases in circulating norepinephrine increase HGO directly by stimulating liver glycogenolysis and gluconeogenesis and indirectly by stimulating

Table 1 Effect of 14 days of BC/SKF treatment on body weight and humoral factors of *ob/ob* mice

Parameter	Treatment group	
	Vehicle	BC/SKF
Body weight (g)	44.7 \pm 0.7	37.8 \pm 0.8*
Blood glucose (mM)	18.6 \pm 1.5	10.7 \pm 1.4*
Plasma insulin (ng/ml)	36.8 \pm 4.3	22.2 \pm 2.3*
Plasma glucagon (pg/ml)	255 \pm 25	106 \pm 15*
Plasma free fatty acids (mM)	1.2 \pm 0.1	0.8 \pm 0.1*
Plasma triglyceride (mg/dl)	271 \pm 27	155 \pm 16*

Values are means \pm s.e.m. of six animals per group. * $P < 0.01$ vs vehicle control.

glucagon secretion (primarily via α_2 receptors) from pancreatic islet α cells.^{16–19}

The stimulatory effect of intravenous NE on plasma insulin level in *ob/ob* mice is curious and worthy of specific discussion. It is well established that under normal circumstances in normal animals, NE inhibits β -cell insulin secretion primarily via interaction with α_2 receptors.⁴² Our data also demonstrate a direct inhibitory effect of NE on islet insulin secretion in lean and *ob/ob* mice which appears more pronounced in *ob/ob* mice. Therefore it is difficult to ascribe the stimulatory effect of intravenous NE on plasma insulin level in *ob/ob* mice to a direct stimulation of β -cell insulin secretion. Hyperglycemia, especially in the presence of elevated plasma FFA,²³ induced by NE infusion could conceivably contribute to the coincident increase in plasma insulin. Raising the plasma glucose level of *ob/ob* mice to a level (30 mM) above that induced by NE infusion and for an equivalent time period did increase the plasma insulin level, although variation in response prevented this effect from reaching statistical significance. A plausible explanation for the NE-induced increase of plasma insulin level may in part be via its stimulation of glucagon secretion which in turn stimulates insulin release. In support of such a tenet is the observation that intravenous yohimbine, a member of potent α_2 antagonists which stimulate insulin release from the β -cell,^{43,44} actually blocked the *in vivo* NE effect on insulin secretion while simultaneously preventing the NE-induced increase in plasma glucagon. Likewise, chronic treatment with the sympatholytic dopamine agonists, BC/SKF, also abrogated both the plasma glucagon and insulin increases in response to NE. Moreover, islet insulin secretory responses to glucagon are more pronounced in *ob/ob* than lean mice and this is blocked by sympatholytic BC/SKF treatment. Therefore in *ob/ob* mice, for glucagon to mediate the stimulatory effect of NE on plasma insulin level, the α -cell glucagon secretory response to NE and/or the β -cell insulin secretory response to glucagon must be greater than the NE inhibitory effect on β -cell insulin secretion. We have recently observed that neither the α -cell number nor the glucagon content of pancreatic islets differ between lean, *ob/ob* and *ob/ob*-BC/SKF-treated mice (Cincotta *et al*, unpublished data). This observation together with the present findings suggest that hyperglucagonemia of *ob/ob* mice may be a function more of α -cell secretory regulation (in part by the SNS) than α -cell morphology or glucagon content. The resultant increase in NE stimulated glucagon secretion of *ob/ob* mice may support increased hepatic glucose output as previously described^{18,19} and evidenced by the yohimbine and BC/SKF glucose responses demonstrated herein. Once again, this indirect effect of glucagon via increased HGO and blood glucose level may contribute to the hyperinsulinemic response to NE infusion in *ob/ob* mice. Although not examined specifically in this study, the possibility that NE exacerbates existing insulin resistance in *ob/ob* mice consequently potentiating increases in plasma insulin is worthy of further investigation.

It has been proposed that concurrent increases in SNS activity and alterations in the hypothalamic–pituitary–adrenal axis potentiate the hyperinsulinemic, insulin resistant, glucose intolerant state,⁴⁵ and recent evidence in humans supports this postulate.⁴⁶ The present findings suggest that such a physiologic neuroendocrine organization may be operative in *ob/ob* mice. While such a pathophysiologic condition may contribute to the hyperinsulinemia of *ob/ob* mice, it clearly is not the only factor, inasmuch as BC/SKF treatment normalizes plasma glucagon level but does not completely normalize the hyperinsulinemia. Interestingly, sympatholytic dopamine agonist BC/SKF treatment of *ob/ob* mice may reduce hyperinsulinemia by reducing plasma glucose and FFA levels^{23,37,38} as well as via its influence on the SNS and glucagon; however, the absence of leptin appears to prevent normalization of plasma insulin in the face of all these metabolic and neuroendocrine improvements.

The physiologic significance of the present findings are best appreciated when viewed in the context of the existing fund of related supportive information as follows. First, this and other reports^{30,31} of increased peripheral noradrenergic responsiveness in *ob/ob* mice are not incongruent with the decreased energy expenditure and decreased sympathetic activation of brown fat observed in these mice.² The hypothalamus can selectively and simultaneously differentially regulate (activate and inhibit) discrete arms of the SNS and mounting evidence now supports this fundamental observation.⁴² For example, NE within the VMH inhibits glutamate stimulated activity therein,⁸ thereby inhibiting SNS stimulated brown fat thermogenesis.^{10,11} However, NE stimulation of the VMH also stimulates increases in plasma glucose, insulin and glucagon via activation of the peripheral SNS.^{18,19,27} Secondly, the hypersensitivity to intravenous NE observed in *ob/ob* mice may be the result of classic counter-regulatory over-compensation to low circulating endogenous NE levels (ie, increased target tissue noradrenergic receptor number and/or affinity). However, available evidence does not indicate reduced peripheral norepinephrine turnover in *ob/ob* vs lean mice.³² In fact, in female *ob/ob* mice (as used in this study), the 24 h urinary NE excretion level was much greater in *ob/ob* vs lean mice.³² Further evidence negating a counter-regulatory mechanism in the over-responsiveness to NE is the fact that the powerful sympatholytic agents, BC/SKF, which markedly reduce circulating norepinephrine levels,^{33–35} also attenuate the hypersensitivity to NE. These data together with other studies provide a physiologic construct wherein SNS activity and tissue responsiveness to NE are both elevated in these mice and the metabolic syndrome in general.^{8,9,13,27,28,30,31} Increases in SNS activity and tissue responsiveness to NE have been described in obese vs lean humans.^{47–49} Available evidence suggests that the increased liver and islet tissue responsiveness to NE infusion in *ob/ob* mice may be due to alterations in hypothalamic modulation of the neuroendocrine axis, which are normalized by BC/SKF treatment to

increase the dopamine:norepinephrine activity ratio therein.^{8,9,13,28}

In summary, intravenous NE infusion in *ob/ob* but not lean mice potentiates hyperglycemia, hyperglucagonemia and hyperinsulinemia. A large body of available evidence indicates that this hypersensitivity to NE in *ob/ob* mice is not due to counter-regulatory compensation of low endogenous NE levels but rather to alterations in hypothalamic systems resulting in potentiation of noradrenergic functions in liver and islet α -cells.^{8,9,13,18,19,24–28,30,31,45,46} The consequent hyperglucagonemia coupled with increased β -cell insulin secretory responsiveness to glucagon in these animals in turn facilitates increased islet β -cell secretion of insulin and hyperinsulinemia. The systemic NE-induced increases in blood glucose may also contribute to its hyperinsulinemic effect in these *ob/ob* mice. These findings further support previous studies^{6,8,9,12–15,18,19,24–28,30,31,45,46} implicating a role for increased SNS and/or peripheral noradrenergic activities in the development and maintenance of the hyperglycemic, hyperglucagonemic and hyperinsulinemic state, characteristic of the obese-type 2 diabetic condition.

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