Immunoreactive Glucagon Levels in Obese-hyperglycemic (ob/ob) Mice

P. U. Dubuc, Ph.D., P. W. Mobley, Ph.D.,
R. J. Mahler, M.D., and J. W. Ensinck, M.D.,† Duarte, California*

SUMMARY

A series of studies examined the effects of various experimental manipulations on the glucose, immunoreactive insulin (IRI), and immunoreactive glucagon (IRG) levels of eight-week old ob/ob mice and their lean littermates. The levels of serum glucose, IRI, and IRG of ob/ob mice were significantly higher than those of the lean mice in the basal state and remained higher throughout an 18-hour period of fasting. Both groups responded to food deprivation with reduced glucose and IRI concentrations, but the IRG levels of obese mice were reduced by fasting while those of lean mice were marginally elevated. The IRG levels of both phenotypes were reduced to a similar degree by glucose administration, and the serum glucose and IRI responses of the two groups were proportional to their preinjection levels. Arginine treatment increased the IRG and IRI of both groups. Arginine also lowered the glucose levels of lean mice, but, notably, it did not affect the serum glucose of ob/ob mice. The simultaneous administration of arginine and insulin to lean mice potentiated the hyperglucagonemia and hypoglycemia that followed arginine alone, but in ob/ob mice combined treatment increased the glucose concentrations while not affecting IRG. These data indicate that ob/ob mice have several abnormalities in their metabolic-endocrine responses to both food deprivation and to stimulation with metabolically active agents. However, from the present results we could not determine a specific disturbance of IRI or IRG regulation that could account for the persistence of their hyperglucagonemia, hyperinsulinemia, or hyperglycemia. DIABETES *26*:841-46, September, 1977.

Recent studies have emphasized the importance of glucagon and of its interactions with insulin on the metabolic derangements of diabetes mellitus in human beings.[1-3] An animal model, the obese-hyperglycemic mouse (ob/ob), has been described that has many metabolic and endocrine abnormalities in common with those of maturity-onset diabetic human beings. These similarities include obesity, elevated food intake, altered basal insulin-glucose relationships, and in-vivo insulin insensitivity.[4] Mayer originally hypothesized that elevated pancreatic α-cell glucagon production may contribute to the altered metabolism of obese mice.[5] Subsequently, in support of Mayer's hypothesis, histologic evidence of increased pancreatic α-cell function[6,7] and in-vivo and in-vitro evidence of abnormal glucagon secretion have been reported in various strains of genetically obese and diabetic mice.[7-11]

In a previous study we showed that young adult ob/ob mice have elevated basal immunoreactive glucagon levels in comparison with their lean littermates.[10] In the present study, these initial experiments were extended to an examination of the effects of fasting and of various metabolic agents on the in-vivo dynamics of glucose, insulin, and glucagon secretion in ob/ob and lean mice.

METHODS

Four-to-five-week-old male ob/ob mice (C57BL/6J) and lean controls(+/?) were purchased from Jackson Laboratories (Bar Harbor, Me.). All mice were housed singly in commercially available cages and, except where noted, had free access to Purina Chow and tap water. The colony room was maintained at 23 ± 2° C. and had a 12:12 light-dark cycle. Between the ages of six and eight weeks all animals were weighed on alternate days and were adapted to handling and the injection procedures.

From the Department of Metabolism and Endocrinology, City of Hope Medical Center, Duarte, California 91010.
*Department of Metabolism and Endocrinology, Eisenhower Medical Center, Palm Desert, California.
†Department of Medicine, University of Washington, Seattle, Washington.
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In the following experiments blood samples for the determination of serum glucose, immunoreactive insulin (IRI), and immunoreactive glucagon (IRG) were taken from the retroorbital plexus under light anesthesia. Glucose was measured by a glucose oxidase procedure (Beckman glucose analyzer) and IRI was determined by the double-antibody procedure described by Hales and Randle.[12] Insulin values are reported in terms of a mouse insulin standard curve (Novo, Copenhagen). Serum samples for IRG measurement were collected in benzamidine (0.05 M) and assayed with Unger's 30-K antiserum and cellulose to separate bound from free hormone.[10,13] The insulin and glucose values reported are from individual animals, while IRG concentrations were determined from 0.5-ml. serum pools (two or three animals).

In experiment 1 the effects of food deprivation on the systemic levels of glucose, IRI, and IRG were determined by taking blood samples from lean and obese mice either three or 18 hours after food removal.

Experiment 2 examined the effects of a glucose challenge on the glucose, IRI, and IRG concentrations of 18-hour fasted ob/ob and lean mice. The experimental groups included (1) ob/ob or lean animals bled after overnight food deprivation only (t_0 values), (2) ob/ob or lean animals bled once, 30 minutes following an intraperitoneal glucose injection of 1 gm./kg. (t_{30} glucose values), or (3) ob/ob or lean animals bled 30 minutes after a distilled water injection (t_{30} sham values).

In experiments 3 and 4 we examined the effects of arginine (L-arginine HCl) and of a combination of arginine and insulin on the serum glucose, IRI, and IRG levels of ob/ob and lean mice. Arginine (1 mmole, i.p.) alone or with crystalline insulin (30 mU.) was administered to either obese or lean mice three hours after food was removed. Control animals received distilled water only (1 ml./100 gm.). All injected animals were bled only once 30 minutes after treatment (t_{30} values). The preinjection levels of glucose, IRI, and IRG (t_0 values) were established by bleeding randomly selected animals after only a three-hour fast.

Unless otherwise indicated the statistical results reported are based on Student's *t*-test for independent groups.

RESULTS

Fasting

The effects of three or 18 hours of food deprivation on the serum levels of glucose, IRI, and IRG of ob/ob and lean mice are presented in figure 1. The results of statistical analysis (two-way analysis of variance) of these data are summarized in table 1. There was a highly significant main effect of phenotype on the levels of glucose, insulin, and glucagon during food deprivation, as ob/ob mice displayed persistently higher values of each variable (all $p < 0.001$). A significant main effect of treatment (three hours vs. 18 hours of a fast) was also noted on glucose and IRI concentrations ($p < 0.01$), but there was no effect of fasting on the IRG levels when both groups were considered together ($p > 0.05$, table 1).

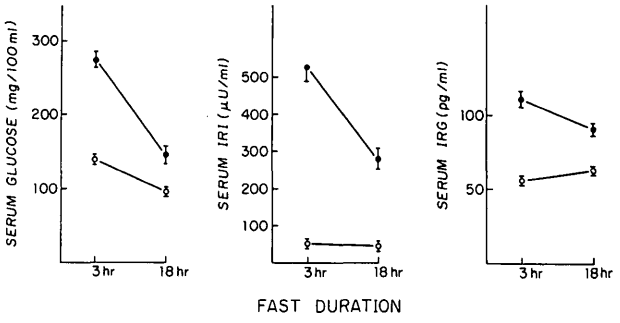

FIG. 1. Serum glucose, IRI, and IRG levels of ob/ob (solid circles) and lean mice (open circles) after three or 18 hours of food deprivation. For glucose and IRI each data point represents the mean ± S.E. of a minimum of 16 observations and for IRG a minimum of nine pooled samples.

As noted previously,[7,14,15] ob/ob mice displayed exaggerated glucose and insulin responses to food deprivation, which appeared to account for the significant P X T interactions in these measures (table 1). It is notable that analysis of the individual group IRG responses during fasting showed that ob/ob mice dis-

TABLE 1

Two-way analysis of variance of the glucose, IRI, and IRG responses to food deprivation presented in figure 1

	Source	M.S. (X 10^3)	df	F	p
Serum glucose	Phenotype (P)	211.2	1	100.0	<0.001
	Treatment (T, fast duration)	188.4	1	89.2	<0.001
	P X T	49.9	1	23.6	<0.001
	Error	2.1	96		
Serum insulin	Phenotype	2,576.2	1	167.4	<0.001
	Treatment	364.0	1	23.6	<0.001
	P X T	314.5	1	20.4	<0.001
	Error	15.4	76		
Serum glucagon	Phenotype	18.3	1	46.2	<0.001
	Treatment	1.3	1	3.3	N.S.
	P X T	1.3	1	3.2	N.S.
	Error	0.40	36		

played a significant diminution of circulating IRG levels ($p < 0.01$) while marginal elevations were noted in the lean animals ($0.05 < p < 0.1$).

Control Injections

The results of previous work[16] indicate that control injections cause significant differences between ob/ob and lean mice in postinjection glucose concentrations. In the present study we examined the glucose, IRI, and IRG responses of the experimental animals to intraperitoneal injections of distilled water after either three or 18 hours of fasting (figure 2). Following an 18-hour fast, control injections had no substantial effects on the 30-minute levels of glucose, IRI, or IRG of either obese or lean mice when compared with preinjection concentrations, nor were there significant differences in the responses between the two phenotypes. However, an injection of distilled water after a three-hour fast led to significant elevations (over t_0 values) of serum glucose ($p < 0.05$) and reduced levels of IRI ($p < 0.05$) in each phenotype. The magnitude of the changes in glucose and IRI of ob/ob mice was substantially greater than those of their lean

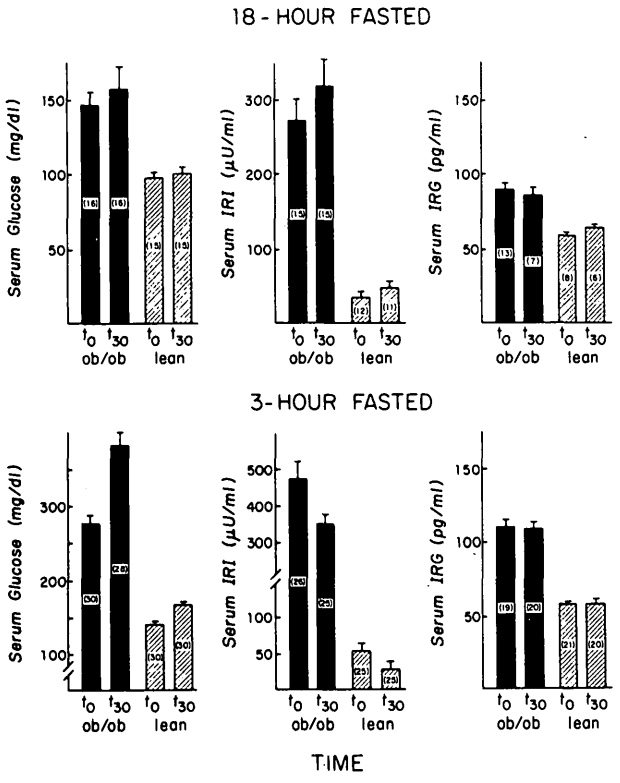

FIG. 2. Effects of distilled-water injections on the t_{30} glucose, IRI, and IRG levels of three- or 18-hour fasted lean and obese mice. All values are presented as group mean ± S.E. with the number of observations in parentheses.

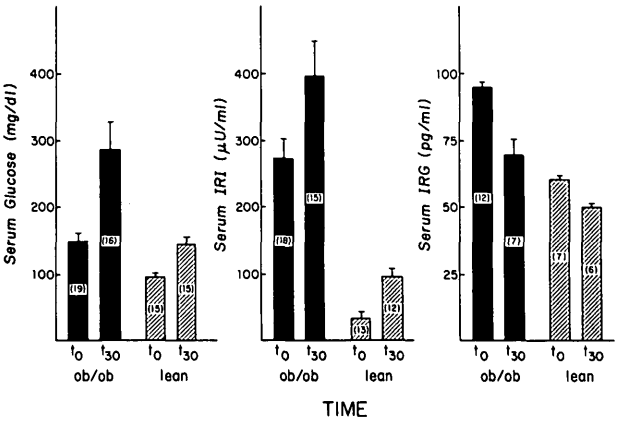

FIG. 3. Thirty-minute (t_{30}) glucose, IRI, and IRG responses of obese and lean mice to glucose injections. Values presented as the drug-treated group means ± S.E. minus that component of response due to sham treatment. The number of animals appears within parentheses.

littermates ($p < 0.05$). In spite of the reciprocal effects of sham treatment on insulin and glucose, however, there were no differences in their serum IRG responses either between phenotypes or within phenotypes 30 minutes after injection.

Since these data indicate some highly significant differential effects of sham treatment on obese and lean mice, the 30-minute glucose, IRI, and IRG responses to a glucose challenge or after arginine or arginine-insulin injections are each presented as the "net" effects of the drug. That is, all poststimulation data are reported as the t_{30} concentrations of glucose, IRI, or IRG minus that component of the response that was due to sham stimulation.

Glucose Administration

The effects of glucose treatment on the glucose, IRI, and IRG levels of obese and lean groups are presented in figure 3. Both ob/ob and lean mice exhibited significantly increased serum glucose and IRI levels 30 minutes following glucose injection, while IRG levels were reduced in the two phenotypes (all $p < 0.05$). Although ob/ob mice displayed greater changes in glucose, IRI, and IRG than did the lean mice ($p < 0.05$), the responses to stimulation of both groups were generally proportional to their t_0 values.

Arginine Administration

The glucose, IRI, and IRG responses of the experimental groups to identical 1-mmole injections of arginine are summarized in figure 4. As before, all data are presented as the drug-stimulated concentrations minus that part of the response due to sham treatment. Arginine caused a substantial reduction of serum glucose in lean mice ($p < 0.01$) while having

no effect on the glucose levels of obese mice. In spite of the differences between phenotypes in their glucose responses to arginine injection, serum IRI levels were significantly increased over the zero-time values in both groups ($p < 0.05$). Also, obese mice displayed greater absolute increases in IRI than did the lean mice, but the changes were approximately proportional to the basal levels of the experimental groups. Arginine treatment caused highly significant increases in IRG in both lean and obese mice ($p < 0.01$), but there were no differences in the magnitudes of the responses between the two phenotypes.

Arginine-insulin Administration

In a final series of experiments, ob/ob and lean mice received identical injections of a combination of arginine and insulin (1 mmole-30 mU.). The results of these treatments are presented in figure 5. Combined treatment caused additional hyperglycemia in ob/ob mice, but reduced levels of serum glucose were noted in the lean mice (both $p < 0.01$ vs. basal values). The differences in glucose responses between obese and lean mice were significant at the $p < 0.1$ per cent level. The IRI concentrations of each experimental group 30 minutes following arginine-insulin administration were above the range of our assay, and no differences between groups could be determined. The IRG levels of both groups after arginine-insulin were significantly elevated ($p < 0.001$ vs. t_0 values), but there were no differences in the responses between obese and lean mice ($p > 0.05$).

It is well known that ob/ob mice display resistance to the metabolic effects of exogenous insulin.[4] Table 2 directly compares the glucose and glucagon responses

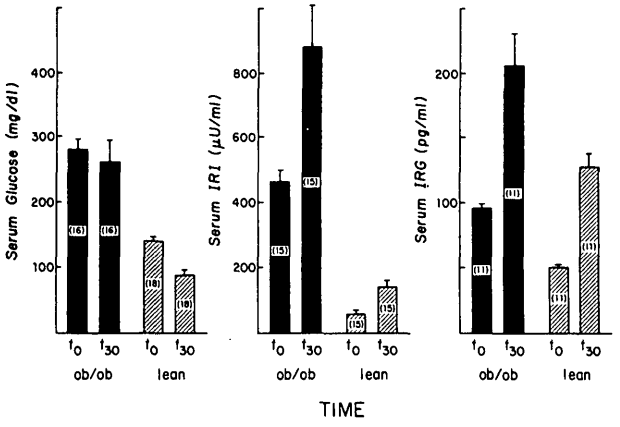

FIG. 4. Effects of arginine injection (1 mmole) on t_{30} levels of glucose, IRI, and IRG of lean and ob/ob mice. Values presented as the group means ± S.E. minus the 30-minute responses to sham treatment. The number of observations is included within parentheses.

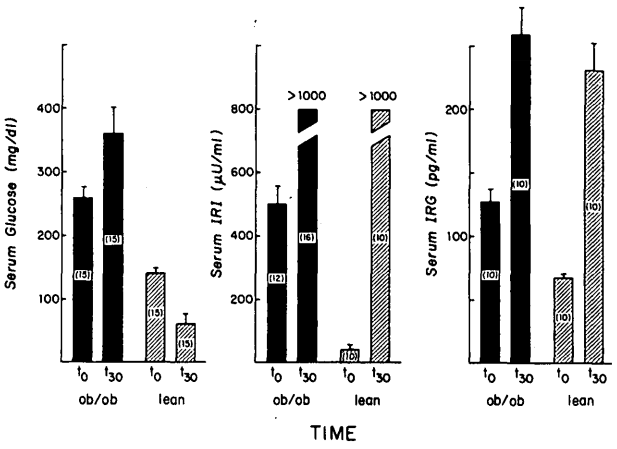

FIG. 5. Thirty-minute (t_{30}) serum glucose, IRI, and IRG responses of obese and lean mice to combined arginine-insulin administration (1 mmole-30 mU.) three hours after food removal. The data are presented as the "net" group means ± S.E. after removing the sham-treatment component of the response. The number of observations appears within parentheses.

of the experimental animals to arginine and to arginine plus insulin administration. In lean mice the addition of insulin potentiated the hypoglycemic responses that followed arginine stimulation alone ($p < 0.05$) while also causing a marked elevation of systemic IRG ($p < 0.05$). Unexpectedly, ob/ob mice responded to combined arginine-insulin treatment with significantly higher serum glucose levels than did ob/ob mice that received only arginine ($p < 0.01$). The paradoxic increase of serum glucose in ob/ob mice, which occurred as a consequence of added

TABLE 2

Comparisons of the glucose and IRG responses (from t_0) of ob/ob and lean mice to arginine and to arginine-insulin treatment

	Arginine treatment	Arginine-insulin treatment	p (rows)
Serum glucose in mg./dl. (vs. t_0 values)			
ob/ob	− 22.8±13.3 (15)	+101.6±16.4 (15)	<0.01
lean	− 54.3± 3.8 (16)	− 79.9± 5.1 (18)	<0.05
Serum glucagon in pg./ml. (vs. t_0 values)			
ob/ob	+111.7± 9.1 (10)	+127.4±17.1 (11)	N.S.
lean	+ 79.5± 8.4 (11)	+164.9±23.8 (10)	<0.01

insulin, did not appear to be a result of increased IRG levels, since no statistical differences in IRG were evident between the arginine and arginine-insulin treatments. Accordingly, the addition of insulin to arginine also did not depress the exaggerated IRG responses of ob/ob mice that were evident after arginine injection alone (table 2).

DISCUSSION

Recently, attention has been focused on the contribution of glucagon to the maintenance of hyperglycemia in human juvenile-onset diabetes[1-3] and in the experimental diabetes of animals.[17-19] Several studies suggest that the elevated systemic glucose concentrations associated with these forms of diabetes mellitus arise from the combination of reduced pancreatic β-cell secretory capacity and persistent glucagon production of pancreatic or gastrointestinal origin. The hyperglycemia that occurs in some forms of adult-onset diabetes and in animals with genetically based diabetes and obesity appears to be more complex, however, as these conditions are characterized by the simultaneous presence of moderate to severe hyperglycemia, relative or absolute hyperglucagonemia, and generally elevated basal insulin levels. The results of the present study exemplify this situation in an animal model of diabetes, the ob/ob mouse, which simultaneously displays hyperglycemia, hyperglucagonemia, and hyperinsulinemia.

It is generally considered that splanchnic glucose concentrations provide the regulatory signals for the release of both pancreatic insulin and glucagon. The results of several recent studies[6,7,10,11] and the data presented here demonstrate that ob/ob mice maintain basal hyperglucagonemia despite abnormally high circulating levels of glucose and insulin. A deficit in either a glucose or glucose-insulin mechanism that inhibits glucagon secretion could account for the hyperglucagonemia of these animals. Evidence consistent with this possibility is provided by in-vitro studies of the dynamics of glucagon secretion in the pancreata of ob/ob and db/db mice.[8,9] In contrast to these in-vitro results, however, we found that exogenous glucose was as effective in obese mice as it was in lean mice in reducing the circulating levels of IRG. It appears therefore that (1) a mechanism other than that of glucose acting directly at the islet is responsible for the glucose-induced reductions of IRG noted in vivo and (2) IRG insensitivity to glucose will not account for the hyperglucagonemia of ob/ob mice.

As an alternative mechanism, the high glucagon concentrations of obese mice might be maintained by an increased responsiveness of the α-cells to endogenous secretagogues. Food deprivation in many species results in compensatory responses of glucose mobilization that are mediated by increased glucagon secretion and reduced insulin release. Overnight-fasted lean mice in the present study and 48-hour fasted lean mice in an earlier work[11] clearly exhibited these response patterns. In contrast, our study showed that ob/ob mice typically displayed exaggerated declines of glucose and IRI with fasting, but these responses were coupled with substantial reductions of systemic IRG. Even after 48 hours of food deprivation, ob/ob mice did not have glucagon levels higher than they did in the fed state.[11] Since these data show that ob/ob mice do not display exaggerated elevations of glucagon to food deprivation, the glucagon hypersecretion that prevails in these animals does not appear to arise solely as a compensatory response to cellular glucoprivation. However, the results of these studies could suggest that glucagon secretion is maintained at maximal levels in ob/ob mice and therefore cannot be elevated in response to the additional peripheral glucose demand of starvation. To investigate this possibility we examined the IRG responses to arginine and combined arginine-insulin stimulation. This model was chosen because previous work has shown that arginine is a potent stimulus for pancreatic glucagon and insulin release in man and in a variety of experimental animals.[3,8,18] Arginine has also been shown to have little activity as a gluconeogenic substrate and thus, in fasted animals, will not result in a sustained hyperglycemia.[20] Consistent with the results of studies of other nondiabetic subjects, both lean and obese mice responded to arginine with substantial elevations of serum IRI and IRG. The similarities between phenotypes in their response patterns to arginine suggest that the ob/ob mice have no major deficit in their α-cell secretory capacity. In fact because these mice displayed greater glucagon responses than did the lean mice to identical arginine loads, while having twice the body weight and larger blood volumes, our results seem to support earlier reports of increased α-cell sensitivity to arginine in obese and diabetic mice.[8,9]

In each phenotype the addition of insulin to arginine had no effect in reducing the IRG responses that followed arginine alone. Lean mice displayed a significant hypoglycemia after combined treatment that, based on previous work, was likely to be responsible for this group's postinjection hyperglucagonemia.[21-23] Obese mice, on the other hand, had elevated glucose levels following arginine-insulin injections. In ob/ob mice, increased α-cell sensitivity to

arginine could also account for the ineffectiveness of added insulin to lower IRG, but at this time we cannot exclude the possibility that the insulin resistance of ob/ob mice extends to the regulatory influence of insulin or insulin plus glucose on glucagon secretion.

In summary, examination of the glucagon responses of ob/ob and lean mice to various experimental manipulations demonstrated substantial differences between phenotypes in many of their endocrine-metabolic responses. Although our in-vivo data appear to support previous in-vitro findings that the pancreata of ob/ob mice have an augmented sensitivity to arginine stimulation even in the presence of hyperglycemia and hyperinsulinemia, we found no evidence that insensitivity to a glucose-inhibitory mechanism contributed significantly to the maintenance of hyperglucagonemia in these animals. In addition, it is reasonable to suspect that the frequently described catabolic effects of glucagon contribute to the hyperglycemia of ob/ob mice. Yet in the present study we frequently observed that these mice displayed either increased or decreased IRG levels without a corresponding alteration in glucose concentrations. Also, we found, as have other researchers, that food deprivation does not elicit the compensatory IRG responses that characterize nondiabetic animals and man. Thus the present data, although demonstrating a number of abnormalities in the dynamics of IRG release in ob/ob mice, do not specify a deficit that could account for the persistent hyperglycemia noted in these animals.

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