Defective Insulin Secretory Response to Glucose in the New Zealand Obese Mouse

Improvement with Restricted Diet

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SUMMARY

Insulin and glucose tolerance tests, and tests of the plasma insulin response to injected glucose were compared in C57B ad libitum fed mice (control strain), NZO ad libitum fed mice, C57B mice with obesity due to induced hyperphagia following gold thioglucose administration, and NZO mice whose weight had been reduced to that of the control strain by dietary restriction. The ad libitum fed NZO mice showed impaired glucose tolerance, increased insulin resistance, basal hyperinsulinemia and absent rise in plasma insulin in response to glucose injection. The gold thioglucose-treated mice also showed impaired glucose tolerance and insulin resistance, but they demonstrated a rise in plasma insulin in response to glucose injection. The NZO mice whose diet had been restricted showed variable glucose tolerance, improved but not normal insulin sensitivity, and a significant rise in plasma insulin in response to injected glucose. It is concluded that the pancreatic islets of obese NZO mice, although very responsive to arginine, are insensitive to glucose, and with weight reduction sensitivity to glucose returns. DIABETES 22:251-55, April, 1973.

In a previous report,[1] a selective defect in insulin secretion has been described in the NZO strain of obese-hyperglycemic mouse. There is a failure of additional insulin secretion in response to glucose, glucagon and tolbutamide, but an accentuated response to the amino acid arginine. In addition, glucose intolerance,[2,3] and insulin resistance[2,4] are well recognized abnormalities in this strain of mouse. However, the relationship of these abnormalities to the obesity is not clear. In this report, the influence of obesity on the development of these characteristics is examined in two ways. Firstly, the NZO mice were subjected to dietary restriction leading to reduction of their body weight to that of a control strain. Secondly, the control strain had their weight increased to that of the NZO mice by induction of hyperphagia with gold thioglucose (GTG).

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METHODS

The NZO mice were derived from the original colony bred by Bielschowsky and Bielschowsky.[4] All mice were maintained on a standard diet containing 10 per cent fat, 20 per cent protein and 50 per cent carbohydrate with added vitamins. C57B mice, obtained from a standard laboratory stock and maintained at the Walter and Eliza Hall Institute of Medical Research, Melbourne, were used as the control strain.

Fifteen male NZO mice aged six to ten weeks, had their food restricted to an amount necessary to reduce their weight to that of the control strain by sixteen weeks of age. They were fed once daily, and required an average of 3 gm. of food per day. In other ways they were treated identically to the ad libitum fed mice.

Initially six male C57B mice were injected intraperitoneally with 0.5 mg./gm. body weight GTG (Schering Corp., Bloomfield, N. J.) in 0.9 per cent saline in a concentration of 20 mg./ml. at the age of forty-six days. They received a further 0.4 mg./gm. thirty-seven days later, since they had gained little weight by then. By the age of forty-eight weeks, four of the males had reached a weight greater than 40 gm. (more than 43 per cent above the mean weight of males not treated with GTG) and these mice were used in subsequent experiments. In addition, a further six male C57B mice received 0.8 mg./gm. body weight GTG at the age of forty days. By thirty-five weeks, one mouse had reached 39 gm. (44.5 per cent above average weight at that age) and it was also used in subsequent experiments. Subsequently the mice will be referred to in four groups: (1) C57B AL—control strain fed ad libitum, (2) NZO AL—NZO strain fed ad libitum, (3) GTG—C57B mice treated with GTC, and (4) NZO RD—NZO mice on restricted diet.

Glucose tolerance tests were performed by injecting 1 mg./gm. body weight glucose intraperitoneally after an overnight fast in male mice aged sixteen to twenty weeks, except for the GTG mice which were aged thirty-five to forty-eight weeks (see above). Blood was obtained from the orbital sinus under light ether anesthesia at 0, 30 and 60 minutes using a 40 μl. Microcap pipette (Drummond Scientific Co.). Blood sugar was measured using a ferricyanide technic[5] on an AutoAnalyzer (Technicon). For comparative purposes, the "glucose intolerance index" was defined as the sum of the blood sugar values before and at thirty and sixty minutes after the glucose injection.

Insulin resistance was assessed by determining the fall in blood sugar following the intraperitoneal injection of 0.5 mU./gm. Actrapid insulin (Novo Industri A/S, Copenhagen) after an overnight fast in the same groups of mice, with blood samples for sugar estimations obtained (by the method described above), just before and at thirty and sixty minutes after the insulin injection. The "insulin resistance index" was defined as the lower blood sugar, reached at thirty or sixty minutes after the insulin injection, expressed as a percentage of the fasting blood sugar.

Plasma insulin was measured by radioimmunoassay using the dextran-coated charcoal method of Herbert et al.[6] Mouse insulin standard (Novo Research Institute, Copenhagen), porcine insulin I-125 tracer, and antiserum to ox insulin obtained in guinea pig (Wellcome Reagents Ltd.) were used. Dilution and recovery experiments established the validity of this assay. The intra-assay coefficient of variation was 3 per cent at 118 μU./ml. and the interassay coefficient of variation was 13 per cent at 161 mU./ml. Fifty microliter aliquots of mouse plasma were assayed in duplicate.

In the experiments to study the insulin release following glucose, 0.2 to 0.4 ml. of blood was obtained from the orbital sinus of each mouse at each time interval for plasma insulin and blood sugar determinations. However, to avoid the possible effects of repeated blood sampling or anesthetics on insulin secretion, blood samples at the successive time intervals were taken on separate days, as described in detail elsewhere.[7] Blood samples were obtained after an overnight fast, and 5, 20 and 40 minutes after the intraperitoneal injection of 2 mg./gm. body weight glucose. Except for the GTG mice, where samples from individual mice were used, blood from two or three litter mates of the same sex was pooled for each determination.

RESULTS

The mean weight of the fifteen NZO male mice fed a restricted diet (NZO RD) at age sixteen to twenty weeks was 25.6 ± 0.5 gm. (S.E.M.) compared with 37.8 ± 1.0 gm. for the nine NZO AL mice at this age. The C57B AL male mice at this age had a mean weight of 25.7 ± 0.5 gm. The four GTG mice aged forty-eight weeks had a mean weight of 45.1 gm. (cf. C57B AL mice mean weight 28.0 gm. at this age) and the GTG mouse aged thirty-five weeks weighed 39.0 gm. (cf. C57B AL mice mean weight 27.5 gm.).

The mean glucose tolerance tests for the four groups of mice are shown in figure 1, and the mean glucose intolerance indices in table 1. It can be seen that the NZO AL mice had impaired glucose tolerance compared to the C57B AL mice. The glucose tolerance of the GTG mice was variable, but the mean glucose intolerance index for this group was also significantly higher than for the C57B AL mice. The glucose tolerance of the NZO RD mice was slightly, though not significantly, better than that of the NZO AL mice, and remained impaired compared with the C57B AL mice.

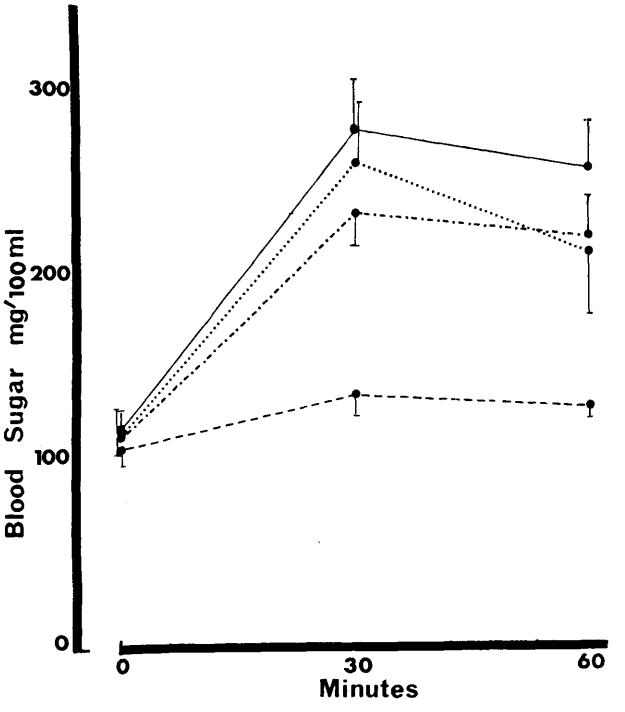

FIG. 1. Intraperitoneal glucose tolerance tests: 1 mg./gm. body weight glucose after overnight fast. Mean ± S.E.M. shown at each time.
● - - - - ● C57B AL (9 mice);
● ——— ● NZO AL (9);
● • • • ● GTG (5);
●-.-.-.● NZO RD (15).

TABLE 1

Mean (± S.E.M.) glucose intolerance indices and insulin resistance indices for male mice of the strain and treatment indicated. Numbers of mice in each group shown in parentheses.

Index	Group of Mice			
	C57B AL	NZO AL	GTG	NZO RD
Glucose intolerance index	372.9 ± 21.2 (9)	656.2 ± 63.5* (9)	593.2 ± 74.4† (5)	575.9 ± 41.1† (15)
Insulin resistance index	25.1 ± 2.8 (12)	65.8 ± 7.0* (9)	63.1 ± 7.1* (5)	48.1 ± 3.5* (15)

*Significance of difference of mean values from those of the C57B AL mice by Student's t test shown—$P < 0.001$.
†Significance of difference of mean values from those of the C57B AL mice by Student's t test shown—*$P < 0.01$.

Figure 2 shows the mean insulin tolerance tests for the four groups of mice, and the mean insulin resistance indices are shown in table 1. The NZO AL mice had increased insulin resistance compared to the C57B AL mice. GTG mice had insulin resistance comparable with that in the NZO AL mice, and significantly greater than that in the C57B AL mice. The NZO RD mice showed a significantly lower mean insulin resistance index than the NZO AL animals ($p < 0.02$), but still significantly higher than the C57B AL mice.

Insulin secretion in response to glucose for the four groups of mice is shown in figure 3. It can be seen that the C57B AL mice showed a prompt rise in plasma insulin evident five minutes after the glucose load. The NZO AL mice showed the completely absent response previously reported for a much larger group of NZO mice of different ages and both sexes.[1] The GTG mice had a significantly raised basal plasma insulin compared to the C57B AL mice ($P < 0.01$), and after glucose a further significant rise occurred. However, compared to

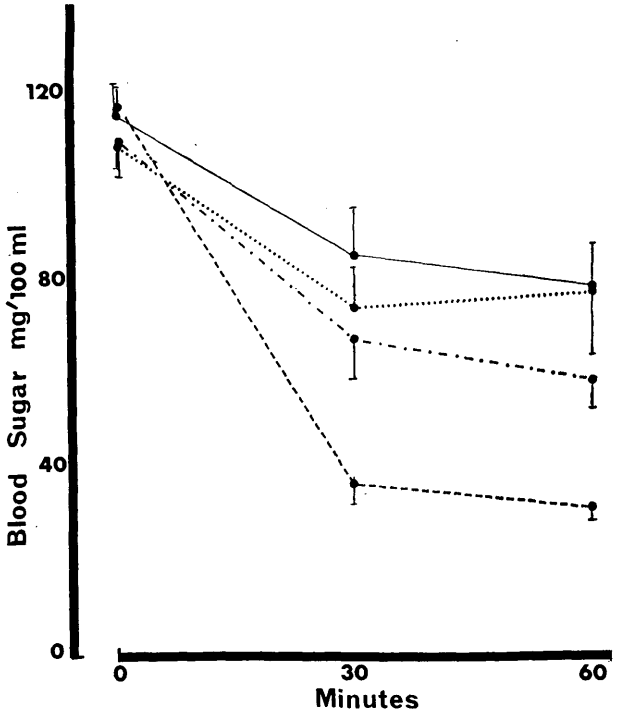

FIG. 2. Intraperitoneal insulin tolerance tests: 0.5 mU./gm. body weight insulin after overnight fast. Mean ± S.E.M. shown at each time.
● - - - - ● C57B AL (12 mice);
● ——— ● NZO AL (9);
● • • • ● GTG (5);
●-.-.-.● NZO RD (15).

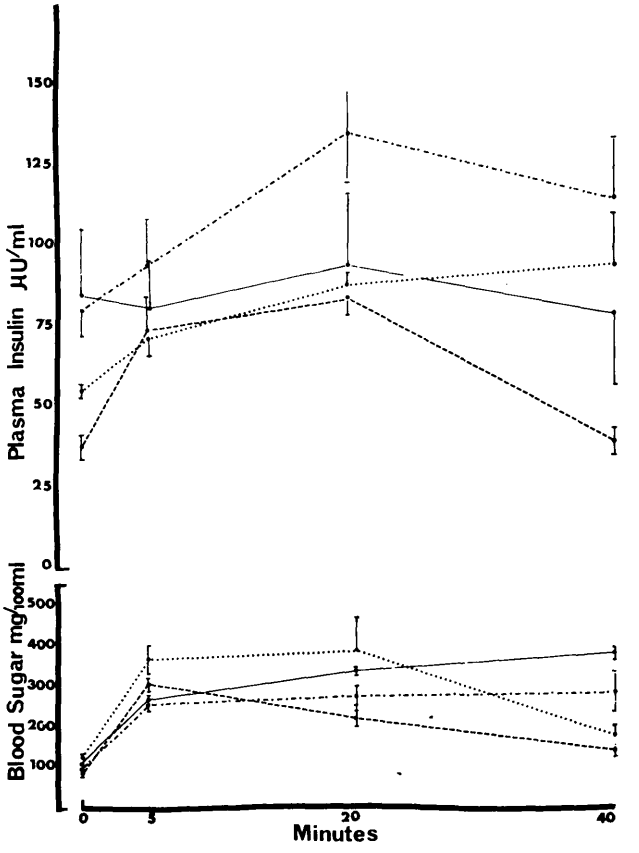

FIG. 3. Plasma insulin and blood sugar responses to 2 mg./gm. glucose intraperitoneally after overnight fast. Mean ± S.E.M. shown at each time.
● - - - - ● C57B AL (12 mice);
● ——— ● NZO AL (10);
● • • • ● GTG (5);
●-.-.-.● NZO RD (15).

the response of the nonobese C57B mice, the maximal response was delayed and prolonged.

Contrasting with the flat curve in the NZO AL mice, a definite rise in plasma insulin occurred in the NZO RD mice, so that the mean plasma insulin at twenty and forty minutes after glucose was significantly greater than the fasting level ($P < 0.05$ at each time). The fasting level was not significantly different from that in the NZO AL mice. The plasma insulin response occurred in spite of a lesser glycemic stimulus in the NZO RD mice.

DISCUSSION

Dietary restriction led to some decrease in the degree of insulin resistance in the NZO mice, but did not restore insulin sensitivity to that of the control strain. However, although body weight was reduced to that of the control strain, it is unlikely that body composition was restored to normal. Indeed, Purves[8] has shown that under such conditions, carcass fat composition in NZO mice remains greater than in the control strain, perhaps accounting for the residual degree of insulin resistance. Periodic hyperphagia, caused by the interrupted feeding regime associated with dietary restriction, may also have influenced the insulin sensitivity after weight reduction.[9] However, this pattern of food intake has been shown not to influence the insulin secretory response to glucose in rats.[10]

Correction of insulin resistance accompanying weight reduction has been reported in the ob/ob mutant strain of obese-hyperglycemic mouse,[11-13] in the Wellesley hybrid mouse,[14] KK mouse,[15] and db/db mouse.[16] However, York et al. found that insulin resistance in the genetically obese fatty rat was not improved by restriction of food intake, although insulin resistance in hypothalamic-lesioned rats was improved.[17]

Induced obesity in the GTG-treated C57B mice led to a comparable degree of insulin resistance with that observed in the NZO mice. However, using technics based on the incorporation of C-14-glucose into diaphragm and epididymal adipose tissue, Stauffacher and Renold have reported qualitative differences between the insulin resistance of NZO mice and GTG-treated mice.[18]

Glucose tolerance was variably affected by dietary restriction in the NZO mice. Overall, there was no significant change, although six of the fifteen RD mice returned to normal glucose tolerance. The persistently impaired glucose tolerance of the remaining mice was probably due to a combination of the still abnormal insulin resistance and delay in the insulin secretory response. The GTG-treated mice showed impaired glucose tolerance. Coleman and Hummel have observed mild hyperglycemia in one strain of mice treated with this agent, but not in another strain.[19] In view of the demonstrated effects of hypothalamic lesions on plasma insulin levels and carbohydrate metabolism independent of effects on appetite or plasma growth hormone,[20,21] the changes due to GTG cannot necessarily be attributed to hyperphagia alone. The necessarily greater age of the GTG mice was probably not significant, as C57B mice did not show altered glucose tolerance, insulin resistance or insulin secretion up to one year of age (unpublished results).

The most significant effect of dietary restriction and weight reduction in the NZO mice was in the pattern of insulin secretion in response to glucose. The lack of rise in plasma insulin in the NZO AL animals reported here was typical of the findings in NZO mice of different ages and both sexes reported previously.[1] Contrasting with this universal lack of response, each group of NZO mice maintained on a restricted diet showed a response to injected glucose. This suggests that the observed failure of plasma insulin response to glucose is not a permanent, irreversible abnormality, and that it is at least in part secondary to obesity or hyperphagia. The concurrent improvement of insulin secretion with improvement of insulin sensitivity raises the possibility that both abnormalities may be due to impaired glucose metabolism associated with obesity or hyperphagia. Reduction in food intake may have led to correction of a block in glucose metabolism, manifesting itself peripherally as improved insulin sensitivity and in the pancreas as improved insulin response to glucose.

Rudnick and Taylor have observed a similar improvement in insulin secretion accompanying two months' dietary restriction in maturity-onset human diabetic patients.[22] They suggested that prolonged hyperglycemia may have led to impairment of the ability of the pancreas to respond to glucose with improvement accompanying the better glucose tolerance following dietary treatment. However, in the NZO mouse, the extreme efficiency of the amino acid arginine as an insulinotropic agent prior to weight reduction[1] suggests that islet cell exhaustion or depletion was not the cause of the defective insulin secretion.

Felig et al. have suggested that increased levels of certain amino acids may provide the stimulus to hyperinsulinemia in obese subjects with normal glucose tolerance.[23] The finding of refractoriness to glucose as a stimulus to insulin release, but an enhanced response to

arginine in obese NZO mice is consistent with a similar mechanism accounting for the basal hyperinsulinemia of these animals. The results after food restriction suggest that under these conditions glucose again becomes an effective stimulus for insulin release. The postulated importance of amino acids in insulin secretion in the experimental obese-hyperglycemic syndrome is supported by a recent report from Chick and Like,[24] who showed in the *db/db* mutant that after a short period of food restriction, protein but not carbohydrate refeeding was effective in producing hyperinsulinemia and increased islet mitotic activity.

It is concluded that obesity in the NZO mouse is accompanied by insulin resistance, glucose intolerance, basal hyperinsulinemia, and impaired insulin response to glucose and other agents, but a heightened response to arginine. Food restriction leads to some improvement in insulin sensitivity, and the acquisition by the islets of Langerhans of the ability to respond to glucose. This suggests that the defective insulin response to glucose is not an immutable genetic characteristic, but is, at least in part, dependent on hyperphagia or obesity.

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