Glucose Tolerance and Insulin Secretion in Neonatal and Adult Mice

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SUMMARY

An ultramicro adaptation of the glucose oxidase method requiring only five microliters of blood was developed to study glucose tolerance of C57BL/Ks mice from one week of age through full maturity. Serum IRI levels were also determined ½ hour following intraperitoneal administration of glucose (2 mg./gm. body weight) and L-leucine (0.2 mg./gm. body weight). Glucose tolerance tests were performed by obtaining tail blood from fed mice immediately prior to (0 time) and ½, 1, 2 and 3 hours following intraperitoneal injection of 2 mg. glucose/gm. body weight. Results indicated that mean baseline (0 time) blood glucose levels were lowest in one-week-old mice (92 mg./100 ml. blood) and reached levels observed in adult animals by five weeks of age (142 mg./100 ml. blood). Despite the low baseline blood glucose at one week of age, these same animals exhibited marked and sustained hyperglycemia following glucose administration with mean levels of 469 mg./100 ml. blood and 401 mg./100 ml. blood at ½ and 3 hours, respectively. By two weeks of age glucose tolerance was improved (½ hour = 306 mg./100 ml. blood, 3 hours = 97 mg./100 ml. blood). By three weeks of age glucose tolerance curves appeared similar to those in adult animals. A significant difference between glucose tolerance in male and female mice was noted as early as eleven weeks of age. The marked glucose tolerance in one-week-old mice was explained, at least in part, by failure of these animals to show a significant rise in circulating serum IRI levels following glucose administration despite having the capability to secrete insulin in response to parenteral L-leucine. DIABETES *20*:134-39, March, 1971.

The glucose tolerance of many newborn mammals is characterized by delayed utilization of a glucose load[1-3] associated with delayed secretion of insulin.[2,4] In contrast, glucose tolerance in the adult is characterized by rapid glucose utilization associated with an almost immediate secretion of insulin.[5] Unfortunately, although small laboratory rodents have been employed in a number of studies dealing with carbohydrate tolerance and insulin release, there have been few reports dealing with the developmental aspects of glucose tolerance and insulin secretion in these animals. Such studies are of obvious importance, for example, in determining criteria for normal glucose tolerance in newborn and young mice for purposes of defining more precisely the earliest appearance of glucose intolerance in strains with inherited diabetes.[6] In addition, they are of value in helping to elucidate the effects of aging on carbohydrate utilization.

Because of the limited blood volumes in neonatal mice, serial studies of carbohydrate tolerance by means of repeated blood glucose determinations following glucose injection have not previously been feasible. An ultramicro adaptation of the glucose oxidase method[7] requiring only five microliter samples of whole blood was therefore developed in our laboratory in order to permit examination of glucose tolerance in animals with very limited blood volumes. The present report deals with the results of glucose tolerance testing in mice from one week of age through full maturity. These data are correlated with studies of serum immunoreactive insulin (IRI) levels in animals of the same age.

METHODS

Animals and diet

Mice from the inbred C57BL/Ks strain were pro-

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duced by matings in our laboratory using animals originally obtained from the production department colony of The Jackson Laboratory, Bar Harbor, Maine. The oldest animals used in this study (thirty-four weeks old), however, were raised at The Jackson Laboratory.

Mice were weaned at three weeks of age and were fed with Old Guilford mouse pellets (7 per cent fat). They were allowed free access to food and water except during the periods of testing of glucose tolerance and insulin secretion.

Glucose tolerance

Serial glucose tolerance tests were performed at weekly intervals in animals between one and seven weeks of age and then again at eleven weeks of age. Studies were also performed in thirty-four week old animals not previously tested. Details of the testing procedure and methods used for glucose analysis have been previously reported.[6] Blood samples were collected from the tail using 5 μl heparinized capillary tubes (Drummond Scientific Company). Following the collection of baseline (0 time) blood samples, mice were injected intraperitoneally with an aqueous solution of 20 per cent dextrose (w/v), 2 mg./gm. body weight. Additional blood samples were then collected ½, 1, 2 and 3 hours following glucose administration.

The heparinized blood samples were added to 70 μl of 2 per cent perchloric acid, thoroughly mixed, and centrifuged to separate the precipitated protein. Fifty μl of supernatant fluid was analyzed for glucose by addition of 1 ml. of 0.2 M phosphate buffer, pH 7.0, containing glucose oxidase (specific activity 14,750 units per gram), 25 mg./100 ml.; horseradish peroxidase (specific activity 200 purpogallin units/mg.), 2 mg./100 ml., and o-dianisidine dihydrochloride, 5 mg./100 ml. Samples were incubated for 30 minutes at 30°C. and optical density was read at 436 mμ. Standards run with each assay consisted of duplicate 5 μl samples containing 0, 62.5, 125, 250, 500 and 1,000 mg. D-glucose/100 ml. double distilled water. A quality control consisting of triplicate samples of pooled sera from six C57BL/Ks mice was analyzed with each assay. The validity of the method was checked by comparing values obtained for several samples of mouse blood with values obtained using the ferricyanide method of Hoffman, as adapted for the Technicon AutoAnalyzer.[8]

Insulin secretion

Insulin secretion was studied in animals one, two, three, four and eleven weeks of age. In order to reduce variability in results presumably related to excitation due to handling, mice were anesthetized by intraperitoneal injection of sodium pentobarbital, 5 mg./100 gm. body weight. Baseline (0 time) samples were obtained by decapitation 10 minutes following administration of sodium pentobarbital. In addition, blood samples were obtained ½ hour following intraperitoneal injection of either glucose (2 mg./gm. body weight), L-leucine (0.2 mg./gm. body weight) or an equivalent volume of 0.9 per cent saline (control). These agents were administered ten minutes after the sodium pentobarbital. In order to obtain sufficient quantities of serum for analysis from animals one to three weeks of age, blood was pooled in the following manner: one week—6 to 8 animals per sample; two weeks—3 to 4 animals per sample; and three weeks—2 to 3 animals per sample.

Blood samples were allowed to clot at room temperature for one-half hour. They were centrifuged for 1 hour at 4°C. and the serum separated and stored at —20°C. for one to two weeks prior to insulin assay. The insulin content was determined in duplicate 100 μl aliquots of serum by the double antibody method[9] using I-125 pork insulin and guinea pig anti-pork insulin serum. Purified mouse insulin was used as a standard.[10]

RESULTS

Glucose oxidase micromethod

Results obtained for the analysis of seventeen specimens of mouse blood by the glucose oxidase micromethod were compared to those obtained for samples from these same animals using the ferricyanide method of Hoffman, as adapted for the Technicon AutoAnalyzer.[8] The mean values for seventeen determinations using these two different methods agreed quite closely (glucose oxidase micromethod—122 mg./100 ml. blood, AutoAnalyzer method —123 mg./100 ml. blood).

In addition, the glucose oxidase micromethod yielded reproducible results from one assay to the next. This was apparent from an analysis of the values obtained for the aliquots of quality control serum analyzed with each assay. The mean for twenty-eight consecutive assays was 155 mg. glucose/100 ml. blood with a range of 149 to 162 mg./100 ml. blood, and a standard deviation of 3.9 mg. glucose/100 ml. blood.

Glucose tolerance

The results of the glucose tolerance tests are summarized in table 1. One week old animals showed marked and sustained elevations in blood glucose following injection. Values rose rapidly to 469 mg. glucose/100 ml. blood at ½ hour; the peak value was observed at 1 hour (511 mg. glucose/100 ml. blood) and then slowly declined to 401 mg./100 ml. blood at 3 hours. By 5 hours (data not shown), blood glucose

TABLE 1

Effect of age on glucose tolerance in mice

Age (weeks)	Blood glucose (mg./100 ml. blood) Time (hrs.) 0	½	1	2	3
1	91 ± 2(14)	469 ± 8(15)	511 ± 7(15)	469 ± 15(15)	401 ± 24(9)
2	108 ± 6(9)	306 ± 9(9)	208 ± 17(9)	111 ± 6(9)	97 ± 4(5)
3	126 ± 4(11)	184 ± 18(12)	150 ± 6(12)	120 ± 5(12)	105 ± 5(12)
4	124 ± 5(11)	202 ± 13(11)	168 ± 7(11)	140 ± 8(11)	107 ± 6(11)
5	142 ± 5(11)	230 ± 9(11)	193 ± 6(12)	151 ± 7(12)	127 ± 5(12)
6	146 ± 7(12)	206 ± 10(12)	185 ± 7(12)	146 ± 6(12)	133 ± 10(4)
7	140 ± 4(10)	222 ± 10(10)	192 ± 9(10)	147 ± 5(10)	137 ± 11(4)
11	118 ± 4(18)	176 ± 7(12)	170 ± 8(13)	138 ± 4(13)	138 ± 9(6)
34	134 ± 6(11)	179 ± 9(11)	157 ± 5(11)	125 ± 4(11)	113 ± 3(8)

Values are means ± SEM. Number of mice in parentheses.

concentrations had essentially returned to control levels (93 mg./100 ml. blood). At two weeks of age, the glucose tolerance curve was still elevated, but appeared improved. Values at both ½ and 1 hour were still quite high. At three weeks of age the ½-hour glucose was 184 mg./100 ml. blood, a level comparable to that of adult animals eleven and thirty-four weeks of age. The glucose tolerance curve was also similar to that of adult animals. It is of interest, however, that this ½-hour glucose value for the three week old animals was significantly less than that observed in five and seven week old mice ($P < .01$ and $P < .05$ respectively). Comparison of values at 1 and 2 hours following glucose injection in animals of various ages tended to maintain the same relative order as the ½-hour values. The 3-hour values, however, tended to be more variable than the others.

The baseline (0 time) blood sugar levels increased with age, reaching a plateau at five weeks. The one exception was noted at eleven weeks of age when the value was 118 mg./100 ml. blood, a level significantly lower than those observed at five, six, seven and thirty-four weeks of age (five-seven weeks, $P < .01$; thirty-four weeks, $P < .05$).

The change in glucose tolerance between one and eleven weeks of age is shown graphically in figure 1. The marked glucose intolerance in one week old animals and the gradual improvement with increasing age is apparent. The rise in baseline blood glucose levels between one and five weeks of age is also evident.

Although there was no apparent difference between the baseline blood glucose levels of male and female mice up to eleven weeks of age, the data were further analyzed to determine whether carbohydrate stress would unmask any sex differences in glucose tolerance. At eleven weeks of age a significant difference in glucose tolerance between male and female animals became apparent (figure 2). Male mice had significantly higher blood glucose levels than females at ½, 1 and 2 hours after glucose administration. It was also noted that the eleven week old male mice were significantly heavier than the female, the respective mean weights being 24.8 gm. for males vs. 20.7 gm. for females ($P < .01$). Interestingly, a significant weight difference between the sexes was noted as early as five weeks of age, but the difference in glucose tolerance was not observed until eleven weeks of age. The thirty-four week old mice also differed in glucose tolerance (figure 2), with the males having significantly higher blood glucose levels ½ and 1 hour following glucose administration. However, unlike the eleven week old mice, the thirty-four week old males had significantly higher mean baseline blood glucose levels than the females (males—143

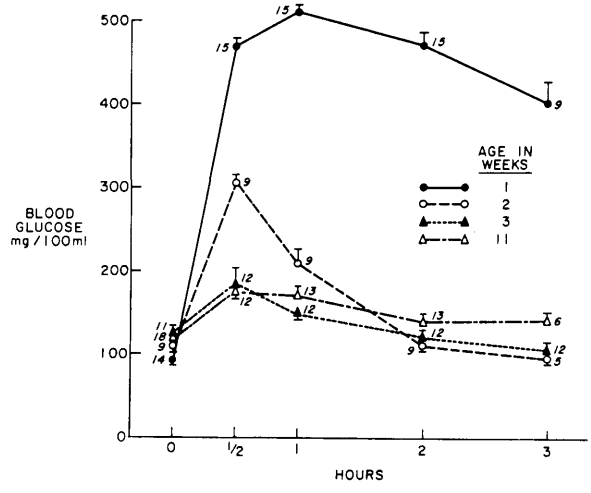

FIG. 1. Effect of age on glucose tolerance in normal C57BL/Ks mice. Values are means ± SEM. The small numerals indicate the number of mice.

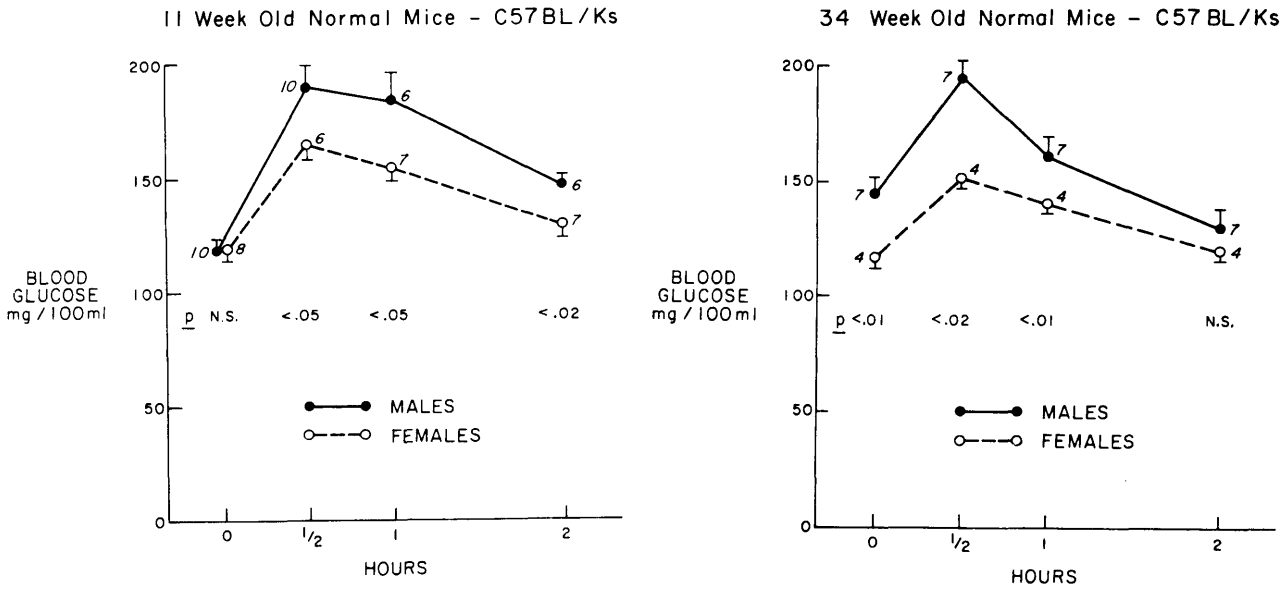

FIG. 2. Glucose tolerance in male vs. female normal C57BL/Ks mice. Values are means ± SEM. The small numerals indicate the number of mice. P is the significance of the difference between glucose values of males and females determined by the Student t-test.
Left panel — 11 week old mice. Right panel — 34 week old mice.

mg./100 ml. blood; females—117 mg./100 ml. blood; $P < .01$), and they did not differ in weight.

Insulin secretion

Serum immunoreactive insulin (IRI) levels were measured to determine whether the markedly abnormal glucose tolerance in one and two week old mice could be explained at least in part by impaired insulin secretion following glucose administration. In addition, serum IRI levels were also measured following the injection of L-leucine, a non-gluconeogenic amino acid known to stimulate insulin secretion. Results were compared both with baseline levels and with those observed following saline injection (figure 3).

At all ages studied there was no significant difference between the baseline IRI and that observed ½ hour following saline. At one week of age glucose administration resulted in no significant increase in IRI levels above baseline or saline control levels. In contrast, however, a definite increase was detected following leucine administration. At two weeks of age a small response

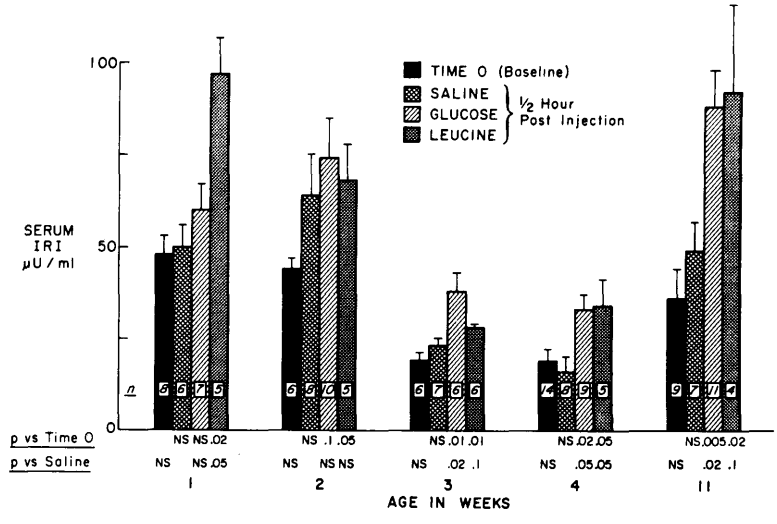

FIG. 3. Effect of age on insulin secretion in response to glucose and leucine in normal C57BL/Ks mice. Values are means ± SEM. The small numerals indicate the number of mice. P values were determined by the Student t-test.

to glucose administration became apparent. By three weeks of age glucose administration resulted in a significant increase in IRI levels above baseline and saline control levels following glucose administration. The IRI response to leucine observed at one week of age persisted in the older mice. An exception to this was seen at two weeks where the leucine induced insulin response was significantly increased compared to baseline ($P < .05$), but not significantly increased compared to the IRI level following saline injection.

The effects of age on the baseline serum IRI levels were also apparent from these data. High levels of serum IRI were observed at one week of age followed by a decline, reaching a nadir at three-four weeks, and then increasing to the high levels again in the older mice. The magnitude of the IRI response following the injection of glucose, leucine and saline also varied with respect to age in a manner roughly parallel to that observed in the baseline IRI levels.

DISCUSSION

The present studies indicate significant glucose intolerance in neonatal mice, with gradual improvement during the first three weeks of life. The most severe, sustained hyperglycemia following parenteral glucose administration was observed in the youngest mice studied, which were one week of age. By two weeks of age, although tolerance had improved, blood glucose levels were still high ½ and 1 hour following injection. By three weeks of age, tolerance curves appeared comparable to those of adult animals.

It is of note that impaired glucose tolerance has previously been reported in other newborn and young mammals including man, monkey, pig and dog.[1-3,11] In the monkey and human this intolerance was associated with failure to observe a prompt, marked rise in serum IRI levels following glucose administration.[2,4] This has been interpreted to reflect a relative inability of the beta cell to secrete insulin in response to glucose.[12,13]

The glucose intolerance in newborn mice is also explained, at least in part, by the failure of serum IRI levels to rise significantly following glucose administration. This poor response to glucose in neonatal mice does not reflect a generalized inability of the beta cell to secrete insulin since serum IRI levels in these animals did rise following L-leucine. It is of interest that a similar response to L-leucine has also been reported to occur in the premature infant.[14] It must be recognized, however, that factors other than changes in insulin secretion may have contributed to the glucose intolerance in one and two week old mice. These include circulating insulin antagonists and decreased tissue sensitivity to insulin. Against these possibilities were the high baseline IRI levels in one and two week old mice which were observed to be associated with low baseline blood glucose levels. This suggested that the circulating insulin indeed was biologically active.

The low baseline blood sugars observed in one and two week old mice deserve comment. This has also been observed in man,[1,15] and a number of hypotheses proposed to explain it. These include an inadequate hormonal control of glycogenolysis during the neonatal period[16] and increased central nervous system glucose consumption[1] due to the relatively high ratio of CNS to total body mass. As already mentioned, the present study suggests that in the mouse, the low baseline blood sugar levels are related to the high baseline serum IRI levels although the other hypotheses mentioned cannot be discounted.

In addition to the findings of glucose intolerance and impaired insulin secretion in newborn mice, it is interesting to note that as early as eleven weeks of age, although mean baseline blood glucose levels were similar, males were less tolerant to injected glucose than females. A similar finding was made in thirty-four week old mice, except that in this case the mean baseline blood glucose levels in males was also greater than that in females. Although eleven week old male mice were significantly heavier than females, it is difficult to ascribe the difference in glucose tolerance to weight alone. First, the difference in weight between the sexes was present prior to eleven weeks and second, the thirty-four week old males and females did not differ significantly in weight. With regard to the difference in glucose tolerance between sexes, it has been reported that testosterone administration results in the deterioration of glucose tolerance in partially pancreatectomized rats.[17]

ACKNOWLEDGMENT

This study was supported in part by U.S. Public Health Service grants AM-14185, AM-12538, AM-09584 and AM-05077. Dr. Chick is the recipient of a U.S. Public Health Service Special Postdoctoral Fellowship, grant F3-AM-36335. Dr. Like is the recipient of a U.S. Public Health Service Research Career Development Award, grant K4-AM-7394.

The authors are indebted to Dr. Philip Poffenbarger for preparing the purified mouse insulin used in this study and to Dr. J. S. Soeldner for his generous help and advice regarding the insulin immunoassay.

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Minerals in Foods

There is a great need for additional information on nutrition composition of foods. Particularly lacking are data on certain vitamins and trace minerals. A. Gormican (J. Am. Dietet. Assn. *56*:397, 1970) has analyzed 128 food items for fourteen inorganic elements. Comparative data are available elsewhere for some of the minerals measured, such as calcium, phosphorus, potassium, sodium, magnesium, and iron. However, the aluminum, strontium, barium, boron, copper, zinc, manganese, and chromium content of several foods is reported for the first time.

While the sample is not exhaustive, it contains several common representative food items in the general categories of: beverages, bread and cereal products, eggs and dairy products, fruits and vegetables, meat and fish, nuts and sugars. The reported values are the result of only duplicate analyses of one sample taken from a hospital kitchen in Wisconsin. Care should be taken, therefore, to avoid overextension of the data. The work is not an attempt to present average values of foods produced under a variety of environmental conditions. However, with these cautions in mind, the data are extremely useful as an order of magnitude estimate of levels of some rarely analyzed nutrients in foods. All measurements were made by use of emission spectroscopy, a very specific method of inorganic analysis.

The author noted general agreement with existing composition data with respect to calcium, phosphorus, and magnesium. Sodium and potassium correlated less well with literature values, perhaps because of differences in methodology. Iron was found to be lower in concentration in most foods than has been reported elsewhere. Copper data were in agreement with some and in disagreement with other reported values. The literature on the other micro-elements is too sparse for comparison.

Mineral data were also presented for composites of several representative routine hospital diets. The recommended dietary allowances of calcium, magnesium, and phosphorus generally were met by all diets except the clear liquid and those diets where protein was restricted. Sodium and potassium were found to be within a range consistent with normal intake in all diets, except those

(*Continued on page* 161)