Studies on Diabetogenic Action of Obesity in Mice: Congenital Insulin Resistance of KK Mice.

HISASHI IWATSUKA AND AKIO SHINO

Biological Research Laboratories, Research and Development Division, Takeda Chemical Industries, Ltd., Osaka.

Synopsis

Inheritance of insulin sensitivity has been studied on C57BL, KK and their F1-hybrids with or without the yellow obese gene (A^y).

Intraperitoneal injection of insulin was followed by a remarkable fall of the blood glucose in C57BL, but a slight fall in KK. In vitro, the adipose tissue of C57BL showed more remarkable response to insulin in glucose-1-14C oxidation than that of KK. F1-hybrid clearly showed intermediate sensitivity to the hormone between the parental strains both in vivo and in vitro.

The yellow hybrid, A^y (the yellow obese gene)-carrying F1-hybrid, showed adiposity and hyperinsulinemia, but did not develop the diabetic symptoms observed in yellow KK, A^y-carrying KK, such as hyperglycemia and degranulation of pancreatic B cells. In the hormone sensitivity of adipose tissue, yellow hybrid was less sensitive than non-yellow hybrid, but more sensitive than KK or yellow KK.

These data suggest that the insulin resistance of KK may be determined by a dosage of polygenes, and may result in higher susceptibility to diabetogenic action of the obesity caused by A^y gene.

Our previous works indicated that KK mice inherited diabetic traits, which were intensified enough to establish overt diabetes by obesity caused by the yellow obese gene (Iwatsuka et al., 1970). We confirmed that the adipose tissue of KK mice showed congenital insulin resistance, which became more prominent with elevation of the blood glucose and plasma insulin. The development of diabetes in this strain is, therefore, likely to be due to increased resistance of peripheral tissue to insulin.

Salans et al., (1968) and Cahill et al., (1967) showed that a decrease in insulin sensitivity of adipose tissue was associated with obesity in human subjects and in Wellesley hybrid mice. These findings support our results described above. Nakamura and Yamada (1963) indicated that the diabetic traits of KK mice, such as glucose intolerance and hypertrophy of pancreatic islets, were inherited by polygenes. The present studies were performed to investigate a possible relation between congenital resistance to insulin and susceptibility to diabetogenic action of obesity, using yellow KK, KK, C57BL and the F1-hybrids between C57BL and yellow KK.

Materials and Methods

Animals: Mice used in the present studies were bred in our laboratory as shown in Figure 1. Male C57BL, non-yellow hybrid (aa: BB: CC or Cc), yellow hybrid (A^ya: BB: CC or Cc), yellow KK (A^ya: BB: Cc) and KK (aa: BB: Cc) were used at the age of 6 to 11 weeks. They were given a laboratory chow (CE2, Clea Japan Inc.) and water ad lib.

Glucose tolerance test and insulin tolerance test:
Mice fasted for 20 hours were injected with glucose (1 g/kg body weight), or insulin (1 or 0.1 U/kg body weight) intraperitoneally. At appropriate time intervals, blood was sampled from orbital veins.

Glucose–1–14C oxidation by adipose tissue: Epididymal adipose tissue from mice was incubated in
1 ml of Krebs-Ringer bicarbonate buffer containing 20 \( \mu \text{moles of glucose-1-}^{14}\text{C} (0.05 \mu \text{Ci}) \) and 2 mg of gelatin with or without insulin. Details of the procedures were described in our previous paper (Iwatsuka et al., 1970).

Histological procedures: Pancreas was fixed in Bouin’s solution, and stained by aldehyde-fuchsin to detect insulin granules in B cells.

Analytical procedures: Blood glucose was determined by a glucose oxidase method (Krebs et al., 1964). Plasma insulin was determined by the double antibody radioimmunoassay method of Hales and Randle (1963). Insulin immunoassay kit and human standard insulin were purchased from The Radiochemical Center.

Results

Diabetic characters of \( F_1 \)-hybrid between C57BL and KK

The blood glucose level of \( F_1 \)-hybrid was comparable to that of the parental strains, C57BL and KK, ranging from 120 to 190 mg\%. There was a difference in glucose tolerance among these types of mice (Fig. 2): i.e. the most impaired in KK, intermediate in the hybrid and least impaired in C57BL, although the difference between the latter two was not statistically significant.

Figure 3 illustrates changes in the blood glucose level after intraperitoneal injection of insulin. Judging from the fall of the blood glucose, KK was defined as “insulin resistant” and C57BL as “insulin sensitive.” The hybrid showed intermediate sensitivity between those of the parental strains.

Enhancing action of insulin on glucose-\( 1^{-14}\text{C} \) oxidation by adipose tissue was observed \textit{in vitro} (Fig. 4). These experiments also indicated the difference in the hormone sensitivity among three types of mice. As summarized

![Graph](image-url)

Fig. 3. Insulin tolerance tests of C57BL, KK and their \( F_1 \)-hybrid. 9 weeks old mice were fasted for 20 hr and administered insulin at doses of 1 U and 0.1 U/kg body weight. (\( \bullet \)) C57BL, (\( \bigcirc \)) KK, (\( \bigotimes \)) \( F \)-hybrid. Mean \( \pm \) s.e. Numbers in parentheses indicate the number of animals.
Fig. 4. Enhancing action of insulin on glucose-1-14C oxidation by adipose tissue. Tissues of 6 to 11 week old mice were used. (●)C57BL, (○)KK, (△)F1-hybrid. Mean ± s.e. Numbers in parentheses indicate the number of animals.

Table 1. Insulin effect on glucose-1-14C oxidation by adipose tissue

<table>
<thead>
<tr>
<th>Insulin Concentration (µU/ml)</th>
<th>10^4 µmoles glucose/100 mg tissue/90 min</th>
<th>10^5 µU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL (a)</td>
<td>0.392 ± 0.025</td>
<td>0.380 ± 0.025</td>
</tr>
<tr>
<td>KK (b)</td>
<td>0.090 ± 0.010</td>
<td>0.123 ± 0.012</td>
</tr>
<tr>
<td>F1-hybrid</td>
<td>0.192 ± 0.014</td>
<td>0.253 ± 0.014</td>
</tr>
<tr>
<td>Mean of Parental</td>
<td>0.241 ± 0.017</td>
<td>0.252 ± 0.018</td>
</tr>
</tbody>
</table>

* Increment of the activity by insulin: µmoles glucose/100 mg tissue/90 min.
**Percent increase of the activity by insulin.

Table 2. General features of non-yellow hybrid, Yellow hybrid and Yellow KK

<table>
<thead>
<tr>
<th></th>
<th>non-yellow hybrid</th>
<th>Yellow hybrid</th>
<th>Yellow KK</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>29 ± 3</td>
<td>29 ± 1</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Blood glucose (mg/100 ml)</td>
<td>177 ± 3</td>
<td>167 ± 9</td>
<td>253 ± 18</td>
</tr>
<tr>
<td>Plasma IRI (µU/ml)</td>
<td>24.3 ± 3.2</td>
<td>58.6 ± 5.8</td>
<td>131.0 ± 4.8</td>
</tr>
<tr>
<td>Adipose Tissue (mg)</td>
<td>289 ± 9</td>
<td>480 ± 61</td>
<td>941 ± 28</td>
</tr>
<tr>
<td>Degranulation of B cell*</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

*Number of mouse with degranulated islets.
8 weeks old mice were used. Mean ± s.e.

in Table 1, the enhancing action of insulin observed in the hybrid agrees with the mean of those of the parental strains, whether the effects are expressed by percent increase or increment of the activity.

**Effects of the yellow obese gene on F1-hybrid**

Figure 4 illustrates the effects of insulin on the glucose oxidation by adipose tissue in four types of mice. Enhancing action of insulin was lower in yellow mice (yellow hybrid or yellow KK) than in their respective non-yellow littermates (black hybrid or KK). The action of the hormone observed in yellow hybrid was much higher, in comparison with that in yellow KK or KK. These results indicate that the yellow obese gene diminished insulin sensitivity of adipose tissue of the hybrid as in the case of KK, and that the difference observed between the hybrid and KK persists in the presence of the yellow obese gene.

Table 2 shows other changes caused by the yellow obese gene in the hybrid. Yellow hybrid showed hypertrophy of the adipose tissue and higher level of the plasma insulin, but did not show any increase in the blood glucose level when compared with the non-yellow hybrid. On the other hand, yellow KK showed advanced diabetic state as indicated by marked increases in adipose tissue weight, the blood glucose and the plasma insulin level. Histological finding that degranulation of B cells occurred in yellow KK, but not in yellow...
Discussion

Several investigators (Nakamura, 1962; Tsuchida, 1966; Dulin and Gerritsen, 1967) have reported on insulin resistance and glucose intolerance of KK strain. These findings were also confirmed in the present studies, using C57BL as the control. F1-hybrid between C57BL and KK clearly showed intermediate insulin sensitivity to those of the parental strains in vivo and in vitro. These results indicate that insulin resistance is subjected to genetic factor(s), presumably polygenes. Polygenic inheritance of insulin resistance in KK mice may be suspected from the genetical analysis of this strain by Nakamura and Yamada (1963) in which glucose intolerance and hypertrophy of pancreatic islets were proved to be determined by polygenes. However, further studies, such as backcrossing between parental strains and F1-hybrid, will be necessary to confirm polygenic inheritance of insulin resistance.

It has been reported that obesity decreases insulin sensitivity of adipose tissue and increases plasma insulin level in human subjects (Salans et al., 1968) and in Wellesley hybrid mice (Cahill et al., 1967). In the present studies, we observed the same actions of obesity due to the yellow obese gene in yellow hybrid as in the case of yellow KK (Iwatsuka et al., 1970). Marked differences in the blood glucose level, the plasma insulin level and degranulation of B cells between yellow hybrid and yellow KK may be accounted for by the difference in insulin sensitivity of adipose tissue: the sensitivity of yellow hybrid was much higher than that of yellow KK. These results suggest that susceptibility of mice to diabetogenic action of obesity may depend on insulin sensitivity of peripheral tissue which is determined genetically.

Hellerström and Hellman (1963) observed hyperglycemia and degranulation of B cells in yellow obese mice, and these results seemed inconsistent to our present findings. The discrepancy may be explained by the effect of age on insulin sensitivity of adipose tissue, because the mice used by Hellerström and Hellman are much older than yellow hybrid in the present studies. De Fronzo et al., (1967) observed age-dependent decrease in insulin
sensitivity of adipose tissue in rats. We also found the same event in KK or yellow KK (Iwatsuka et al., 1970).

In conclusion, KK strain carries genetically determined impairment in insulin sensitivity, which may cause higher susceptibility to such diabetogenic factors as obesity or age, lowering the hormone sensitivity of periphery.

Acknowledgements

We wish to thank Mr. E. Ishikawa for his technical assistance, and Mr. K. Furuno and Mr. K. Shimakawa for their supply of mice for the present studies. Our thanks are also due to Dr. Z. Suzuoki and Dr. K. Shimamoto for their guidance and encouragement.

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