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Pathogenetic Aspects of the Obese-Hyperglycemic Syndrome in Mice (Genotype *obob*): I. Function of the pancreatic B-cells*

S. Westman

Department of Histology, University of Uppsala, Uppsala, and Department of Pathology, Medical School of Linköping Linköping, Sweden

Summary. The insulin secretion has been studied in both fed and starved obese-hyperglycemic mice (genotype *obob*) and their lean siblings. Groups of mice, 2—15 months old, were injected intravenously with either glucose or glucagon, and the serum levels of glucose and immunoreactive insulin measured. Injection of glucose, 3.75 g per kg body weight, caused a prompt increase of the serum insulin in all *obob* mice except for the group of 4 months old animals with free access to food. In contrast to the lean siblings the obese mice displayed an enhanced disappearance rate of glucose from serum with increasing age. Administration of glucagon effected a marked and prompt increase of the serum insulin concentration in all animals. Hence the delayed and low insulin response previoulsy observed in human diabetics was not found in mice with the obese-hyperglycemic syndrome.

Aspects de la pathogénèse du syndrome obésité-hyperglycémie chez la souris (génotype obob). I. Fonctions des cellules B du pancréas

Résumé. La sécrétion de l'insuline a été étudiée chez la souris obèse-hyperglycémique (génotype *obob*) nourrie et à jeun ainsi que chez les animaux normaux des mêmes nichées. Des groupes de souris âgées de 2 à 15 mois ont reçu des injections intraveineuses de glucose ou de glucagon et les taux sanguins de glucose et d'insuline immunoréactive ont été mesurés. L'injection de 3.75 g glucose/kg de poids corporel est suivie d'une augmentation immédiate de l'insulinémie chez toutes les souris *obob*, à l'exception du groupe d'animaux âgés de 4 mois nourris *ad libitum*. A l'opposé de ce qui se passe chez les souris normales, le taux de disparition de glucose du sérum chez la souris obèse augmente avec l'âge. L'administration de glucagon entraîne un accroissement prompt et marqué de l'insulinémie chez tous les animaux. La riposte retardée et faible, décrite précédemment dans le diabète humain n'a donc pas été observée chez la souris obèse-hyperglycémique.

Zur Pathogenese des obes-hyperglykämischen Syndroms der Maus (Genotyp obob) I. Die Funktion der B-Zellen des Pankreas

Zusammenfassung. Die Insulinsekretion 2—15 Monate alter *obob* Mäuse und deren gleichaltriger normalgewichtiger Geschwister wurde nach intravenöser Injektion von Glucose (3.75 g/kg) oder Glucagon untersucht. Mit Ausnahme der 4 Monate alten *ad libitum* ernährten Tiere stieg die Insulinkonzentration im Serum aller *obob* Mäuse schnell an. Im Gegensatz zu den normalgewichtigen Tieren nahm die Abbaurate der Glucose bei *obob* Tieren mit steigendem Alter zu. In allen Fällen stieg nach Glucagoninjektion die Insulinkonzentration sofort stark an. Die beim diabetischen Menschen festgestellte verminderte und verspätet erfolgende Insulinsekretion ist somit im Fall des obes-hyperglykämischen Syndroms der *obob* Mäuse nicht nachzuweisen.

Key-words: *obob* mice, insulin secretion *in vivo*, serum glucose, serum insulin, glucose tolerance, glucagon effect, islet B-cell function.

The recessively inherited obese-hyperglycemic syndrome in mice (gene symbol *ob*) has been studied from various aspects during the 19 years that have elapsed since it was first described [4]. The first sings of a disturbed carbohydrate metabolism have been observed in the homozygous animals at the age of about 20 days, when they display both increased serum insulin levels and an increased tolerance towards exogenous insulin. In somewhat older obese animals the serum glucose level also rises. After some months the increased serum insulin and glucose concentrations gradually decrease again [12]. A peripheral defect of the carbohydrate metabolism has been assumed to cause the long-term hyperglycemia associated with an excessive insulin production [9]. However, up to now there seems to be no data available on the insulin secretion following short term stimulation of the islet B-cells in the obese-hyperglycemic animals. Information on this point would further clarify the question of whether a defective B-cell function is of etiological significance in the development of the syndrome. In the present study the variations in serum immunoreactive insulin and glucose concentrations have been measured after intravenous administration of glucose and glucagon.

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Materials and Methods

Animals. Altogether 95 male *obob* mice and 81 of their lean littermates, 2—17 months old, were used. The animals belonged to the colony bred at the Department of Histology, University of Uppsala, Sweden. They were given a commercial pelleted food fortified with vitamins (Protovit®, Roche) and tap water ad libitum (cf. 12).

Glucose injections. Immediately after a blood sample had been obtained by puncture of the orbital vein plexus with a thin walled Pasteur pipett glucose was injected into a tail vein. The vein was visualized by transilluminating the pigmented tail. For the glucose tolerance tests 3.75 g glucose per kg body weight was given as a 30% solution. Blood samples were then taken at regular intervals between 4 and 64 min. The blood

was collected in dry test tubes without anticoagulants, allowed to coagulate and centrifuged immediately afterwards. The serum was then removed and stored at −18° C until assayed. Serum glucose determinations were performed on duplicate 5 µl samples by a glucose oxidase method as described by Hjelm and DeVerdier [3]. A separate blank without added glucose oxidase was run for each sample. The immunoreactive serum insulin was determined by the double antibody radioimmunoassay according to Hales and Randle [2] using the kit obtained from The Radiochemical Centre, Amersham, England; crystalline mouse insulin (23 IU/mg) being used as a standard.

Glucagon injections. Each animal received an intravenous injection of 0.6 mg glucagon (Lilly) per kg body weight dissolved in dilute HCl, pH 2.6. A group of control animals were injected with dilute HCl only. Serum glucose and insulin concentrations were measured at regular intervals up to 128 min afterwards as described above. In one experiment a second injection of glucagon was given immediately after the 32 min blood sample had been withdrawn.

Results

The results of the i.v. glucose tolerance tests are given in Figs. 1 and 2. It is evident that in the fed mice (Fig. 1) the glucose disappearance changed with age

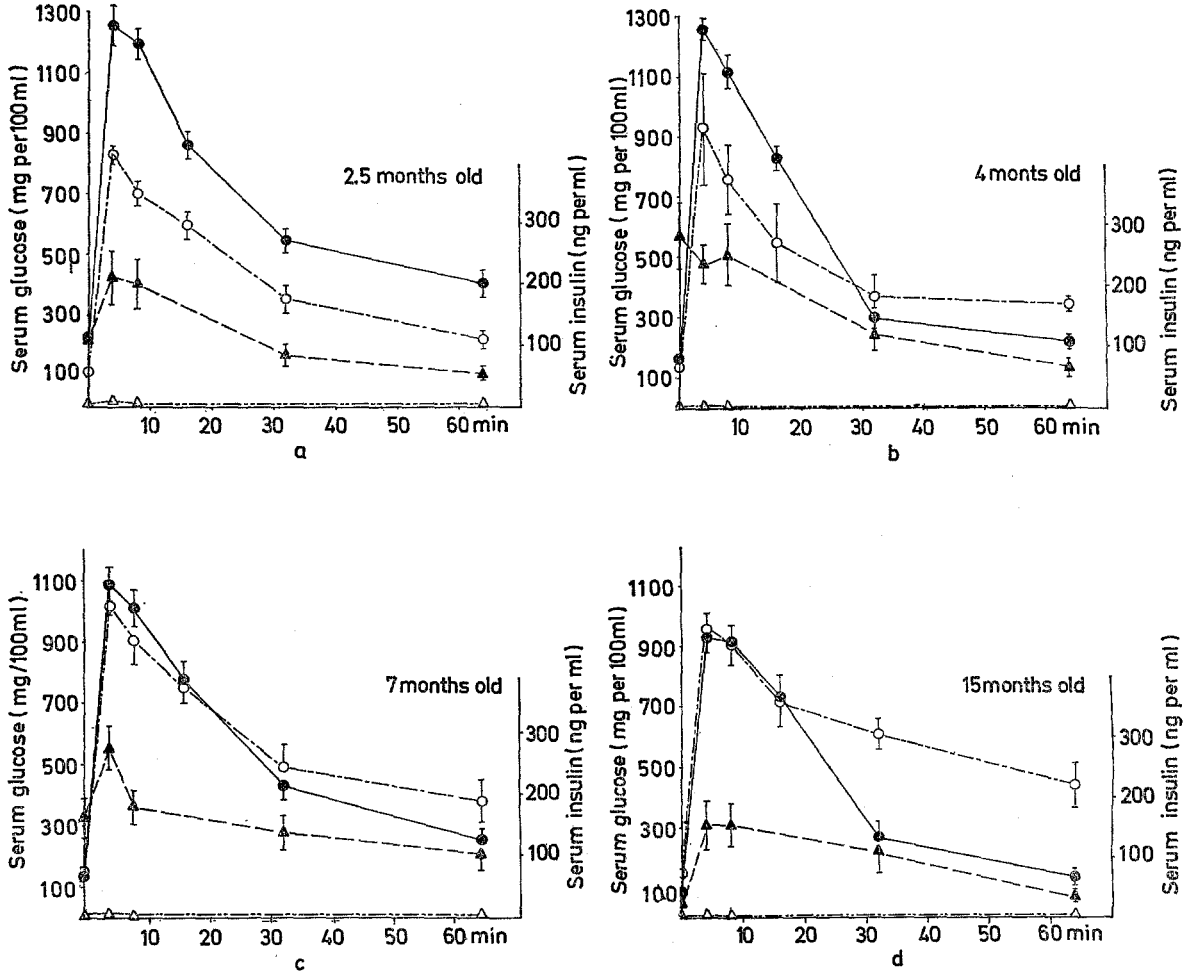

Fig. 1. I. v. glucose tolerance tests in obese and lean mice of different ages and with free access to food. Mean values obtained in 8 to 11 obese and 3 to 9 lean animals

Lenght of vertical lines indicates S.E.M. Serum glucose in obese (●) and lean (0) mice. Serum insulin in obese (▲) and in lean (△) mice.

both in the obese and the lean animals. Whereas in the former there was an increased glucose disappearance rate in the older animals, the lean littermates rather showed the reverse, which is particularly evident from a comparison between Figs. 1a and 1d.

Differences between the two types of animals were found also with respect to the insulin secretion patterns In the obese mice there was a marked insulin response to the glucose injections in all age groups except for the 4 months old animals. At this age the pre-injection

insulin level was very high and gradually decreased after glucose administration. The lean animals showed a small or absent insulin response in all age groups; only at 2.5 months of age was the increment statistically significant ($P < 0.01$).

As can be seen in Fig. 2 the general pattern of the serum glucose curves in the starved obese and lean animals were similar to those of the fed animals. Hence, in the 7 and 15 months old obese mice the glucose disappearance rate appeared increased as compared to the lean animals. As expected, the pre-injection insulin levels were lower in the starved than in the fed obese mice. At all ages the serum insulin levels of the former animals increased in response to the glucose injection. There was a particularly strong stimulation of the B-cell function in the 15 months old mice. By contrast, there was no significant increase of the serum insulin concentration in any of the lean animal groups after the starvation period.

Administration of glucagon to the fed animals caused a marked rise in the serum glucose concentrations, which increased to more than twice the initial levels (Fig. 3). Both the obese and the lean animals displayed a marked increase of the serum immunoreactive insulin concentration. In the 2.5 months old obese animals (Fig. 3a) the insulin had increased at 4 min to a value about 4 times that of the baseline level and in the lean littermates about 2.5 times. For the 4 months old animals (Fig. 3b) the corresponding increases were 3.5 and 2.5 times respectively.

When the mice were given the same amount of glucagon after 18 h starvation, the relative increase of the serum insulin content in the 2.5 months old mice was similar to that observed in the fed animals (Fig. 4a). The effect in the 4 months old obese animals was found to be significantly greater; the mean serum insulin level was in fact increased more than 10 times (Fig. 4b). Administration of HCl alone did not affect

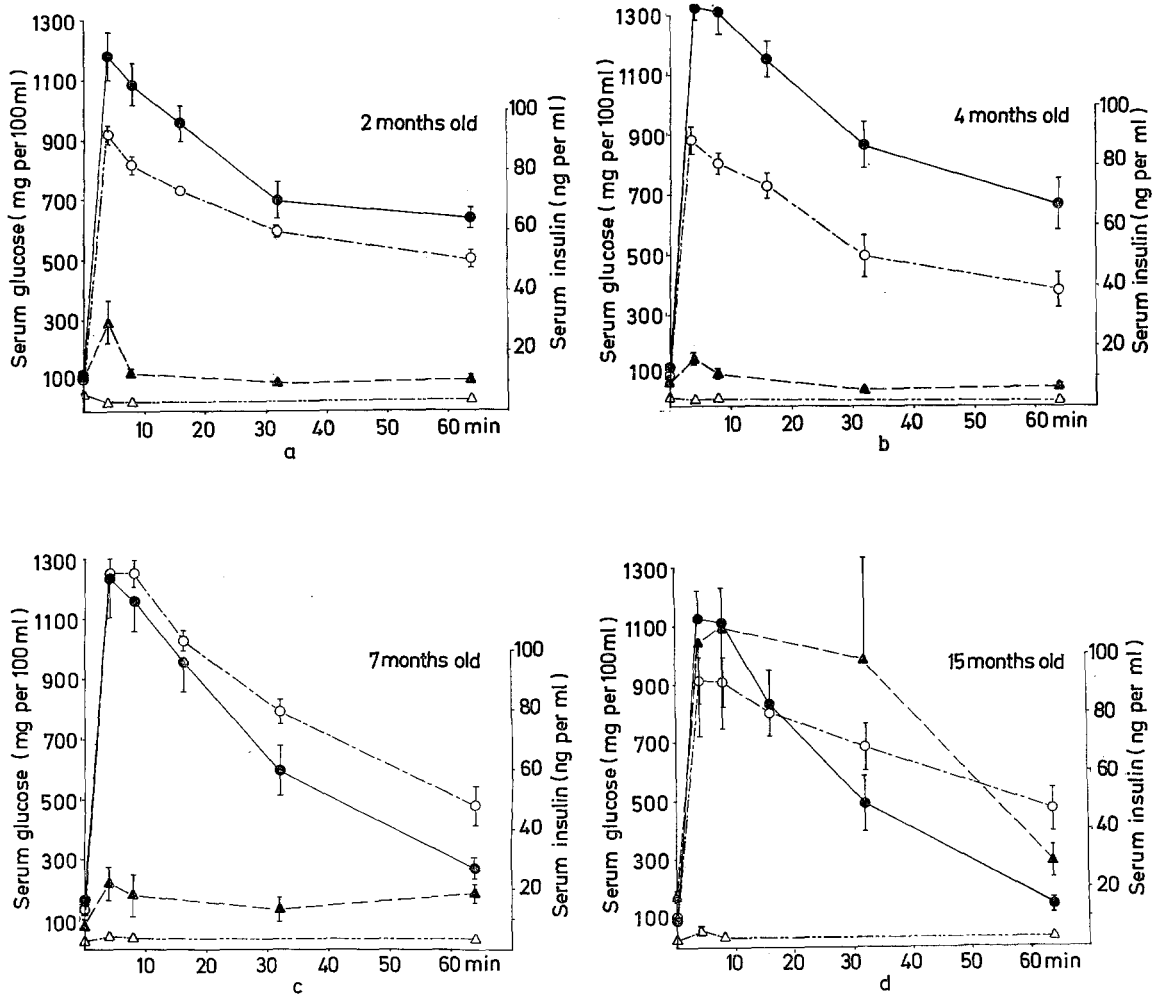

Fig. 2. I. v. glucose tolerance tests in obese and lean mice of different ages and after 18 h starvation. Mean values obtained in 6 to 10 obese and 5 to 9 lean animals. Symbols as in Fig. 1.

the serum insulin level to any considerable extent. When two intravenous glucagon injections were given, the second dose was also found to result in a significant increase of the serum insulin level in the *obob* mice (Fig. 5).

Discussion

From a previous study on the development of the obese-hyperglycemic syndrome in mice it appeared that in the younger animals the increased serum insulin levels were not sufficient to normalize the high blood glucose concentration [12]. The present results agree well with this conclusion in that the peak serum glucose levels were particularly high in the 2.5 and 4 months old obese mice. Alternatively, it seems possible that a prolonged blood circulation time because of the high fat storage at these ages, might be of significance in this context. A further possibility that apparent differences in glucose disappearance reflect differences in extracellular fluid volumes between obese and lean animals should also be considered. There is recent evidence to suggest that the extracellular volume as expressed per unit body weight may be somewhat smaller in the obese animals [13]. The observation that the obese animals were able to eliminate their blood glucose at a near normal rate appears somewhat puzzling in view of their marked insulin resistance [9, 12].

In the 4 months old obese animals with free access to food an extra glucose load did not appear to stimulate the pancreatic B-cells to further insulin secretion. Possible explanations for this lack of secretory responsiveness to an acute glucose load might be a depletion of intracellular insulin stores, or alternatively an adaptation to the prevailing high levels of circulating serum glucose [12]. The chronic hyperinsulinemia of these animals might also contribute to make glucose a less effective stimulus for insulin output. Administration of sub-hypoglycemic doses of insulin to rats for up to 4 days has recently been found to inhibit the effect

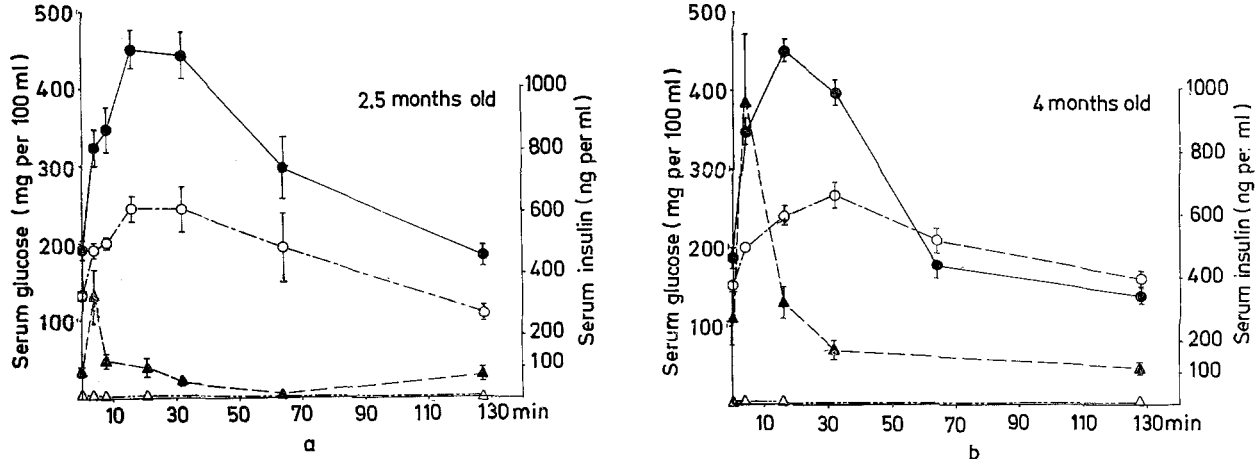

Fig. 3. I. v. glucagon tests in obese and lean mice of different ages and with free access to food. Mean values obtained in 10 obese and 10 lean animals. Symbols as in Fig. 1.

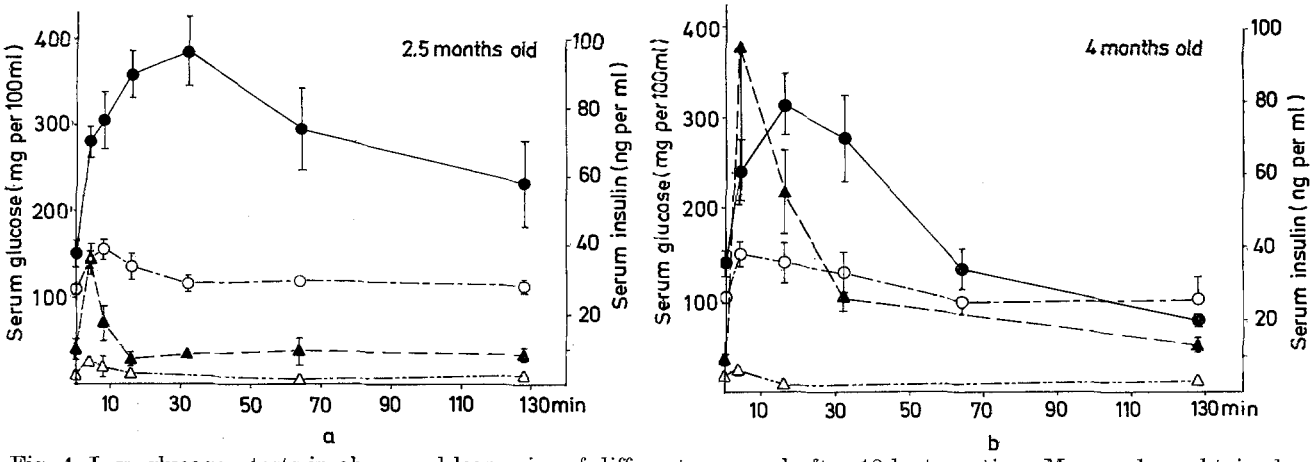

Fig. 4. I. v. glucagon tests in obese and lean mice of different ages and after 18 h starvation. Mean values obtained in 5 to 9 obese and 5 to 10 lean animals. Symbols as in Fig. 1.

of glucose on the insulin release from pancreatic slices [7].

The 4 months old fed obese-hyperglycemic mice retained the capacity to respond to glucagon with a significant increase. It appears therefore that glucagon and glucose affect the insulin secretion mechanism of the B-cells in different ways. Moreover also a second dose of glucagon was effective in the 4 months old obese-hyperglycemic mice although the resulting insulin peak was considerably smaller. These findings agree well with current concepts indicating a direct glucagon stimulation of the insulin secretion both *in vivo* and *in vitro* [5, 10, 11].

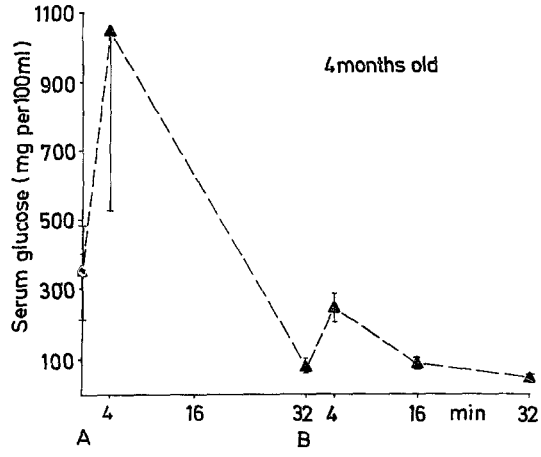

Fig. 5. The effect of 2 successive i. v. injections at A and B of glucagon (0.6 mg/kg body weight) on the serum insulin level of 4 months old obese-hyperglycemic mice with free access to food (▲). Mean values ± S.E.M. based on observations in 6 animals

An increased insulin release in mice with the obese-hyperglycemic syndrome (except for the 4 months old fed animals), is in conformity with previous observations on both pancreatic slices and isolated islets from these animals [6, 8]. The insulin response displayed by the homozygous mice was generally prompt and there was a fast return to the initial, or lower than initial, serum insulin levels. This is in marked contrast to the delayed and low insulin response to glucose which has been recorded in diabetic humans and which has been suggested to be of etiological significance for the development of the disease [1].

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S. Westman, M. D.
Department of Histology
University of Uppsala
V. Ågatan 26 A
S-752 20 Uppsala 1