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CHANGES IN PLASMA GLUCOSE, INSULIN AND GLUCAGON LEVELS, GLUCOSE TOLERANCE TESTS AND INSULIN SENSITIVITY WITH AGE IN THE RAT *

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SUMMARY

Glucose and insulin relationships with aging were studied in fed rats. Levels of basal circulating glucose did not change while those of RIA-insulin increased and RIA-glucagon decreased lineary with animal weight. The oral glucose tolerance test revealed a greater increase in blood glucose in adult and old rats than in prepuberals, while the rise in plasma insulin was faster and greater in the oldest group. After intravenous glucose load, plasma insulin increase was greater in adult than in prepuberal and old rats, and in the latter group values remained elevated for a longer period. The hypoglycemic response to i.v. insulin was greatest in the prepuberals with no difference between adult and old rats. In prepuberals, the augmented insulin sensitivity was counteracted by retarded insulinotropic glucose action and an enhanced basal glucagon level, while in the old animals normoglycemia was maintained due to an augmented secretory response of B cells, counteracted by reduced sensitivity to endogenous insulin.

Key Words : Aging. Glucose tolerance tests. Insulin sensitivity. Glucagon. Glycemia.

RÉSUMÉ

Modifications du taux plasmatique du glucose, de l'insuline et du glucagon, du test de tolérance au glucose et de la sensibilité à l'insuline chez le rat en fonction de l'âge.

Les relations du glucose et de l'insuline ont été étudiées en fonction de l'âge chez les rats normalement nourris. Le taux basal de la glycémie n'était pas modifié tandis que celui de l'insuline s'élevait et celui de la glucagonémie s'abaissait d'autant plus que le poids était plus élevé. Le test de tolérance au glucose par voie orale montrait une élévation de la glycémie supérieure chez les rats adultes ou âgés que chez les rats prépubères, tandis que l'élévation de l'insulinémie était plus précoce et plus marquée chez les rats âgés. Après charge glucosée intra-veineuse l'élévation de l'insulinémie était plus marquée chez les rats adultes que chez les rats prépubères ou âgés, mais chez ces derniers elle persistait plus longtemps. La réponse hypoglycémique à l'insuline injectée par voie veineuse était plus marquée ch z ' s rats prépubères que chez les autres. Chez les ra pubères, l'hypersensibilité à l'insuline était annulée par le retard de l'effet insulinotrope du glucose et l'élévation de la glucagonémie basale ; chez les animaux âgés la normoglycémie était maintenue par la réponse sécrétoire accrue de la cellule B qui s'opposait à la sensibilité réduite à l'insuline endogène.

Mots Clés : Age. Tests de tolérance au glucose. Sensibilité à l'insuline. Glucagon. Glycémie.

Previously reported changes in carbohydrate tolerance with age are contradictory. Abnormally high plasma insulin levels have been observed in basal conditions and during oral glucose tolerance tests in old subjects (1-7) and senile rats (8), while other studies have indicated a decrease in tissue responsiveness to insulin in old subjects (1, 3, 9-11). Conversely, reduced

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(12) or unaltered (13) levels of blood insulin in old humans as well as persisting insulin sensitivity (14) in old rats have been reported.

Glucagon and insulin are basic elements in the continuous regulation of glucose homeostasis, providing signals for glucose generation and utilization respectively (15). Basal and arginine stimulated levels of blood glucagon are not significantly altered during advancing years in man (16) but, to our knowledge, no studies on glucagon levels in the aging rat have been reported. In the present study, the changes with age of basal levels of glucose, insulin, and glucagon were investigated in male and female rats. Oral and intravenous glucose tolerance tests and the response to exogenous insulin were also compared in three groups of male rats of different ages in order to determine whether gastrointestinal insulinotropic factors and variations in sensitivity to endogenous and exogenous insulin contribute to alterations in insulin/glucose relationships with age.

MATERIALS AND METHODS

Wistar rats from our colony were fed purine chow diet and housed in a temperature (22 ± 1°C) and light cycle (12 h on-off) controlled room. Animals of different ages were sacrificed by decapitation without anesthesia and blood was collected from the neck into heparinized beakers. Aliquots of whole blood were deproteinized with Ba $(OH)_2$-Zn SO, (17), analyzed for glucose (18) and aliquots of plasma were assayed for insulin (19) with an insulin radioimmunoassay kit (Amersham Radiochemical Center) and with rat insulin as standard, generously supplied by Novo Industri A/S. Another series of rats was sacrificed in the same manner, using serum instead of plasma, for the assay of glucagon (20) after ethanol extraction (21), employing the total rat glucagon radioimmunoassay kit also provided by Novo Industri A/S.

For the study of glucose tolerance and insulin sensitivity, male animals were divided into three groups : *prepuberals*, weighing 80-100 g (about four weeks old), *adults* of 180-200 g (eight to ten weeks old) and *old animals* of 400-500 g (over one year old). When glucose tolerance tests were performed, the animals received glucose (2 g/kg b.w.) by gastric intubation or by i.v. injection through a tail vein. At different times following treatment, blood samples were collected from the tip of the tail into heparinized porcelain plates. The entire procedure was performed without anesthesia. Aliquots of whole blood and plasma were used for the assays of glucose and insulin, respectively.

Glucose disappearance rates were calculated in the i.v. glucole tolerance tests from the slope of the $\log_e$ of the glucose level at 3, 7.5 and 15 min. The insulin sensitivity test was performed by injecting i.v. 10 IU/kg b.w. of monocomponent porcine insulin into a tail vein. Blood was collected at different times for the assay of glucose as described above. In all tests, control animals from each age group received the corresponding medium and blood was treated as for experimental animals. Control values were pooled since there were no significant age effects on blood glucose or insulin levels. Statistical analysis of the data was performed with the Student " t " test.

RESULTS

Basal values :

Circulating levels of glucose did not change while those of insulin showed a significant positive correlation to body weight ($p < 0.001$) (*Figs 1a* and *1b*), with no differences between male and female rats. Total glucagon values in plasma were correlated significantly and negatively to weight (females, $p < 0.001$ and males, $p < 0.01$) (*Fig. 1c*), values in males being higher than those in females.

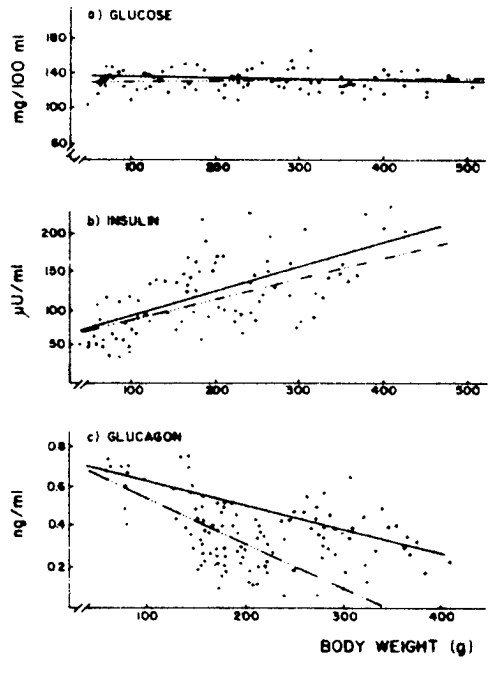

FIG. 1. — *Blood glucose and plasma RIA-insulin and — total glucagon levels in female and male rats as related to their body weight.*

a) Glucose :
♂ ——— , $y = 137.8 - 0.0121\ x$, $r = -0.130$, p = N.S.
♀ - - - - , $y = 129.0 + 0.0132\ x$, $r = 0.144$, p = N.S.

b) Insulin :
♂ ——— , $y = 56.5 + 0.255\ x$, $r = 0.585$, $p < 0.001$
♀ - - - - , $y = 57.5 + 0.265\ x$, $r = 0.635$, $p < 0.001$

c) Glucagon :
♂ ——— , $y = 0.715 - 0.00113\ x$, $r = 0.474$, $p < 0.01$
♀ - - - - , $y = 0.739 - 0.00206\ x$, $r = 0.623$, $p < 0.001$

Glucose tolerance tests :

Oral and intravenous responses to 2 g/kg glucose were studied in three groups of male rats : *prepuberals*, weighing 80-100 g (about four weeks old), *adults* of 180-200 g (eight to ten weeks old), and *old animals* of 400-500 g (over one year old). Blood glucose levels after the oral glucose load (*Fig. 2a*) revealed a greater increase in adult and old rats than in prepuberals. In this latter group, the glucose values 15 min after glucose administration were significantly lower than those of adults ($p < 0.01$) and old ($p < 0.05$) rats, and the values did not differ from those in controls receiving saline up to 30 min after glucose administration. Plasma insulin levels in old animals (*Fig. 2b*) peaked 7.5 min after glucose load. At this time these were higher than levels in adults ($p < 0.05$) while the peak in prepuberal rats did not appear up to the 15 min time point, its size being not different than that observed in the old animals at 7.5 min. Plasma insulin values 60 min after oral glucose load in old rats and in adults did not differ but were significantly higher ($p < 0.01$) than in the prepuberals.

After i.v. glucose administration, the 3 min blood glucose level was highest in prepuberals (*Fig. 3a*) ($p < 0.01$, vs. adults) followed by the old and adults groups while at 60 min it was lowest in the youngest group ($p < 0.05$ vs. adults). Calculated as glucose disappearance coefficients, Kg was 5.13 ± 0.26 for prepuberals, 6.04 ± 0.34 for adults (no significant difference when compared with prepuberals : $p > 0.05$) and 3.70 ± 0.40 for olds ($p < 0.05$, vs. prepuberals and $p < 0.01$ vs. adults). Plasma insulin (*Fig. 3b*) increased more in the adult than in prepuberal and old rats, as early as 3 min after glucose load ($p < 0.01$ and $p < 0.05$ respectively vs. the values in the adults) and remained higher in the older group 60 min after glucose administration ($p < 0.01$ vs. both adults and prepuberals).

Insulin Sensitivity Test :

The drop in blood glucose levels after i.v. administration of monocomponent porcine insulin (10 IU/kg b.w.) was faster and greater in prepuberals than in

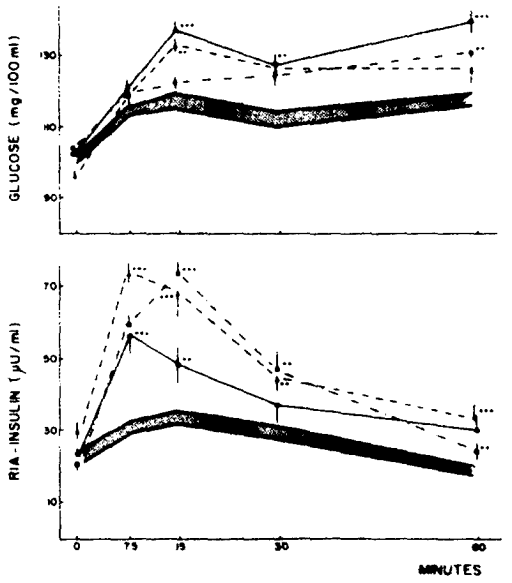

FIG. 2. — Blood glucose (a) and plasma RIA-insulin (b) concentrations in prepuberal (80-100 g, b.w.) (■—■), adult (180-200 g, b.w.) (●——●) and old rats (400-500 g) (▲- - -▲) after oral glucose administration (2 g/Kg), compared with controls (shadowed area). P values vs controls : * = $p <$ 0.05, ** = $p < 0.01$, *** = $p < 0.001$ Means ± SEM of 7-10 rats/group. Control values are from 7 rats from each age group, receiving saline.

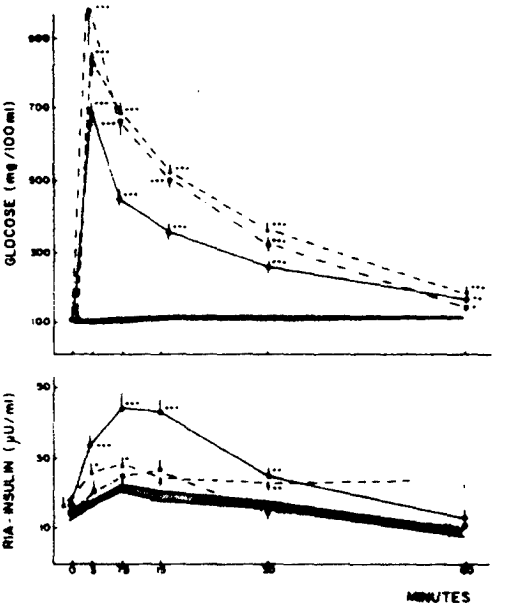

FIG. 3. — Blood glucose (a) and plasma RIA-insulin (b) concentrations in prepuberal (80-100 g, b.w.) (■—■), adult (180-200 g, b.w.) (●——●) and old rats (400-500 g) (▲- - -▲) after intravenous glucose administration (2 g/Kg), compared with controls (shadowed area). P values vs control : * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Means ± SEM of 6-8 rats/group. Control values are from 6 rats from each age group, receiving saline.

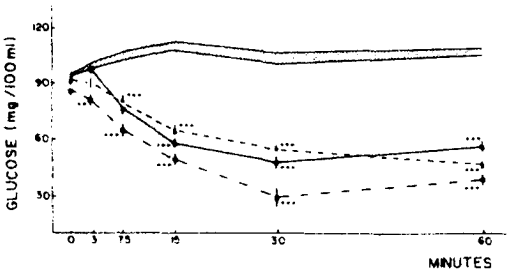

FIG. 4. — Blood glucose concentrations in prepuberal (80-100 g, b.w.) (■—■), adult (180-200 g, b.w.) (●——●) and old rats (400-500 g) (▲- - -▲) after intravenous monocomponent porcine insulin administration (10 IU/Kg, b.w.), compared with controls (shadowed area). P values versus controls : * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Means ± SEM of 6-8 rats/group. Control values from 6-8 rats from each age group, receiving saline.

adult and old rats *(Fig. 4)*, their blood glucose values being significantly higher ($p < 0.05$) than those of the first group at all time points, with no significant differences between the last groups.

DISCUSSION

In the present study, body weight has been used as an index of age in the rat for it increases regularly in the species up to one year of age. Although the changes with body weight in circulating glucagon, insulin and glucose levels observed in our experiments agree qualitatively with comparable values for young, adult, and old subjets reported in the rat (22), dog (23), and in man (1,16), the significant correlation found between plasma insulin and total glucagon in relation to weight or age has not been previously reported. This could either be due to the limited number of samples taken or to the use of fasting samples to secure those parameters, as in the study of Dudl and Ensinck (16), producing an apparent decrease unless related to age, as already reported for plasma glucagon levels in the dog (23).

Plasma insulin levels are significantly and positively correlated to body weight of the animals. The low circulating insulin levels in the young rats coincide with the decreased insulinotropic action following i.v. glucose load and with the delayed insulin peak after oral glucose administration observed in prepuberals compared with adult levels. This difference may be due to increased hepatic insulin extraction (24) and/or reduced response of the pancreas to the insulinotropic effect of glucose in young animals. The *in vitro* insulinotropic effect of glucose concentration is reportedly similar in young and old rats although the pancreatic insulin pool in young animals is much greater (25) ; this finding indicates a higher prepuberal threshold for insulin response. Plasma immunoreactive glucagon levels are also significantly but negatively correlated to body weights. The high plasma glucagon levels in the young animals could contribute to a diabetogenic situation, but it is known that tissue sensitivity to glucagon is reduced in young versus adult animals due to lowered affinity to receptor sites (26). This effect, combined with the augmented insulin sensitivity in these animals, may counteract their augmented diabetogenic factors, producing normal glycemia.

In old rats, the high circulating levels of insulin correspond to their normal or enhanced response to the glucose load, a situation that has also been observed in older humans (1, 6, 27). The elevated levels of circulating insulin in the presence of normal glycemia in old animals signify either an augmented sensitivity to the direct glucose insulinogenic stimulus (contrary to the conclusions in another *in vitro* study) (25) ; or to an enhanced response of gastrointestinal, insulinotropic factors. Present results support the second possibility because, under the same oral glucose stimulus, old rats produced a greater peak of circulating insulin than did the younger animal, while this was not the case after i.v. glucose administration. A prolonged half-life of circulating insulin may also influence the maintained increase of this hormone in plasma under basal, fed conditions. The prolonged enhancement of plasma insulin levels after glucose administration (both *per os* and intravenously) observed in this study and the reduced insulin-degrading activity in different tissues recently reported (28) in old rats would support this explanation. On the other hand, augmented circulating levels of insulin would cause reduced glucose concentrations which, in confirmation of an earlier report (14), were not found in our old rats in spite of their normal response to exogenous insulin. These results, in the presence of the normal or reduced carbohydrate tolerance in the aging process, may well be attributed to reduced activity of the endogenous hormone, as proposed by several investigators (1, 10, 11, 13). The mechanism by which this phenomenon develops may be the result of alterations in the nature of circulating insulin antagonists, or other yet unknown mechanisms. Thus the prolonged half-life of the insulin together with the low basal plasma glucagon levels seem to be counteracted by reduced sensitivity to endogenous insulin, allowing maintenance of normoglycemia in old animals. This balanced equilibrium may, however, be broken down when the pancreas B-cell is unable to continue augmented activity, in which case hyperglycemia would appear, as is frequently the case in humans.

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