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COMPARATIVE CHANGES WITH AGE OF THE FASTING RESPONSE IN CIRCULATING KETONE BODIES, GLUCOSE AND INSULIN AND ORAL GLUCOSE TOLERANCE TEST IN THE RAT

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Abstract—1. Body weight loss in 48 hr fasted rats decreased with age.
2. Blood glucose and plasma RIA-insulin levels correlated negatively and positively respectively with body weight in fed rats. Fasting produced a greater fall in blood glucose and a smaller decrease in RIA-insulin in young than in old rats.
3. Blood ketone bodies correlated negatively with body weight after 48 hr fasting.
4. In oral glucose tolerance tests, blood glucose rose more in adult and old rats than in prepuberals when both fed and fasted. RIA-insulin levels rose more in prepuberals than in older rats when fed but not when fasted.
5. Changes in body composition and reduced insulin sensitivity with age are discussed.

INTRODUCTION

Oral glucose tolerance is reduced in old animals (Chlouverakis *et al.*, 1967; Gommers & Gasparo, 1972; Gommers & Genne, 1975; Codina *et al.*, 1980) as a consequence of decreased responsiveness to insulin (Himsworth & Kerr, 1939; Frolkis *et al.*, 1971) as well as an impaired islet secretory sensitivity to the glucose stimulus (Crockford *et al.*, 1966), although these changes have not always been reported (Codina *et al.*, 1980). Fasting also produces a glucose intolerance (Oyama *et al.*, 1963; Cahill *et al.*, 1966; Bosboom *et al.*, 1973; Turner & Young, 1973), and both decreased insulin responsiveness (Knopp *et al.*, 1970) and islet secretory sensitivity to glucose overdose (Malaisse *et al.*, 1967; Hahn & Gottschling, 1977) are evident. Ageing alters the fasting effect on the responsiveness of the insulin-secreting mechanism of the islets to glucose (Coddling *et al.*, 1975). In the present work we studied the effects of fasting in the ageing rat, comparing modifications in oral glucose tolerance with responses in other metabolic parameters such as body weight change and circulating glucose, RIA-insulin and ketone body levels which are known to be greatly altered by food deprivation.

MATERIALS AND METHODS

Wistar rats were fed a purina chow diet and housed in a temperature (22 ± 2°C) and light cycle (12 hr on-off) controlled room. Animals of different ages fed *ad libitum* or after a 48 hr fast were sacrificed by decapitation without anesthesia and blood was collected from the neck into heparinized beakers. Aliquots of whole blood were immediately deproteinized with $Ba(OH)_2$–$ZnSO_4$ (Somogyi, 1945) and the protein-free supernatants were stored frozen for later analysis of glucose (Huggett & Nixon, 1957), acetoacetate (Mellanby & Williamson, 1974) and beta-hydroxybutirate (Williamson & Mellanby, 1974) by enzymatic methods. Other aliquots of blood were used for plasma separation in which insulin was assayed (Hales & Randle, 1963; Heding, 1972) with an insulin radioimunoassay kit for the rat, generosly provided by Novo Industri A/S (Denmark).

Oral glucose tolerance tests were performed in three groups of male rats of different ages: prepuberals, weighing 80–100 g (about 4 weeks old); adults, weighing 180–200 g (8–10 weeks old), and olds, weighing 400–500 g (over 1 year old). Glucose (2 g/kg body wt) was given by gastric intubation and 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 or plasma were analyzed for glucose and insulin respectively by the methods indicated above.

RESULTS

Body weight loss with fasting

The loss of body weight with 48 hr of food deprivation decreased progressively with the age of the animals. As shown in Fig. 1, that parameter was correlated significantly and negatively with animal weight, the slope of the line being significantly greater in females than in males.

Blood glucose and plasma insulin

Blood glucose levels showed a significant and negative correlation with body weight in animals when fed (Fig. 2), with no differences between females and males. The correlation between blood glucose concentration and body weight appeared positive when the animals were studied after a 48 hr fast (Fig. 2). Fasting produced a greater fall in blood glucose concentrations in young than in old animals (Fig. 2) and values in fasted rats were higher in old than in young animals. Plasma RIA-insulin levels showed a significant and positive correlation to body weight in fed animals (Fig. 3) while the change with age was much less when animals were fasted (Fig. 3). The decrease of insulinemia with fasting was greater in o[illegible] than in

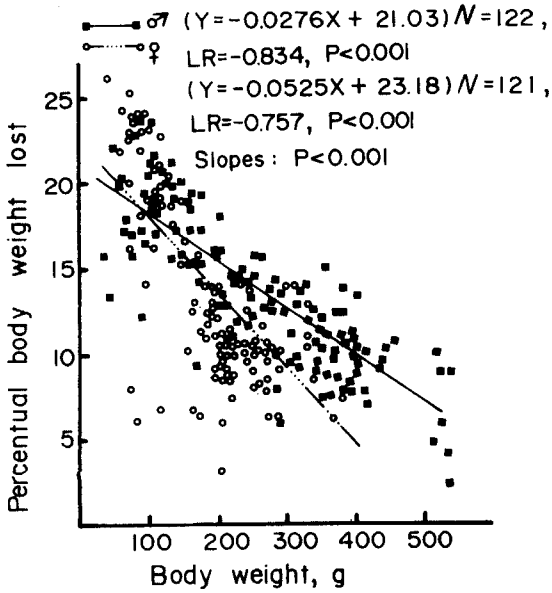

Fig. 1. Effect of 48 hr fasting on body weight loss in male and female rats. Values are expressed as percentual body weight loss compared with absolute body weight of the animals at the onset of fasting. P value of the slopes represent the statistical comparison between the lines of males and females.

young animals. Here again there were no differences between males and females.

Blood ketone body levels in fasted animals

The blood levels of acetoacetate and beta-hydroxybutirate in 48 hr fasted rats were linearly and negatively correlated to the weights of both males and females (Fig. 4). Although the correlation coefficient was not significant for acetoacetate in female rats, there were no significant differences between males and females.

Oral glucose tolerance tests

Oral glucose tolerance tests were performed in fed and 48 hr fasted prepuberal, adult and old male rats. As shown in Fig. 5, the administration of 2 g/kg of glucose produced a rapid rise in blood glucose level in all animals. When they were fed, this rise was greater in adult and old rats than in prepuberals at 15 min after glucose administration, values being significantly higher in the former two groups than in the latter. After 48 hr deprivation, the increase in blood glucose level after glucose load was much greater than in fed animals in all groups (Fig. 5). Values at 30 min in the fasted adult and old rats were significantly higher than those of prepuberals.

Plasma RIA-insulin values in this same experiment are summarized in Fig. 6. In the fed animals, plasma insulin rise after glucose administration was greater in the fed prepuberal rats than in adult and old animals, values at 15 min being significantly higher in the former group than in the two others (Fig. 6). At 60 min, the plasma insulin levels decreased in all groups but levels attained were significantly higher in adult and old rats than in prepuberals (Fig. 6). The response to 48 hr fasting was quite different among the groups. In the prepuberals, fasting produced an important reduction in plasma insulin levels after glucose load, with levels significantly lower than those in fed animals of the same group at 7.5, 15 and 30 min. In fasted adults, the change in plasma insulin levels did not differ from that observed when the animals were fed, although the peak was less pronounced than in fed rats and appeared retarded. In old fasted rats, plasma insulin levels were significantly lower than in fed subjects at 7.5 min after glucose load but this result did not differ at the other times studied. In these old rats, the peak in plasma insulin clearly appeared at 30 min while in the fed rats it occurred at 7.5 min.

DISCUSSION

Present discussion will be focussed mainly on the comparative response to fasting in animals of different

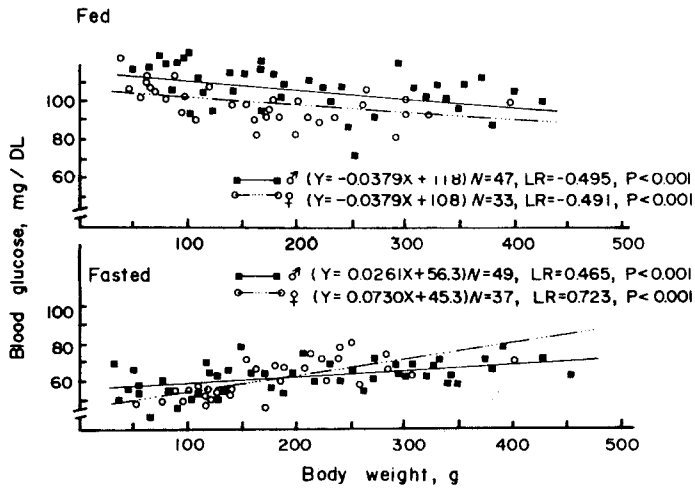

Fig. 2. Blood glucose levels coincident with body weight changes in fed and 48 hr fasted male and female rats. Differences between the slopes of values in males and females were not significant ($P > 0.05$) in fed or fasted animals.

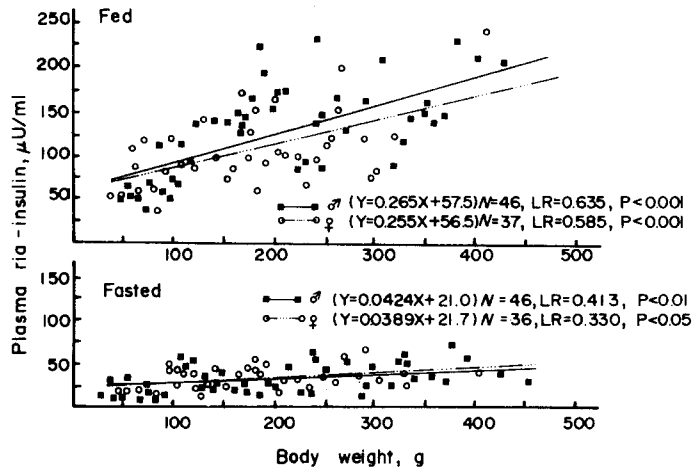

Fig. 3. Changes with body weight of the plasma levels of RIA-insulin in fed and 48 hr fasted male and female rats. Differences between the slopes of values in males and females were not significant ($P > 0.05$) in fed or fasted animals.

ages, as changes observed with age in fed rats have already been reported (Codina *et al.*, 1980). The progressive reduction of body weight with 48 hr of fasting as animals aged may be related with their body composition. It is known that with ageing there is reduced lipid synthesis, but lipids are also metabolized less, resulting in age-related increased tissular lipid accumulation (Kritchevsky, 1972). The caloric store is more efficient in lipid than in other metabolic forms (Ball, 1973) thus even if the same endogenous resources were mobilized per unit of body weight with fasting, it is not surprising that the mass lost is smaller as animals age. The more rapid body weight loss with fasting in female than in male rats may be due to several factors: (i) a different body composition with greater lipidic accumulation in females, causing less body weight loss than in males. It is known that triglyceride concentration in the liver of normal female rats exceeds that of males (Breen *et al.*, 1975), probably as a result of augmented uptake and esterification of fatty acids and reduced oxidation (Soler-Argilaga & Heimberg, 1976); (ii) a reduced sensitivity to the accelerated breakdown of endogenous stores caused by fasting. (This possibility appears less likely since our results show that circulating ketone bodies, the final products of fatty acid oxidation, were similar in males and females, and it has been demonstrated that fasting produces similar effects on hepatic metabolism in rats of both sexes (Berdanier *et al.*, 1979)); (iii) an important factor may be that when adult female rats weigh less than males of the same age (Rice *et al.*, 1976), they will be older than the males when they do attain the same body weight. It is known, for example, that lipidic metabolism in female rats of 200 g is closer to that of males of 320 g than to males of their same body weight (Hansen *et al.*, 1980). Thus ageing itself, aside from differences in body composition, is probably the main factor responsible for greater preservation of body weight mass with fasting in old female rats as compared with males.

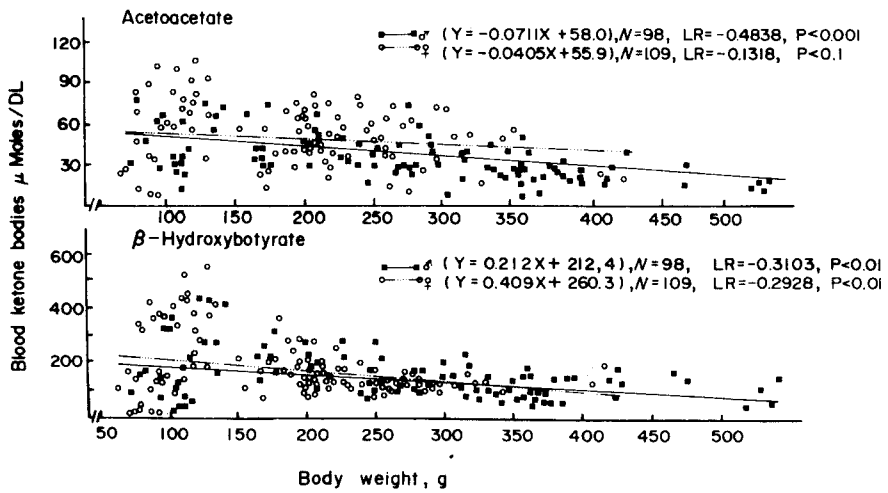

Fig. 4. Changes with body weight of the blood levels of ketone bodies in 48 hr fasted male and female rats. Differences between the slopes of values in males and females were not significant ($P > 0.05$).

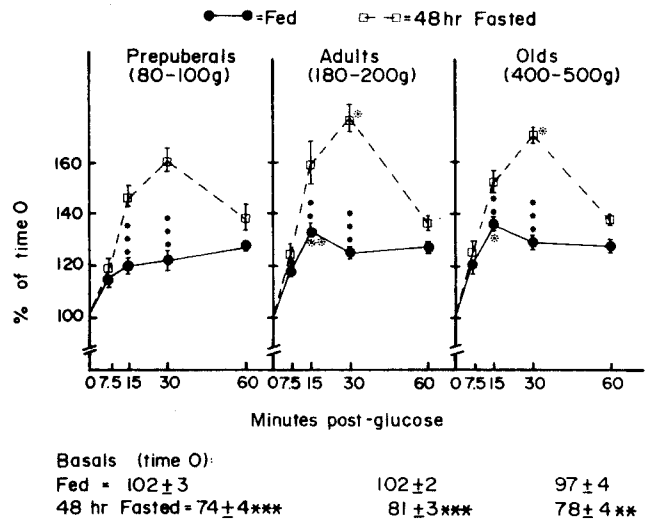

Fig. 5. Blood glucose level changes after oral glucose administration (2 g/kg body weight) in fed (●—●) and 48 hr fasted rats (□- - -□) of different ages. Values are expressed as percentage of amounts present before the glucose load (time 0). Absolute values at time 0 (basals) are expressed as mg/dl and are shown below each corresponding graph. $N = 6$–7 animals/group. Statistical comparisons between groups are shown as follows. P values of fed vs fasted in each age category: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. P values of each group vs prepuberals: $P < 0.05$, $P < 0.01$, $P < 0.001$.

In spite of augmented lipid stores, circulating levels of ketone bodies during fasting decreased as rats aged. Ketone bodies are the main products of fatty acid breakdown in the fasted state (McGarry & Foster, 1980). One of the main factors known to be responsible for the enhanced ketogenesis during fasting is the fall in circulating insulin (Foster, 1967; Herrera & Freinkel, 1968). It has been shown in the present study that the fall in plasma RIA-insulin with fasting is greater in old than in young animals which indicates a reduced sensitivity in the old subjects to the lipolytic stimulus of the insulin reduction in fasting. In agreement with this interpretation, adipocytes from old animals have reduced sensitivity to the stimulus of different lipolytic factors (Manganiello & Vaughan, 1972; Macho & Kolena, 1975), probably as a consequence of their difficulties in enhancing the endogenous cyclic-AMP levels (Macho & Kolena, 1975). Reduced metabolic sensitivity must also be caused by diminished fall in blood glucose levels with fasting, observed in old animals. This condition is not produced by an alteration of stored hepatic glycogen for subsequent mobilization, because this value is not affected by ageing in the rat (Codina, 1978) and there is no reported evidence of greater enhancement of gluconeogenetic activity during fasting in old vs young animals. The main cause of smaller reduction in blood glucose in fasted old animals is probably de-

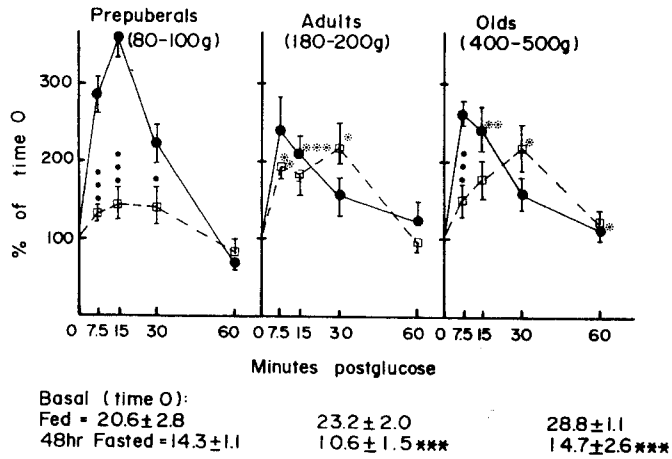

Fig. 6. Plasma RIA-insulin change after oral glucose administration (2 g/kg body weight) in fed (●—●) and 48 hr fasted rats (□- - -□) of different ages. Values correspond to the same animals of Fig. 5 and are expressed as percentage of amounts present before the glucose load (time 0). Absolute values at time 0 (basals) are expressed as μU/ml and are shown below each corresponding graph. Number of animals and statistical comparisons are as indicated for Fig. 5.

creased insulin sensitivity, as previously demonstrated in old fed rats (Codina *et al.*, 1980).

Our results show that fasting produced a greater insulinotropic response to oral glucose load in old than in young animals although blood glucose levels rose more in the former group, indicating that fasting also reduced sensitivity to endogenous insulin in old more than in young rats.

The greatly reduced insulinotropic effect of oral glucose produced by fasting in young animals coincides with the reported reduced capacity of pancreatic secretion in response to the glucose stimulus in fasting animals (Feldman & Lebovitz, 1973; Turner & Young, 1973; Joost & Beckmann, 1980). This may be caused either by a defect in the cyclic-AMP metabolism (Selawry *et al.*, 1972; Howell *et al.*, 1973) or by the reduced insulin content of the pancreas (Malaisse *et al.*, 1967; Joost & Beckmann, 1980). The latter possibility seems unlikely as it is known that fasting also produces reduction in pancreatic insulin content in old rats (Coddling *et al.*, 1975), while our findings show that the insulinotropic effect of glucose was much less affected with fasting in older than in younger rats. As the insulin content of the pancreas is not homogeneous and the fraction of released insulin is only a minor percentage of the total (Joost & Beckmann, 1980), the readily available compartment of insulin may be less depleted during starvation in old than in young animals. This situation is, however, metabolically ineffective due to the reduced sensitivity of the released hormone, causing a diabetogenic situation when the pancreas of the old subject is unable to maintain its augmented activity as frequently occurs in elderly people.

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