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Effect of Fasting on Circulating Glucose, Ketone Bodies and Insulin Levels in the Suckling Rat *

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Suckling rats from 5 to 30 days of age were subjected to fasting in a 37° C chamber to avoid possible metabolic effects from low environmental temperature. The percentage of body weight loss in 24 h fasting increased along with the age of the rats. Blood glucose levels were the same in 5, 10, 20 and 30 day old animals when fed, whereas fasting produced a fall in all the groups which was minimal in the 20 day old animals. Plasma insulin levels were rather low in 10-day-old fed animals; the maximal decrease in this parameter was reached by 5-day-old rats under fasting. Both beta-hydroxybutyrate and acetoacetate levels were higher in animals of 5 and 10 days of age than in those of 30 days, but fasting did not produce any changes in the former while those parameters augmented in the 20 and 30-day-old animals. The results are discussed in relation to the high fat content in the mother's milk, which affects the metabolic situation of the suckling rats when fed and their response to the fasting situation.

The high-fat content of the rat milk (10) is the main factor responsible for the elevated levels of circulating lipids (7) and ketosis (2) in the suckling rat because fasting produces a greater fall in serum lipids (7) and a smaller rise in blood ketone bodies (2) than weaning and adult animals. The metabolic picture of the suckling animal is also affected by the progressive adaptation of its endocrine system to extrauterine life, especially the changes of circulating levels of glucagon and insulin (20) as well as adaptation to a cooler environment.

In our previous studies with suckling rats (1, 2, 4) fasting was effected by main-

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taining the pups separated from their mothers at room temperature, which is lower than that of fed control rats warmed by their mothers. This decreased environment temperature should accelerate the breakdown of endogenous stores to increase thermogenesis and thus could mask the metabolic effects of food deprivation. To overcome this possible artifact, in the present study, we investigated the effects of fasting on body weight loss and plasma insulin, glucose, beta-hydroxybutyrate and acetoacetate levels in suckling rats kept at 37° C while they were separated from their mothers.

Materials and Methods

Newborn Wistar rats were kept with their mothers and maintained in a temperature (22 ± 1° C) and light (12 h on-off) controlled environment. During the fasting period the animals were maintained in an open cage placed in a 37° C controlled water bath and were given tap water periodically for drinking. They were weighed daily on a fine torsion balance and decapitated without anesthesia. Blood was collected from the necks into heparinized beakers. After centrifugation in the cold plasma aliquots were kept at —27° C until the evaluation of glucose with glucose oxydase (11), β-hydroxybutyrate and acetoacetate by an enzymatic-fluorimetric method (17) and insulin by radioinmunoassay (18) using the Radiochemical Center (Amersham) kit with rat Novo insulin as standard. Statistical comparisons were carried out by the Student «t» test (15) with a 445-Compucord electronic calculator.

Results

The average weight of the rats increased as the suckling period progressed and the growth rate appeared more rapid after the 20th day of age (fig. 1). The percent of

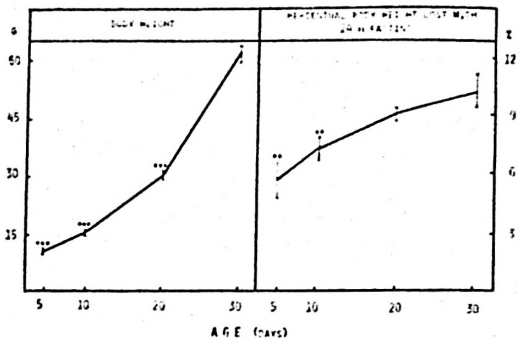

Fig. 1. *Changes in body weight and percentual body weight lost with 24 h fasting during suckling in the rat.*
Asterisks correspond to the statistical comparison between each group and the value in the 30 day old animals: ** $p<0.01$; *** $p<0.001$; no asterisks = $p>0.05$ (not significant).

body weight lost during 24 h of fasting increased progressively in rats aged 5, 10, 20 and 30 days (fig. 1). Blood glucose levels did not differ in 5, 10 and 20 day old rats as compared to the 30 day old group (table I). Fasting for 24 h produced a significant reduction in blood glucose levels in all the groups (table I) but the decrease was minimal in the 20 day old rats and this value was statistically different to that of the 30 day animals. Plasma insulin levels were similar in the 5, 20 and 30 day old animals but were significantly reduced in 10 day old rats (table I). Fasting for 24 h produced a maximal reduction in plasma insulin levels in the 5 day old rats. The fasting insulin levels increased as the animals became older but at all ages studied the effect of fasting producing a reduction in the plasma insulin levels was significant (table I). Circulating glucose/insulin ratio did not differ among the groups when fed but when fasted it was maximal at 5 days of age after which it decreased progressively (table I). Both beta-hydroxybutyrate and acetoacetate levels were higher in the fed 5 and 10 day old rats than in the 30 day old animals while the 20 day old ones

Table I. *Effect of 24 h fasting on blood glucose and plasma immunoreactive insulin in suckling rats.*

P values correspond to the statistical comparison between fed and fasted animals at each age while asterisks correspond to the statistical comparison between each group and the values in the 30 day old animals: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. No asterisks = $p > 0.05$ (not significant).

AGE (days)	BLOOD GLUCOSE (mg/100 ml) FED	FASTING	PLASMA INSULIN (μU/ml) FED	FASTING	GLUC/INS RATIO (mg/100 μU) FED	FASTING
5	98.52 ± 3.76	44.37 ± 4.53	41.84 ± 7.16	7.10 ± 2.09**	2.85 ± 0.44	8.25 ± 2.22*
	$p < 0.001$		$p < 0.01$		$p < 0.05$	
10	99.98 ± 4.28	54.51 ± 3.69	35.95 ± 5.70*	12.42 ± 1.24**	3.87 ± 1.07	4.59 ± 0.40*
	$p < 0.001$		$p < 0.001$		NS	
20	100.80 ± 6.45	87.47 ± 1.65***	43.62 ± 4.49	22.21 ± 1.64*	2.76 ± 0.45	4.36 ± 0.49*
	$p < 0.05$		$p < 0.001$		$p < 0.05$	
30	107.62 ± 3.28	54.62 ± 3.08	51.08 ± 4.07	32.83 ± 4.82	2.23 ± 0.18	2.51 ± 0.65
	$p < 0.001$		$p < 0.01$		NS	

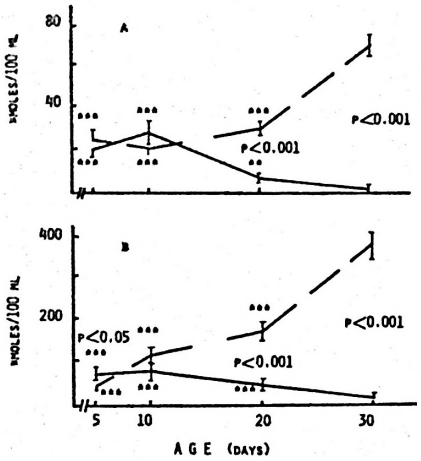

Fig. 2. *Effect of 24 hr fasting on blood acetoacetate (A) and beta-hydroxybutyrate (B) levels in suckling rats.*
Asterisks correspond to the statistical comparison between each group and the values in the 30 day old animals: $^{**}p<0.01$; *** $p<0.001$. The statistical comparison between fed (——) and fasted (— —) animals at each age is shown by the P values.

showed significantly higher values than the 30 day ones in the beta-hydroxybutyrate levels but not in the acetoacetate (fig. 2). Fasting for 24 h did not change the blood level of either ketone body in 5 and 10 day old rats while in 20 day old animals both beta-hydroxybutyrate and acetoacetate levels were higher than when fed. The greatest increase in the blood ketone bodies levels with fasting was observed in 30 day old rats and this value differed significantly from those of all other groups studied including the 20 day old rats.

Discussion

In spite of the reduced availability of carbohydrate from mother's milk (11) normal blood glucose levels are maintained during the suckling period, due to enhanced gluconeogenesis (4, 6, 14, 16, 19) and reduced peripheral glucose utilization (16). The insulinotropic response to glucose administration is also reduced during the suckling period (7) and thus, a balanced equilibrium is established between glucose and insulin which allows maintenance of normal glucose/insulin ratio in the fed suckling animals. The fed suckling animal is mainly sustained with lipids coming from the mother's milk as in spite of their reduced lipogenesis (3, 5) they are able to maintain elevated levels of circulating ketone bodies, as observed previously (2, 9, 12, 13) and confirmed in the present work. Fasting produces a higher glucose/insulin ratio in suckling rats of 5 and 10 days of age than in 30 day old animals. This condition is not maintained by an enhanced gluconeogenesis in the former animals because we have previously shown that contrary to what happens in the 30 day old rats and adults, fasting does not produce a change in the rate of *in vivo* gluconeogenesis in 5 and 10 day old rats (3). Thus the preservation of fasting glycemia must be caused by reduced utilization of glucose due to augmented fat breakdown; very probably the lack of increase of blood ketone bodies in these fasting animals is due to enhanced utilization of lipidic products. Although fat storage must be lower in 5 and 10 day old rats than in older ones, we have previously seen that when the metabolic stress of fasting is added to the thermogenetic stimulus caused by the lower temperature when fasting pups are maintained at room temperature (2) their circulating ketone bodies levels are significantly augmented compared to that of fed animals. This suggests the presence of enough substrate for maximal ketogenetic stimulus. In the present situation where body temperature in fasting pups was preserved at 37 ° C, the lack of augmented ketosis may be the result of enhanced utilization of ketone bodies in the presence of slightly augmented ketogenesis. At the age of 20 days rats are already sampling solid diet and thus their carbohydrate intake is augment-

ed. This gives them a special metabolic condition enhancing their rate of growth as well as augmenting storage of liver glycogen when fed as compared with the youngest rats (8). They also show an adequate response to fasting as seen by their enhanced gluconeogenesis and augmented handling of lipids (4) permitting a maximal preservation of blood glucose levels and increase in circulating ketone bodies concentration.

Resumen

Ratas lactantes se someten a ayuno colocándolas en una cámara a 37° C, para evitar los posibles efectos metabólicos de la baja temperatura ambiental. El porcentaje de pérdida de peso corporal con 24 horas de ayuno aumenta con la edad de las ratas de 5 a 30 días. La glucosa en sangre es igual en ratas de 5, 10, 20 y 30 días de edad cuando están alimentadas, mientras que el ayuno produce un descenso en todos los grupos, siendo mínimo en los animales de 20 días. Los niveles plasmáticos de insulina están bajos en las ratas de 10 días alimentadas y el ayuno produce un máximo descenso en este parámetro en las ratas de 5 días. Tanto los niveles de β-hidroxibutirato como acetoacetato son más altos en los animales de 5 y 10 días que en los de 30; el ayuno no produce cambio en los primeros, mientras que aumentan en las ratas de 20 y 30 días. Los resultados se discuten en función del elevado contenido de grasa en la leche materna que influye en la situación metabólica de los animales lactantes y su respuesta al ayuno.

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