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Liver Components, Blood Glucose and Ketone Bodies in Fed and Starved Suckling Rats

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Summary

Rats of 5, 10, 20 and 30 days of age were compared with adults. In the fed state liver total fatty acids and blood ketones concentrations are high in the youngest animals and decrease throughout the suckling period. Liver citrate does not differ among the groups and the same is true for acetyl CoA with the exception of the 20 days old animals whose liver concentration is lower than in the others. Liver glycogen and blood glucose are low in the youngest rats increasing during the suckling period to attain the adult levels at the age of 20 days. After 24 h of starvation the liver total fatty acids concentration does not change in the young animals or even decreases in the rats of 20 days of age while in those of 30 days and the adults there is a significant rise. Liver acetyl CoA and blood ketones increase with fasting in all the groups while the concentration of citric acid rises in the livers from rats of 5 and 10 days of age, does not change in those of 20 days and falls in the older animals. The fall in blood glucose with fasting is less pronounced in the young rats, and it is even not shown in the animals of 20 days of age. Thus the high lipid availability in the newborn rats allows them a preservation of blood glucose which is specially seen in the fasted state. This is maximized in the rats of 20 days of age thanks to a concomitant increase in the carbohydrate intake as the animals start sampling solid food.

Key-Words: *Suckling – Starvation – Liver Metabolism – Acetyl-CoA – Citrate*

Introduction

As rat milk can be regarded as a high-fat, high-protein and low-carbohydrate diet (*Dymsza, Czajka and Miller* 1964), it is well accepted that fat is the main source of energy for the newborn rat. About the 14th day of age the rat starts replacing the milk diet of suckling by the laboratory diet, which usually has a high-carbohydrate/low-fat content. Gluconeogenesis and lipogenesis are altered when the carbohydrate-lipid ratio in the diet varies and actually these metabolic changes are present during suckling, where it is known that hepatic gluconeogenesis is enhanced (*Ballard and Oliver* 1963, *Yeung and Oliver* 1967, *Philippidis and Ballard* 1969, *Vernon and Walker* 1972) and lipogenesis inhibited (*Ballard and Hanson* 1967). Liver acetyl-CoA and citrate have been evaluated in the fed and fasted rat (*Herrera and Freinkel* 1968, *Herrera, Knopp and Freinkel* 1969, *Aranda, Montoya and Herrera* 1972) to study their regulatory effects upon those pathway during starvation. To gain a better understanding of the carbohydrate and lipid interactions during suckling we have studied the steady state of circulating glucose and ketone bodies and of liver acetyl-CoA, citrate and other components in fed and 24 h-starved rats after different times of birth.

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Methods

Animals. Virgin female Wistar rats of approximately three months of age (adults) maintained on standard pellets were compared with suckling rats of both sexes. At the beginning of starvation the animals were transferred to clean cages which provided unrestricted access to drinking water. Rats were killed between 10 and 11 a.m. by decapitation and without anesthesia. Blood was extracted from the neck of the animals in heparinized porcelain and a piece of liver was placed in liquid N_2 within 15 sec after killing.

Blood components. Immediately after collection, an aliquot of blood was placed in cold distilled water, deproteinized with Ba $(HO)_2$-$ZnSO_4$ (*Somogyi* 1945) and analyzed for glucose (*Huggett and Nixon* 1957) and total ketones (*Bessman and Anderson* 1957).

Analyses of liver. Frozen liver was powdered and extracted with 6% HC10 (*Herrera and Freinkel* 1968) for the estimation of acetyl-CoA (*Herrera and Freinkel* 1967) and citric acid (*Moellering and Gruber* 1966).

Aliquots of the frozen liver were also analyzed for glycogen (*Good, Kramer and Somogyi* 1933), DNA-phosphorus (*Fiske and Subbarow* 1925, *Schmidt and Thannhauser* 1945) and total fatty acids (*Ho and Meng* 1969), as described elsewhere (*Herrera and Freinkel* 1968, *Aranda* et al. 1972).

Results

Changes in weight of whole body and liver and liver DNA P, fatty acids and glycogen concentration during suckling in fed and 24 h-starved rats

Results are summarized in Table 1. Body weight increases progressively during the suckling period while the rate of growth of the liver in the neonatal rat lags behind that of the whole body. After 24 h-starvation liver weight diminishes in all the groups, and the percentual fall in liver weight is maximal in the younger rats and it decreases progressively to have the smaller fall in the adults.

These changes in the liver size prompted a study of some index of liver cellularity. On the basis of concentration per unit of fresh liver weight, the DNA-phosphorus concentration in the fed animals is maximal in the 5 days old rats (Table 1) and it decreases progressively with age to reach the lowest level in the 30 days old rats where the DNA-phosphorus concentration in the liver does not differ from that in the adults. After 24 h-starvation the DNA-phosphorus per gm of liver rises significantly in all the groups as on other occasions (*Herrera and Freinkel* 1968, *Herrera* et al. 1969, *Aranda* et al. 1972). Liver total fatty acids concentration per gm is maximal in the fed 5 days old rats, after which it falls to reach a level in the 10 days old rats the same as that in the adults. In the 20 and 30 days old rats this parameter increases in their liver to become higher than in the adults (Table 1). 24 h-starvation does not alter the concentration of total fatty acid in the liver of the 5 and 10 days old rats, but it causes a decrease in the rats of 20 days and an increase in those of 30 days and the adults.

The percentage of liver glycogen is minimal in the 5 days old rats (Table 1) and it rises later on to become the same in the 20 days old animals as in the adults. 24 h-starvation makes the liver glycogen concentration fall in all groups (Table 1). The percentual diminution with fasting as smaller in the younger rats than in the adults, and this is especially seen in the 20 days old rats (Table 1).

Effects of suckling and starvation on liver acetyl-CoA and citric acid

The results are summarized in Table 2. When the rats are fed the steady state concentration of acetyl-CoA in the liver is the same in the 5, 10, 30 days old rats and the adults, but this parameter is significantly lower in the 20 days old rats than in the adults. After 24 h-starvation acetyl-CoA increases in all the groups but the highest value is obtained in the adults. Liver citrate concentration is the same in all the groups when fed (Table 2). The response to fasting was however very different among the groups. While in the 30 days old and adult rats there is a decrease in the level of citrate with fasting as happened on other occasions (*Herrera and Freinkel* 1968, *Herrera* et al. 1969, *Aranda* et al. 1972), in the 20 days old animals it does not change. The 5 and 10 days old rats show however a significant increase in the steady state concentration of liver citrate with 24 h fasting.

Circulating ketone bodies and glucose in fed and starved suckling rats

Blood ketone bodies concentration is higher in the fed suckling rats than in the adults (Table 2). After 24 h of fasting, all the animals increase their circulating levels of ketone bodies but this rise is greater in the young rats than in the adults and thus the differences with this group are made greater. Blood glucose concentration is lower in the fed 5 days old rats than in the adults (Table 2) and the level rises in the 10 days old rats, the difference with the adults disappearing. Blood glucose rises from 10 to 20 days old rats (Table 2) attaining levels higher than in the adults; this difference disappears in the rats of 30 days of age. Blood glucose falls with fasting in the 5 and 10 days old rats less than in the adults, the difference with this group being highly significant in both cases ($P < 0.001$) (Table 2). Different from the other groups, blood glucose does not change with fasting in the 20 days old rats, maximising the difference with the adult controls.

Discussion

Up to about the 20th day of life the metabolism of lipids in the rat plays an important role in the whole metabolic economy, which allows a maximal preservation of glycemia when food is withheld, as shown here. In the animals of 5 days of age the concentration of total fatty acids in the liver is twice that in the adult animals and this is probably due to the mixture of the remaining lipids accumulated in the liver during the foetal life and those coming from the mothers milk. Lipogenesis does not seem to partake in this innundation of lipids as it is negligible in the liver of the animals of this age (*Ballard and Hanson*

Table 1. Changes in body and liver weights and liver components in the fed and 24 h-starved suckling rat

Age of the rats	Dietary status	Body wt[a] (g)	P[b]	Liver wt (g)	P'
5 days	Fed.	9.1 ± 0.8 (9)	<0.001	0.30 ± 0.03 (9)	<0.001
	24h-starved	8.2 ± 0.6 (9)	<0.001	0.19 ± 0.02 (9)	<0.001
	P[b]	N.S.		<0.01	
10 days	Fed.	16.9 ± 0.6 (10)	<0.001	0.49 ± 0.02 (10)	<0.001
	24h-starved	14.2 ± 0.7 (7)	<0.001	0.34 ± 0.01 (8)	<0.001
	P	<0.01		<0.001	
20 days	Fed.	27.8 ± 1.4 (11)	<0.001	1.02 ± 0.05 (11)	<0.001
	24h-starved	22.9 ± 0.8 (14)	<0.001	0.70 ± 0.03 (14)	<0.001
	P	<0.01		<0.01	
30 days	Fed.	49.9 ± 4.0 (10)	<0.001	2.03 ± 0.25 (10)	<0.001
	24h-starved	44.9 ± 4.6 (9)	<0.001	1.53 ± 0.18 (9)	<0.001
	P	N.S.		N.S.	
Adults	Fed.	177.1 ± 3.0 (8)		6.36 ± 0.29 (8)	
	24h-starved	168.1 ± 3.2 (10)		5.10 ± 0.20 (10)	
	P	N.S.		<0.01	

[a]Mean ± S.E. ( ) = number of animals/groups
[b]P refers to the differences between fed and fasted groups and P' to the differences between each group and the adults under the same dietary status. N.S. = not significant (P or P' > 0.05).

Table 2. Effects of suckling and starvation on liver acetyl-CoA and citric acid and blood ketone bodies and glucose

Age of the rats	Dietary status	Liver acetyl-CoA[a] (nmoles/g)	P' [b]	Liver citrate (μmoles/g)
5 days	Fed.	39.0 ± 4.4 (8)	N.S.	0.296 ± 0.042 (8)
	24h-starved	58.2 ± 5.3 (8)	<0.001	0.441 ± 0.034 (7)
	P[b]	<0.05		<0.05
10 days	Fed.	40.9 ± 4.0 (9)	N.S.	0.412 ± 0.042 (10)
	24h-starved	57.5 ± 4.2 (8)	<0.001	0.776 ± 0.050 (18)
	P	<0.05		<0.001
20 days	Fed.	28.0 ± 2.0 (11)	<0.001	0.360 ± 0.040
	24h-starved	56.8 ± 4.8 (18)	<0.001	0.353 ± 0.24 (18)
	P	<0.001		N.S.
30 days	Fed.	38.2 ± 4.7 (8)	N.S.	0.493 ± 0.090 (10)
	24h-starved	69.9 ± 5.9 (7)	<0.05	0.226 ± 0.014 (9)
	P	<0.001		<0.05
Adults	Fed.	50.3 ± 3.2 (8)		0.343 ± 0.043 (7)
	24h-starved	88.9 ± 4.5 (9)		0.200 ± 0.024 (10)
	P	<0.001		<0.01

[a]Mean ± S.E. ( ) = number of animals/groups.
[b]P refers to the differences between fed and fasted groups and P' to the differences between each group and the adults under the same dietary status. N.S. = not significant (P or P' > 0.05)

1967). The abundance of lipids in the animals of 5 days of age allows them to maintain elevated circulating levels of ketone bodies in the presence of normal steady state concentration of liver acetyl CoA and citric acid. In these animals the concentration of glycogen in the liver is lower than in the other groups probably as a consequence of a preponderance of glycogenolysis over glycogenesis in some way facilitated by the low plasma insulin levels (*Blásquez, Montoya and López Quijada* 1970). The glucose utilization is decreased (*Vernon and Walker* 1972) and gluconeogenesis is augmented in the suckling rat (*Ballard and Oliver* 1963, *Yeung and Oliver* 1967, *Philippidis and Ballard* 1969, *Vernon and Walker* 1972), which together with the abundance of lipids and the breakdown of glycogen should be sufficient to compensate the low availability of carbohydrate in the diet. All of this have not been however sufficient for the 5 days old rat to maintain the blood glucose at the levels of the adult animal. 24 h of fasting changes the whole metabolic picture as the glycemia is better preserved than in the adults. This is probably due to a maximal utilization of lipids and not to an augmented gluconeogenesis since we have found that this parameter does not increase with fasting in the suckling rat as in the adults (*Aranda and Herrera*, un-

Liver DNA P (μg/g)	P'	Liver total fatty acids (μmoles/g)	P'	Liver glycogen (%)	P'
350 ± 12 (8)	< 0.001	159 ± 19 (8)	< 0.01	1.45 ± 0.17 (7)	< 0.001
426 ± 14 (8)	N.S.	130 ± 18 (9)	< 0.05	0.23 ± 0.05 (6)	N.S.
< 0.01		N.S.		< 0.001	
306 ± 10 (10)	< 0.001	94 ± 9 (10)	N.S.	2.02 ± 0.33 (9)	< 0.01
344 ± 13 (5)	N.S.	106 ± 18 (6)	< 0.01	0.11 ± 003 (8)	N.S.
< 0.05		N.S.		< 0.001	
272 ± 12 (11)	< 0.001	109 ± 4 (12)	< 0.01	2.98 ± 0.21 (12)	N.S.
367 ± 9 (10)	N.S.	92 ± 4 (10)	< 0.001	1.05 ± 0.11 (12)	< 0.001
< 0.001		< 0.05		< 0.001	
197 ± 11 (9)	N.S.	105 ± 3 (9)	< 0.01	3.70 ± 0.45 (8)	N.S.
300 ± 19 (8)	N.S.	151 ± 21 (8)	N.S.	0.64 ± 0.10 (6)	< 0.001
< 0.001		< 0.05		< 0.001	
197 ± 9 (8)		87 ± 2 (7)		3.89 ± 0.47 (8)	
352 ± 39 (9)		196 ± 19 (9)		0.15 ± 0.05 (9)	
< 0.01		< 0.001		< 0.001	

P'	Blood ketones (μmoles/l)	P'	Blood glucose (mg/100 ml)	P'
N.S.	1806 ± 202 (9)	< 0.01	101.2 ± 4.5 (8)	< 0.05
< 0.05	3526 ± 370 (8)	< 0.001	82.1 ± 3.0 (7)	< 0.001
	< 0.001		< 0.01	
N.S.	1186 ± 81 (10)	< 0.05	118.7 ± 2.9 (9)	N.S.
< 0.001	2343 ± 76 (6)	< 0.001	89.4 ± 5.7 (7)	< 0.001
	< 0.001		< 0.001	
N.S.	1437 ± 149 (9)	< 0.05	134.6 ± 3.4 (14)	< 0.01
<0.001	3211 ± 245 (12)	< 0.001	130.9 ± 4.5 (18)	< 0.001
	< 0.001		N.S.	
N.S.	1280 ± 198 (9)	N.S.	129.1 ± 5;5 (9)	N.S.
N.S.	2699 ± 304 (9)	< 0.01	91.3 ± 4.5 (9)	< 0.001
	< 0.01		< 0.001	
	834 ± 86 (5)		116.8 ± 5.9 (7)	
	1630 ± 72 (7)		68.1 ± 1.8 (10)	
	< 0.01		< 0.001	

published observation). This interpretation would fit in with the elevated levels of liver citrate in these animals which would mean that the renewal of oxalacetate for gluconeogenesis is not augmented and there is sufficient amount of substrates for the citrate synthese reaction, which is different from what happens in the adults as shown here and in other occasions (*Herrera and Freinkel* 1968, *Herrera* et al. 1969, *Aranda* et al. 1972). The increase in the liver content of total fatty acids with fasting in some way might be used as an index of the fat mobilized by lipolysis from peripheral depots. In the suckling rat this increase is not seen. This would suggest either that lipolysis is not augmented with fasting in the suckling rat or that there are not enough stores of fat to compensate the tremendous breakdown of lipids in the liver of these animals.

The metabolic situation in the rats of 10 days of age is not very much different from that discussed above for the animals of 5 days of age although minor changes might be considered. Among them is the fall in liver total fatty acids concentration to the values in the adult rats which could be the result of the augmented use of lipids without its proper replacement since lipogenesis is still negligible in these animals, as reported by *Ballard and Hanson* (1967).

At the age of 20 days the rat is already sampling solid diet and thus its carbohydrate intake is augmented. Liver lipogenesis is already increasing (*Ballard and Hanson* 1967) which could be contributing to the increase in the liver concentration of fatty acids. This mixed situation allows the concomitant presence of ketosis, hyperglycemia and a concentra-

tion of glycogen in the liver which is no longer below that of the adult animals. The liver concentration of acetyl CoA in these animals is lower than in any of the other groups studied. This might be the result of an enhanced utilization of this metabolite for lipogenesis (*Ballard and Hanson* 1967) in the presence of still augmented ketogenesis (*Lockwood and Bailey* 1971, *Drahota, Hahn, Kleimzeller and Kostolanská* 1964, *Page, Krebs and Williamson* 1971). The response to fasting is quite different in this group than in the others as blood glucose levels do not change in relation to the postpandrial ones. This can not be explained by a greater breakdown of liver glycogen as the percentual fall in its concentration is smaller than in the animals of the other ages studied. The preservation of fasting glucose might in same way be facilitated by a maximal utilization of lipids and actually the blood levels of ketone bodies increase to a value double to those in the controls. This would also agree with the fall of total fatty acids found in the liver of these animals in the presence of low levels of acetyl CoA and high of citrate, suggesting that whatever lipids that come to the liver from peripheral fat depots are used to the highest rate.

The whole metabolic situation is not quite normal in the rats of 30 days of age, which time the animal is not yet fully adapted to the solid diet, although its carbohydrate intake is higher than in the younger animals. As we have seen here, these animals still maintain liver fatty acids and blood ketones concentrations which are higher than in the adults. Different from the other young animals studied, liver fatty acids concentration increase in the rats of 30 days with fasting as it happens in the adults and would indicate that the arrival of lipids to the liver from peripheral resources is greater in these animals than in the younger ones. In any case, blood glucose still falls with fasting in the rats of 30 days less than in the adults, which is consistent with their still elevated availability of lipids, as the greater elevation of blood ketones would suggest.

It is evident that not all these metabolic changes might be ascribed to the changes in the composition of the diet that occur during the suckling period. Changes in body temperature regulation, in endocrine situation and other factors might partake in the establishment of all this metabolic picture. Further investigation are clearly required to elucidate the precise interrelationships among these factors during suckling specially in the fasted state.

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Adipose Tissue Metabolism in Six Week Old Fatty Rats*

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Summary

This study reports measurements of lipolysis and lipogenesis by adipose tissue of 6 week fatty rats and age and sex matched lean controls. At this age fatty rats had enlarged adipocytes and increased concentrations of circulating insulin. Basal release of glycerol was significantly higher in fat from obese rats. The lipolytic effects of epinephrine were greater in these animals. Lipogenesis was also enhanced in adipose tissue from fatty rats. The quantity of radioactivity from glucose incorporated into CO_2 fatty acids and glyceride-glycerol by adipose tissue from fatty rats was higher in the presence on insulin but the percentage increment was smaller than in lean animals. Epinephrine abolished the stimulatory effect of insulin on fat from fatty rats but not in fat from lean animals. These findings are contrasted to previous results using older animals.

Key-Words: *Fatty Rats – Adipocytes – Insulin – Epinephrine*

Introduction

Genetically obese 'Fatty' rats inherit their obesity on a single recessive gene (*Zucker and Zucker* 1961). Although many metabolic and endocrine changes have been observed in these animals, it has not been clear which of these are primary and which secondary to the obesity (*Hartman, Cohen, Richane and Hsu* 1971, *Hubbard and Matthew* 1971). The excess weight is primarily fat and is stored by both an increase in adipose cell size (*Bray* 1969, *Johnson, Zucker, Cruce and Hirsch* 1971) and an increase in cell number (*Johnson* et al. 1971). The adipose tissue shows an increased total lipogenesis (*Bray, Barry and Mothon* 1970, *York and Bray* 1973, *Bray* 1968) and impaired lipolysis (*Bray, Mothon and Cohen* 1970, *Zinder and Shapiro* 1971). Several other studies have suggested insulin insensitivity (*York and Bray* 1973, *Bray* 1968, *York, Steinke and Bray* 1972, *Zucker and Antoniades* 1972) and hypothalamic dysfunction (*Bray and York* 1971, *York, Hershman, Utiger and Bray* 1972, *York and Bray* 1971, *Bray and York* 1971, *Bray, York and Swerdloff* 1973). Until now no detailed *in vitro* studies have been performed on young animals to investigate the role of altered adipose tissue metabolism in the onset of obesity although *Zucker and Antoniades* (1972) have reported increased lipogenesis *in vivo* at 6 weeks of age. This paper reports the results of an *in vitro* study of lipolysis and lipogenesis by adipose tissue of 6 week old 'Fatty' rats.

Material and Methods

Six female rats and six female lean littermates were obtained at 6 weeks of age from the Harriet G. Bird Memorial Labs., Stow, Massachusetts. They were starved for 48 hours and refed Purina Laboratory Chow for 24 hours prior to experimentation. Food intake was not measured in the refeeding period.

This experiment employed a 2^4 factorial design with each factor at 2 levels (Table 1). This allowed an investigation of the effects of genetics, epinephrine, insulin and glucose and their interactions upon lipolysis and lipogenesis using eight experimental conditions. Tissue obtained from each

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