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Comparative Changes in Liver Composition, Acetyl-CoA and Citric Acid during Fasting in Genetically Obese Hyperglycemic Mice (ob/ob) and Lean Littermates*

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Summary

Liver mass, cellularity, composition and content of acetyl-CoA and citrate were compared in obese hyperglycemic (ob/ob) mice and their lean littermates in the fed state and following 6, 15 and 72 hr of fasting. Hepatocytes from ob/ob animals were larger and more abundant at all times although they also sustained cytoplasmic shrinkage during fasting. As judged by sequential changes in the hepatic content of regulatory metabolites, transition from the fed to the fasted state occurs more slowly during dietary deprivation in livers of ob/ob mice than in their lean littermates.

Key-Words: *ob/ob Mice – Fasting – Liver Cellularity – Citrate – Acetyl-CoA – Hyperglycemia*

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Introduction

The liver is the major site for the biosynthetic transformation of endogenous fuels during the transition

from the fed to the fasted state. We have previously characterized the sequential changes in liver composition and in the content of certain regulatory metabolites that may influence hepatic gluconeogenesis and lipogenesis in the fed and fasted rat under different conditions (*Herrera and Freinkel* 1968, *Herrera, Knopp and Freinkel* 1969, *Aranda, Montoya and Herrera* 1972). In the male rat, we observed that a transitory rise in acetyl-CoA, and a fall in citrate and glycogen per hepatocyte can be demonstrated within 6 hr after the withdrawal of food and that these changes precede the reduction in the size of liver cells that occurs as the fast is extended (*Herrera and Freinkel* 1968). The present study was performed to determine whether similar changes obtain in other species and whether they are modified when obesity and hyperinsulinism precede the period of dietary deprivation. Towards these objectives, the genetically obese, hyperglycemic Bar Harbor strain of mice (ob/ob) and their lean litter mates were examined. The concurrence of increased adiposity, hyperphagia, hepatomegaly and hyperinsulinism in adult ob/ob animals has been well documented (*Cameron, Stauffacher and Renold* 1972).

Materials and Methods

Genetically obese, hyperglycemic male mice (ob/ob) and lean littermates were purchased from the Jackson Memorial Laboratory (Bar Harbor, Maine, USA) and maintained in our laboratory on commercial pellet diet. Studies of fasting were initiated by transferring paired lean and obese, 3-4 month old (adult) mice to cages with unlimited access to drinking water only. Animals were sacrificed between 9:00 am and 10:00 am. The duration of the fast was equated with the prior period of food deprivation.

Animals were stunned by cervical fracture and then decapitated. Blood was collected from the neck into receptacles containing dried heparin and precipitated with $(Ba(OH)_2$-$ZnSO_4$ (*Somogyi* 1945). Supernatants were analyzed for glucose (*Huggett and Nixon* 1957) and total ketones (*Bessman and Anderson* 1957). Livers were excised immediately following cervical fracture and directly frozen in liquid nitrogen. Appropriate aliquots were analyzed for citrate by the method of *Moellering and Gruber* (1966) and for DNA phosphorus (DNA P), water, protein, phospholipid phosphorus (phospholipid P), esterified fatty acids of neutral lipids (equivalent principally to triglyceride fatty acids), glycogen, and acetyl-CoA as detailed previously (*Herrera and Freinkel* 1967, 1968). Nuclei were counted in aliquots of fresh liver by modifications of the technique of *Weber and Cantero* (1957) described elsewhere (*Herrera* et al. 1969).

Results

Lean mice: As body and liver weights fell with fasting (Table 1), the decrements were proportionally greater for liver. Significant reductions of liver mass (averaging 19.3% of the wet weight of livers from fed animals) were already manifest after only 6 hr of food deprivation. Concentrations of DNA P/g wet liver weight increased in parallel (Table 1). On the other hand, the total micromoles of DNA P per liver, the total number of nuclei per liver, and the DNA P/nucleus did not change even after 72 hr fasting (Table 1). Thus, total liver cellularity was preserved and the early (i.e. 6 hr) as well as the subsequent (i.e. 15 and 72 hr) reduction of liver weight during fasting could be ascribed wholly to decreases in the size of hepatocytes.

The effect of food deprivation on selected aspects of liver composition in lean mice are summarized in Table 2. To correct for the shrinkage of cells and to provide an index of the content per hepatocyte, results have been expressed per micromole hepatic DNA P. On this basis, liver protein, phospholipid P, and citrate did not change significantly during the first 6 hr of food deprivation whereas meaningful decrements in glycogen ($P < .05$), and increments in acetyl-CoA ($P < .05$), and esterified fatty acids of neutral lipids ($P < .01$) were already evident at this time (Table 2). As the fast was prolonged for 72 hr, the hepatic content of protein, phospholipid P, and glycogen decreased 31.3%, 31.9% and 88.4% respectively below values in the fed state whereas esterified fatty acids of neutral lipids increased an average of 536%. Concomitantly, after 72 hr fasting, water per hepatocyte had diminished an average of 34.9% (Table 2). Acetyl-CoA and citrate per μmole hepatic DNA P displayed biphasic patterns as the fasts were extended: Acetyl-CoA returned to fed levels after 15 hr and then fell below fed levels after 72 hr. On the other hand, citrate per hepatocyte fell to nadir values at 15 hr and then began to rise by 72 hr (Table 2).

The values for blood glucose and ketones are shown in Table 3. The changes in liver composition after 6 hr fasting were not accompanied by significant alterations in blood glucose although ketones were already increased ($P < .01$). Blood sugar remained within normoglycemic ranges throughout the fast although significant decrements were seen after 15 hr ($P < .01$) and further reductions ($P < .01$) were manifest by 72 hr. Blood ketones increased progressively throughout the fast (Table 3).

Obese mice: The obese mice weighed twice as much as their lean littermates in the fed state and that differential was preserved throughout 72 hr fast (Table 1). Differences in liver mass between the lean and obese mice were even more pronounced: the liver accounted for a greater percentage of total body weight in obese animals and contained more DNA P and more nuclei at all times (Table 1). Ratios for hepatic DNA per nucleus were similar in lean and obese mice whereas the amounts of DNA P and nuclei per gm of liver were consistently less (Table 1). Thus, hepatocytes from obese animals were larger and more abundant than those of their lean littermates in the fed state as well as throughout 72 hr fasting. Indeed, by this criterion, the fed state was prolonged in obese animals. Their DNA P per mg wet liver weight after 15 hr fasting

Table 1. Effect of starvation on body and liver weights and liver cellularity in normal and genetically obese mice. Means ± S.E.M. The Number of paried litter mates examined at each time point are denoted in parentheses. P refers to the differences between fed and starved groups and P' to the difference between each group and its respective lean control under the same dietary status.

Group	h of fast	Body wt. (g)	P	Liver wt. (g)	P	Liver DNA Phosphorus µmol/g	P	Liver DNA Phosphorus µmol/liver	P	Liver Nuclei 1×10^8/g	P	Liver Nuclei 1×10^8/liver	P
Lean	0	23.9±3.1 (15)	–	1.50±0.22 (15)	–	6.58±0.21 (13)	–	9.61±0.19 (13)	–	1.79±0.08 (6)	–	2.65±0.09 (6)	–
	6	22.8±1.3 (15)	N.S.*	1.21±0.09 (15)	<0.05	8.42±0.10 (11)	<0.001	10.7 ±0.53 (11)	N.S.	–	–	–	–
	15	21.6±1.0 (15)	N.S.	1.11±0.03 (15)	<0.01	9.01±0.12 (14)	<0.001	9.48±0.31 (14)	N.S.	–	–	–	–
	72	18.0±1.3 (15)	<0.001	0.82±0.02 (15)	<0.001	9.92±0.18 (13)	<0.001	9.63±0.19 (13)	N.S.	2.89±0.21 (6)	<0.001	2.82±0.20 (6)	N.S.
Obese	0	47.6±2.1 P' <0.001	–	3.95±0.59 P' <0.001	–	4.25±0.16 P' <0.001	–	14.6±0.13 P' <0.001	–	1.07±0.09 (6) P' <0.001	–	3.43±0.17 (6) P' <0.001	–
	6	43.0±3.2 P' <0.001	N.S.	2.99±0.30 P' <0.001	N.S.	5.31±0.11 P' <0.001	<.01	15.4±0.63 P' <0.001	N.S.	– –	– –	– –	– –
	15	37.0±4.1 P' <0.001	<0.001	2.41±0.31 P' <0.001	<0.05	6.27±0.63 P' <0.001	<0.01	15.5±0.51 P' <0.001	N.S.	–	–	–	–
	72	35.9±2.0 P' <0.001	<0.001	1.69±0.12 P' <0.001	<0.001	7.24±0.28 P' <0.001	<0.001	14.3±0.21 P' <0.001	N.S.	1.86±0.18 (6) P' <0.001	<0.001	3.77±0.19 (6) P' <0.001	N.S.

*N.S. denotes "not significant" (i.e. $p > 0.05$)

Table 2. Effect of starvation on liver composition in normal and genetically obese mice. Means ± S.E.M. The number of paried litter mates examined at each time point are denoted in parentheses. P refers to the differences between fed and starved groups and P' to the difference between each group and its respective lean control under the same dietary status

Group	h of fast	Water (mg/µmole DNA P)	P	Protein (mg/µmole DNA P)	P	Phospholipid (mg/µmole DNA P)	P	Esterified fatty acids of neutral lipids (µmol/µmole DNA P)	P	Glycogen (mg/µmole DNA P)	P	Acetyl-CoA (nmol/µmole DNA P)	P	Citrate (nmol/µmole DNA P)	P
Lean	0	106±3 (6)	–	25.2±1.1 (5)	–	4.64±0.44 (6)	–	4.1±1.2 (6)	–	6.9±0.8 (7)	–	6.3±0.9 (7)	–	92±7 (7)	–
	6	–	–	21.4±2.0 (5)	N.S.*	4.52±0.44 (5)	N.S.	9.5±1.3 (5)	<0.01	3.8±0.9 (5)	<0.05	10.1±0.7 (6)	<0.05	74±9 (6)	N.S.
	15	–	–	17.6±1.5 (5)	<0.001	3.20±0.24 (8)	<0.05	20.2±3.7 (9)	<0.001	1.4±0.2 (8)	<0.001	7.3±0.9 (9)	N.S.	26±3 (9)	<0.001
	72	69±1 (6)	<0.001	17.3±1.5 (5)	<0.001	3.16±0.16 (7)	<0.05	22.0±7.9 (7)	<0.001	0.8±0.1 (7)	<0.001	3.4±0.3 (6)	<0.001	53±4 (6)	<0.001
Obese	0	139±7 P' <0.01	– –	29.1±2.0 P' <0.05	– –	7.64±0.32 P' <0.001	– –	131.7±31.0 P' <0.001	–	12.1±1.4 P' <0.01	– –	15.3±2.4 P' <0.001	– –	112±12 N.S.	– –
	6	–	–	23.2±2.1 N.S.	N.S.	6.20±0.36 P' <0.02	<0.05	100.2±48.8 P' <0.001	N.S.	6.8±0.6 P' <0.05	<0.01	12.8±2.2 N.S.	N.S.	141±12 P' <0.001	N.S.
	15	–	–	19.2±1.3 N.S.	<0.001	6.36±0.32 P' <0.001	<0.05	71.9±9.0 P' <0.001	N.S.	3.1±0.4 P' <0.01	<0.001	10.8±1.0 P' <0.001	N.S.	45±5 P' <0.001	<0.001
	72	86±3 P' <0.001	<0.001	20.2±1.8 N.S.	<0.01	4.56±0.08 P' <0.001	<0.001	67.7±10.9 P' <0.01	N.S.	2.5±0.3 P' <0.001	<0.001	6.5±0.6 P' <0.001	<0.001	68±6 P' <0.05	<0.05

*N.S. denotes "not significant" (i.e., $p > 0.05$)

Table 3. Effect of starvation on blood components in the normal and genetically obese mice. Means ± S.E.M. The number of paired litter mates examined at each time point are shown in parentheses. P refers to the differences between fed and starved groups and P' to the difference between each group and its respective lean control under the same dietary status.

Group	h of fast	Blood glucose (mg/100 ml)	P	Blood ketone bodies (μmol/ml)	P
Lean	0	128 ± 6 (7)	–	0.4 ± 0.0 (6)	–
	6	130 ± 9 (8)	N.S.*	0.8 ± 0.1 (6)	<0.01
	15	95 ± 6 (7)	<0.01	1.3 ± 0.1 (6)	<0.001
	72	62 ± 2 (6)	<0.001	3.4 ± 0.3 (6)	<0.001
Obese	0	313 ± 23	–	0.7 ± 0.1	–
		P' <0.001		P' <0.001	
	6	163 ± 15	<0.001	0.6 ± 0.0	N.S.
		P' <0.05		N.S.	
	15	116 ± 16	<0.001	1.1 ± 0.1	<0.05
		N.S.		N.S.	
	72	87 ± 10	<0.001	3.3 ± 0.4	<0.001
		P' <0.05		N.S.	

*N.S. denotes "not significant" (i.e., p >0.05)

(6.27 ± 0.63; Table 1) was not different than in lean animals with 0 fast (6.58 ± 0.21; Table 1).

In obese as in lean animals, meaningful decrements in the content of glycogen per hepatocyte were already evident after 6 hr fasting (Table 2) and, on a relative basis, the reductions were of similar magnitude in both groups (i.e. 43.8% vs 44.9% below the values for 0 fast in obese and lean respectively). However, on an absolute basis, the obese again displayed some prolongation of the fed state and their average glycogen content per hepatocyte after 6 hr fast (6.8 ± 0.6; Table 2) was not different than in lean animals with 0 fast (6.9 ± 0.8; Table 2). Similarly, in contrast to lean animals, the acetyl-CoA and esterified fatty acids of neutral lipids in hepatocytes of obese animals did not change during 6 hr fasting and actually tended to fall, though not significantly at 6 hr. However, since the values for citrate tended to increase in the obese and to fall in the lean (Table 2), the differences between the two groups were highly significant after 6 hr dietary deprivation (P < .001). After 15 and 72 hr fasting, obese animals displayed the same directional changes in acetyl-CoA and citrate as lean animals although absolute values for both regulatory metabolites were higher at all time points. By contrast, directional changes for esterified fatty acids of neutral lipids were totally different; in obese animals, the fatty acids fell progressively throughout the fast (Table 2).

As summarized in Table 3, blood glucose was about three-fold greater in fed obese than in fed lean mice. Although blood glucose fell promptly in response to dietary deprivation, and normoglycemia was achieved within 15 hr, the absolute values remained significantly greater than in lean littermates throughout the fast. Blood ketones were also significantly greater in fed obese (Table 2). However, blood ketones failed to increase further during the first 6 hr of fasting whereas ketonemia became manifest in lean animals. Thus, after 6 hr, blood ketones in obese and lean animals ceased to be significantly different (Table 3).

Discussion

Our present efforts reaffirm that the size of liver cells must be considered in estimates of the regulatory content of metabolites during fasting. We found that in mice, as in rats, total hepatic DNA and numbers of liver nuclei remained constant during 72 hr of dietary deprivation whereas total liver weight, water and protein diminished. Presumably, therefore, fasting did not alter the total number of hepatocytes although a pronounced contraction of the cytoplasmic mass of individual hepatocytes supervened. The same phenomenon was evident in genetically-obese (ob/ob) animals as in their lean littermates. However, liver cells were more abundant and larger in the obese and the relative differences between the two groups persisted throughout the fast. By extrapolation from the shrinkage of liver cells that occurs, when food is withheld, it is tempting to speculate that the larger hepatocytes of the obese animals may reflect the hyperphagia and hyperinsulinism that characterizes their metabolic behavior when access to food is unrestricted. In the least however, these features may have contributed to some of the differences in the response of intrahepatic regulatory metabolites to fasting in the two groups.

In lean mice, as in normal rats (*Herrera and Freinkel* 1968), we found that the content of acetyl-CoA per hepatocyte increased within the first 6 hr following

withdrawal of food and declined thereafter. We have previously suggested that this transitory early increment in acetyl-CoA may reflect an evanescent "pile-up" of acetyl-CoA within mitochondria as fatty acid oxidation is initiated while removal of acetyl-CoA for citrate synthetase has become attenuated, and ketogenesis has not yet been fully established. Our findings in lean animals are compatible with this proposal. Hepatocyte citrate tended to fall during 6 hr of fasting (although not significantly) whereas two-fold increments in blood ketones were becoming manifest. The concurrent observations in obese animals are also consistent. Acetyl-CoA *did not* rise during 6 hr of fasting in the obese whereas hepatocyte citrate tended to increase, and no significant changes in blood ketones supervened.

The present studies may furnish some additional insights into the determinants of hepatic citrate. Elsewhere we have noted that liver citrate appears to be acutely responsive to insulin and glucose flux (*Herrera and Freinkel* 1968). In the present studies, absolute values for the hepatic content of glycogen were the same in the obese after 6 hr of fasting as in the lean animals when fed (Table 2). At the same time, blood glucose did not change during the 6 hr fasting in the lean whereas it declined from an initial value of 313 mg% to 163 mg% in the obese (Table 3). Although inferences concerning gluconeogenic activation are not warranted from the available data, the glycogen and blood sugar findings would suggest that continued utilization of glucose may have prevailed during the fist 6 hr of fasting in the obese animals. This would be consistent with the known reduced capacity of obese animals to mobilize lipids during dietary deprivation (*Marshall and Engel* 1960, *Hellman and Westman* 1964, *Westman and Hellman* 1963). Conceivably, the postulated prolonged reliance upon glucose during fasting in the obese may have been implicated in their significantly greater hepatic content of citrate after 6 hr of fasting (141 ± 12 vs 74 ± 19 nmoles/μmole DNA P in obese vs lean; $P < .001$).

Insofar as regulatory metabolites may delimit the "fed" or the "fasted" state in liver, all the above findings would also suggest that transition to the "fasted" state in response to dietary deprivation occurs more slowly in the livers of obese than lean animals.

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