EFFECT OF PROLONGED STARVATION AND REFEEDING ON FUEL METABOLISM IN RATS

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Summary : Body and liver weights, Liver lipids, glycogen, aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2) and blood glucose levels were determined in starved and starvedrefed rats. Decrease in body and liver weights was rapid during the initial stage of starvation and slowed down thereafter. Water was the major liver constituent lost in early fast. Following 10 days of starvation, body weight was reduced by nearly 20%, liver weight 43%, liver glycogen 93% and blood glucose 34%. Liver lipids and the activities of the two transaminases however, were increased by about 30-50%. On refeeding body weight and its water content increased and became nearly double of the initial fasting value on day 2. Blood glucose, liver glycogen, liver lipids and transaminases were significantly altered and got normalised within 5-8 days.

Key words : starvation, refeeding, fuel metabolism, liver glycogen, blood glucose, aminotransferases

INTRODUCTION

Metabolic response to starvation with respect to fuel metabolism has been described as biphasic, changes differening in early and late stages of fasting (4,5). On prolonged starvation, multiple adaptations occur which result in conservation of first glucose then proteins and finally ketone bodies. However, the length of time that a rat can survive depends upon its adipose mass. An old obese rat was able to survive total starvation for 20-30 days whereas age matched non-obese rat survived for about 12 days only (8). The effect of refeeding after prolonged starvation however, has not been studied in detail, as yet. In the present study fuel metabolism was studied during starvation and on refeeding in rats.

MATERIAL AND METHODS

Male albino rats weighing nearly 200-250 *g* were selected and fed on a diet containing (*g/kg*)- potato starch 580; lactose 120; casein 200; groundnut oil 50; salt mixture 40 ; and vitamin mixture 10 (14) Food was given *ad libitum* before starvation and du-

ring refeeding. Animals had free access to water. During starvation rats were studied on day 2,5,8,10,12 and 16. To see the effect of refeeding, after 10 days of starvation, rats were fed *ad libitum* and were studied on day 2,5,8, and 16. At each stage a group of 5-6 rats was studied.

Abdominal cavity was opened, whole liver was removed blotted and weighed immediately. Liver glycogen was estimated by the method of Kemp and Kitsvan Heijningen (9). Total lipids were estimated according to Folch *et al.* (7). For estimating aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, one per cent tissue homogenate was prepared in 0.25 M sucrose, using potter Elvehjem homogenizer fitted with a teflon pestle. The homogenate was centrifuged at 500xg for 10 min at 0°C and the supernatent was used. The activities were assayed according to Reitman and Frankel (13) and were expressed as μM of pyruvate formed/*hr*/*mg* of protein. Blood glucose was determined using O-toluidine reagent (3).

RESULTS

On day 10 of starvation body weight was reduced by nearly 20%, wet liver weight 43%, glycogen 93% and blood glucose 34%, however, liver lipids (56%), ALT (55%) and AST (30%) levels were increased (Tables I and II).

On refeeding 10 days starved rats though there was some gain in body weight yet it did not reach the initial control value even by day 16 (Table I). On day 2 of refeeding wet liver weight was significantly increased. Liver glycogen was also increased and be-

TABLE I : Changes in body and liver weights and liver lipids in starved and starved-refed rats. (Percentage of the mean control value for 5-6 rats in each group)

Duration (days)	*Body weight*	*Liver weight*		*Liver lipids*
		Wet weight	*Dry weight*	
During starvation				
2	92.5	71.0	87.0	126.0
5	87.7	65.7	81.0	155.0
8	82.8	57.1	78.8	149.0
10	80.4	57.0	76.7	156.0
12	73.6	54.2	77.0	151.0
16	69.0	55.0	76.5	152.0
On refeeding				
0	80.4	57.0	76.7	256.0
2	82.9	117.4	93.4	136.0
5	84.5	119.1	100.0	118.0
8	78.5	100.9	100.0	109.0
16	83.7	123.2	100.0	108.0

came comparable with the control value on day 16 while mean value for blood glucose became comparable to the control value on day 5. Liver lipids and ALT and AST levels were normalised within 8 days (Tables I and II).

TABLE II : Changes in blood glucose, liver glycogen, aspartate aminotransferase and alanine aminotransferase activities in starved and starved-refed rats. (percentage of the mean control value for 5-6 rats in each group).

Duration (days)	*Blood glucose*	*Liver glycogen*	*Aspartate amino transferase*	*Aianine aminotransferase*
During starvation				
2	75.2	16.4	113.0	102.9
5	70.0	8.7	119.0	116.0
8	66.6	6.3	123.0	150.0
10	66.0	7.3	130.0	155.0
12	64.9	5.2	130.0	158.0
16	64.0	4.9	133.0	160.0
On refeeding				
0	66.0	7.3	130.0	155.0
2	75.0	82.0	121.0	105.0
5	100.0	95.0	108.0	102.0
8	101.0	95.0	100.8	100.0
16	100.0	100.0	100.0	100.0

DISCUSSION

The results of the present study suggest that compared to body weight liver weight was lost more rapidly during starvation. It was interesting to note that water formed nearly 65-70% of the liver weight loss. The percentage of water in the total body weight loss however, was not calculated. In a study by Passmore *et al.* (11), water loss varied from 10 to 23% of the total weight loss in human subjects on a weight reducing diet receiving 400 Kcal/day for 45 days. It appears that greater the amount of water in an organ more may be the per cent loss of water on starvation. One of the major changes in the liver during starvation which resulted in loss of water was the loss of glycogen from the liver since glycogen stored in the liver traps a considerable amount of water which is responsible for diuresis seen when human subjects are put on starvation/semi starvation schedules.

A fall in blood glucose was also observed. Liver lipids however, were significantly increased. Hyperlipidaemia during starvation may be due to the increased mobilisation of free fatty acids from the adipose tissue (12).

A generalised decline in plasma amino acid levels has been reported in starvation in which fall in alanine is more prominent (1). Further during fasting decrease in plasma alanine has been interpreted as a reflection of enhanced neoglucogenesis (1,6). Greater increase in hepatic ALT compared to AST observed in the present study do suggest that alanine may be the most important amino acid involved in neoglucogenesis during starvation. Alleyne and Young (2) reported that in starvation plasma cortisol levels were elevated because of a slowed rate of turnover. Since there was reduction in liver weight along with the lowering of liver glycogen and blood glucose, the changes observed in present study may not be the result of increased levels of corticoids alone, as in a pure corticoid response the overall action is catabolic while in liver their influence is primarily anabolic. In starvation however, some other influences do appear to operate e.g. plasma insulin levels are reported to be reduced while glucagon levels are increased (2,10).

On refeeding 10 days starved rats liver weight increased. The increase in liver weight however, was mainly due to the accumulation of water. Within 5-8 days extra lipids from the liver disappeared. Blood glucose and liver AST and ALT levels were also normalised by this time.

REFERENCES

1. Adibi, S.A. and A.L. Drash. Hormone and amino acid levels in altered nutritional states. *J. Lab. Clin. Med.*, **76** : 722 1970.
2. Alleyne, G.A. and V.R. Young. Adrenocortical function in children with severe protein calorie malnutrition. *Clin. Sci.*, **33** : 189, 1967.
3. Dubowski, K.M. An O-toluidine method for body glucose determinations. *Clin. Chem.*, **8** : 215, 1962.
4. Felig, P., O.E. Owen, J. Wahren and G.F. Cahill, Jr. Amino acid metabolism in prolonged starvation. *J. Clin., Invest.*, **48** : 584, 1969a.
5. Felig, P., E. Marlise, O.E. Owen and G.F. Cahill, Jr. Blood glucose and gluconecgenesis in fasting man. *Arch. Intrn. Med.*, **123** : 293, 1969b.
6. Felig, P., T. Pozefsky, E. Marliss and G.F. Cahill, Jr. Alanine: key role in gluconeogenesis. *Science*, **167** : 1003, 1970.
7. Folch, J., M. Less and G.H.S. Stanley. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226** : 497, 1957.
8. Goodman, M.N. P.R. Larsen M.M. Kaplan, T.T. Aoki, V.R. Young and N.B. Ruderman. Starvation in the rat II. Effect of age and obesity on protein sparing and fuel metabolism. *Am. J. Physiol.*, **239** : 277, 1980.
9. Kemp, A. and A.J.M. Kitsvan Heijningen. A colorimetric micromethod for determination of glycogen in tissues. *Biochem. J.*, **56** : 646, 1954.
10. Marliss, E.B., T.T. Aoki, R.H.Ungen, J.S. Soeldner and G.F. Cehill Jr. Glucagon levels and metabolic effects in fasting. *J. Clin. Invest.*, **49** : 2256, 1970.
11. Passmore, R., J.A. Stron and F.J. Ritchie. The chemical composition of tissue lost by obese patients on a reducing regimen. *Br. J. Nutr.*, **12** : 113, 1958.
12. Ramalingaswami, V. and M.G. Deo. In calorie deficiencies and protein deficiencies (R.A. McCance & E.M. Widdowson, editors). London : J. & A. Churchill, 1968.
13. Reitman, S. and S. Frankel. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Path.* **28** : 56, 1957.
14. Shanker, V., K., Chugh, H. Lal and A.S. Saini. Effect of dietary restriction with and without excess leucine on hepatic tryptophan oxygenase, 3-hydroxyanthranilate oxygenase and leucine amino-transferase in rats. *Ann. Nutr. Metab.*, **26** : 227, 1982.