Carcass Glycogen as a Potential Source of Glucose during Short-Term Starvation

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In small rats deprived of food for 19h (or 43h), 36% (or 39%) of the glycogen that disappeared was lost from the carcass and 64% (or 61%) from liver. Carcass glycogen is potentially a substantial source of glucose during short-term starvation via the Cori cycle.

Muscle glycogen can be converted into blood glucose in the liver by way of glycolysis and blood lactic acid (Cori, 1931). It is also theoretically possible for some 8% of the glucose residues in muscle glycogen to be released as glucose during glycogenolysis by the action of debranching enzyme. The same arguments are presumably applicable to other tissues that contain glycogen but lack glucose 6-phosphatase. It has been shown on many occasions that the glycogen concentration in skeletal muscles in the rat decreases during short-term starvation (e.g. Funabiki *et al.*, 1975). Nevertheless the potential role of carcass glycogen as a source of blood glucose in short-term starvation appears to have been overlooked in texts, reviews and papers on glucose homeostasis. For example, in a review of glucose homeostasis in man during brief and prolonged fasting, glycogen outside of the liver is not mentioned (Felig, 1975). In order to ascertain the potential contribution of carcass glycogen to glucose homeostasis in short-term starvation, we have measured liver and carcass glycogen concentrations in rats fed *ad libitum* or deprived of food for 19 or 43h.

Experimental

Male albino rats fed on diet 41B (Oxoid, London S.E.1, U.K.) were divided into two groups of identical weight at 15:00h on day 1 and food was withdrawn from one group. At 10:00h on day 2 or 3 the animals were anaesthetized with intraperitoneal Nembutal. The liver was removed into liquid N₂ (<10s), the viscera and kidneys were removed, and the carcass was put into acetone/solid CO₂ (<10s). The frozen carcass was then dried on paper towel and stored in liquid N₂. Livers were weighed and approx. 0.5g was dissolved in 2ml of 5.4M-KOH at 90°C. The carcasses were weighed, broken into small pieces and dissolved in 5.4M-KOH at 90°C (approx. 1.7ml/g). The resultant solution was clarified by centrifugation and 2 or 3ml of the

clear aqueous layer taken for glycogen analysis. Glycogen was precipitated at -10°C following addition of 0.2 vol of 8% (w/v) Na₂SO₄ and 2.3 vol. of ethanol. The precipitated glycogen, separated by centrifugation, was hydrolysed with 2ml of 0.5M-H₂SO₄ for 3h at 100°C. After neutralization (to pH 6–7, measured with a glass electrode) with 1M-KOH and 6ml of 0.2M- or 2ml of 1M-triethanolamine/HCl (adjusted to pH 7 with 1M-KOH), glucose was measured spectrophotometrically on small samples, with hexokinase and glucose 6-phosphate dehydrogenase (Slein, 1963).

Results and Discussion

The concentrations of glycogen in liver and carcass and the losses during starvation are shown in Table 1. The reproducibility of the technique used is shown by the nearly identical concentrations of glycogen/g of tissue in liver and carcass in the fed controls in the two experiments. Liver glycogen fell by 96% during 19h of deprivation of food and by 99% during 43h. The corresponding losses of carcass glycogen were 47 and 70% respectively. In terms of the total loss of glycogen from liver plus carcass, liver contributed 64% and the carcass 36% during deprivation of food for 19h. The corresponding values over 43h were 61% for liver and 39% for the carcass. The loss of glycogen from liver plus carcass over 19h could give rise to an average rate of glucose oxidation of 2.57mg of glucose/min per kg of rat. The corresponding value for 19–43h is 0.74mg of glucose/min per kg of rat. In a study of glucose oxidation rates in larger rats (320g), using isotope methods, Freminet *et al.* (1976) obtained values of 3.2 and 1.1mg of glucose/min per kg of rat respectively for rats fed *ad libitum* or starved for 24h. If comparisons between rats of different weight are valid then the breakdown of liver and carcass glycogen may account for a substantial proportion of glucose oxidation in short-term starvation. The technique

Table 1. *Effects of starvation on concentration of glycogen in the liver and carcass of rats*

Rats were anaesthetized with Nembutal, the livers were excised and frozen, viscera and kidneys were removed, and the carcasses were frozen (see the Experimental section). After solution of liver and carcass in KOH, glycogen was precipitated with ethanol, hydrolysed in acid, the hydrolysate neutralized and glucose measured spectrophotometrically with glucose 6-phosphate dehydrogenase and hexokinase (see the Experimental section). There were six rats in each group.

* $P < 0.01$ against fed control.

Rats	Rat wt. at time of selection (g)	[Glycogen] (mean $\pm$ S.E.M.)			
		(μ mol of glucose per 100 g of rat)		(μ mol of glucose/g fresh wt. of tissue)	
		Liver	Carcass	Liver	Carcass
Fed <i>ad lib.</i>	115	1100 $\pm$ 110	1250 $\pm$ 80	270 $\pm$ 27	17 $\pm$ 1
Starved (19 h)	115	49 $\pm$ 8*	661 $\pm$ 41*	15.4 $\pm$ 2.5*	9.1 $\pm$ 0.7*
Fed <i>ad lib.</i>	67	1410 $\pm$ 140	1250 $\pm$ 160	296 $\pm$ 27	15 $\pm$ 3
Starved (43 h)	67	15.7 $\pm$ 7.6*	373 $\pm$ 58*	5.2 $\pm$ 2.7*	5.8 $\pm$ 0.9*

	Change in [glycogen] (mean $\pm$ S.E.M. of difference)			
	(μ mol glucose/100 g of rat)		(μ mol glucose/g fresh wt)	
	Liver	Carcass	Liver	Carcass
Starved (19 h)	-1050 $\pm$ 110	-585 $\pm$ 90	-256 $\pm$ 27	-7.9 $\pm$ 1.2
Starved (43 h)	-1400 $\pm$ 140	-879 $\pm$ 172	-291 $\pm$ 27	-9.2 $\pm$ 2.7

Scheme 1. *Glucose production during starvation*

used for analysis of carcass glycogen is not readily applicable to larger rats.

According to current concepts (see Scheme 1) much of the glucose formed by gluconeogenesis in short-term starvation is derived from lactate, pyruvate (and alanine) formed from glucose in extrahepatic tissues by glycolysis (and transamination (e.g. see Felig, 1975). This recycling of glucose through the Cori and glucose-alanine cycles (Cori, 1931; Mallette *et al.*, 1969; Felig *et al.*, 1970; Felig, 1973) involves no net synthesis of glucose. The glucose-alanine cycle has been questioned because

of evidence for total synthesis of alanine from other amino acids in skeletal muscles (Garber *et al.*, 1976; Goldstein & Newsholme, 1976). Such a process of alanine formation would lead via gluconeogenesis to net synthesis of glucose. There seems no reason *a priori* why lactate, pyruvate (and perhaps alanine) formed from carcass glycogen by glycolysis (and transamination) may not give rise to glucose via the circulation and hepatic or renal gluconeogenesis (see Scheme 1). The degradation of glycogen and of glucose is by a common pathway, i.e. glycolysis. The basic question to which no answer is available

is what proportion of carcass glycogen degraded in starvation is oxidized in its tissue of origin and what proportion may be converted into blood glucose. The present experiments show that carcass glycogen is a substantial factor in carbohydrate metabolism during short-term starvation and a potentially important source of blood glucose.

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