BIOCHEMICAL LETTERS

Starvation of phosphorylase kinase deficient rats is accompanied by partial hepatic glycogen depletion

A recent report compared the effect of hyperthyroidism upon glucose output by the livers of starved Wistar (normal) or phosphorylase kinase-deficient rats [1]. The latter rats, termed *gsd/gsd*, have a lowered hepatic phosphorylase *b* kinase activity resulting in the inability to activate glycogen phosphorylase [2,3]. As a result, the hepatic glycogen concentration of the rats is much higher than that of normal rats ([2–10] and Table 1). These rats have allowed the effects of the apparent absence of glycogen phosphorolysis to be studied in a number of situations including anaerobic perfusion [7,8], ischaemia [7], anoxia [8], starvation [1,3–6,9] and post-mortem ([4] and P. C. Calder & R. Geddes, unpublished work). In each of these situations the decline in the liver glycogen concentration of the *gsd/gsd* rats has been much less than in normal (*GSD/GSD*) rats. Indeed, in this latest report it is stated that ‘liver glycogen concentrations in (48 h starved) *gsd/gsd* rats were only slightly lower than those found in the fed state’ [1].

The *gsd/gsd* rats show hepatomegaly (Table 1) and when both the enlarged liver size and the excess glycogen store are taken into account the liver glycogen content of the affected rats is approx. 3.2 times that of *GSD/GSD* rats (Table 1). Upon 40 h starvation of *GSD/GSD* rats almost all (98%) of the hepatic glycogen store is mobilized (Table 1). However, the change in hepatic glycogen concentration of the *gsd/gsd* rats upon starvation is relatively small (approx. 20%; Table 1). Starvation

is also accompanied by a reduction in liver size (Table 1) and when this is taken into account it is clear that the hepatic glycogen store is reduced by about 25% by 40 h starvation. Indeed the total glycogen depletion from the 40 h starved *gsd/gsd* rat liver is 545 mg, 80% of that of the *GSD/GSD* rat liver (676 mg; Table 1). Quite clearly *gsd/gsd* rats can, and do, deplete a significant amount of their hepatic glycogen store during starvation (and during the other metabolic perturbations mentioned above), although the change in glycogen concentration may be relatively small. Indeed the error of measuring glycogen concentration alone after such treatment was shown by Clark *et al.* [5] who reported that after 24 h starvation of *gsd/gsd* rats 17% of the hepatic glycogen store was depleted, although the glycogen concentration of the livers actually increased.

It is found that upon starvation of the affected rats the blood glucose concentration is maintained [3–6]. It has been suggested that blood glucose maintenance by the starved, affected rats is a result of increased gluconeogenesis but it has been shown, using the gluconeogenic inhibitor 3-mercaptopicolinate, that during starvation *gsd/gsd* rats are less reliant upon gluconeogenesis than are normal rats [5]. Recognizing that a significant amount of the *gsd/gsd* rat hepatic glycogen store is mobilized during starvation explains the blood glucose maintenance. Clearly much of the blood glucose originates from liver glycogen, as is the case in *GSD/GSD* rats.

The remaining question then is how do the *gsd/gsd* rats mobilize hepatic glycogen and maintain blood glucose levels if they cannot activate glycogen phosphorylase? It has been suggested that mobilization may

Table 1. Liver weights and glycogen concentrations of fed and starved normal (*GSD/GSD*) and phosphorylase-deficient (*gsd/gsd*) rats

Rats were either fed *ad libitum* or starved for 40 h. After killing, livers were removed, weighed and quickly frozen in liquid nitrogen. Liver glycogen concentrations were determined by the method of Kemp & van Heijningen [11]. Data are expressed as the mean ($\pm$ S.D.) of three determinations on each of six animals. ^aCalculated from mean of total glycogen store fed versus starved. Statistically significant effects are indicated; ^b $P < 0.001$ for *GSD/GSD* fed versus starved; ^c $P < 0.001$ for *GSD/GSD* fed versus *gsd/gsd* fed; ^d $P < 0.005$ for *gsd/gsd* fed versus starved; ^e $P < 0.01$ for *gsd/gsd* fed versus starved.

	Liver wt. (g)	Hepatic glycogen			
		Concentration (% tissue wet wt.)	Total store (mg)	Relative store ^a	Net loss (mg) ^a
<i>GSD/GSD</i> fed	10.27 $\pm$ 0.97	6.83 $\pm$ 1.01	692 $\pm$ 156	100	—
<i>GSD/GSD</i> starved	5.98 $\pm$ 0.57 ^b	0.26 $\pm$ 0.13 ^b	16 $\pm$ 5 ^b	2	676
<i>gsd/gsd</i> fed	15.36 $\pm$ 1.22 ^c	14.80 $\pm$ 1.42 ^c	2262 $\pm$ 384 ^c	327	—
<i>gsd/gsd</i> starved	14.40 $\pm$ 0.79	12.07 $\pm$ 0.91 ^d	1717 $\pm$ 172 ^e	248	545

be due to residual phosphorylase *a* activity [7], although this is less than the basal phosphorylase *a* activity of *GSD/GSD* rats, at which no glycogenolysis occurs [7,8]. Allosteric activation of phosphorylase *b* is a possibility [7], but the levels of activators present are much lower than those required to activate the enzyme *in vitro* [12]. Finally, lysosomal glycogen hydrolysis has been suggested [5,7–10] and it has been shown that the lysosomes of the affected rats contain glycogen [9], as do the hepatic lysosomes of humans affected with phosphorylase kinase deficiency [13]. Starvation is known to induce autophagy [14,15], activate lysosomal enzymes [16,17] and stimulate lysosomal proteolysis [18]. As such, during starvation a significant proportion of the cytosolic glycogen in the *gsd/gsd* rat liver may be autophagically engulfed and digested hydrolytically.

In view of the attention given to the small change in *gsd/gsd* rat hepatic glycogen concentration following starvation [1–6] and other metabolic perturbations [7,8] it must be appreciated that a significant proportion of the glycogen is degraded (Table 1). As such the validity of the use of these animals as ‘non-glycosylating controls’ [1] is questionable. Although a mechanism for the glycogen degradation is not known, lysosomal hydrolysis is the most likely possibility.

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Suppression of hepatic glucose output after glucose re-feeding in the *gsd/gsd* rat

In their Letter [1], Calder & Geddes reiterate earlier findings of Clark *et al.* [2,3] demonstrating loss of hepatic glycogen upon starvation in the phosphorylase *b* kinase-deficient (*gsd/gsd*) rat. Based on measurements of total liver glycogen contents of fed or 40 h-starved (*gsd/gsd*) rats, they suggest (i) that hepatic glycogen mobilization contributes to the maintenance of glycaemia in the starved (*gsd/gsd*) rat; and (ii) that lysosomal glycogen hydrolysis involving acid α -glucosidase may be involved. The authors cite as support for their suggestion the results of experiments performed by Clark *et al.* [2] involving the use of 3-mercaptopicolinate, an inhibitor of gluconeogenesis. Acute inhibition of gluconeogenesis resulted in less marked decreases in glycaemia in *gsd/gsd* rats than in the normal (i.e. *GSD/GSD*) controls. There was a significant decline in glycogen concentrations in livers of *gsd/gsd* rats in response to 3-mercaptopicolinate, although changes in liver glycogen content did not attain significance.

These results, together with measurement of glycogen pool sizes, have been taken by Calder & Geddes to indicate that non-phosphorolytic degradation of glycogen may be of physiological significance for glucose homeostasis in starvation in *gsd/gsd* rats. This may well be the case, as we shall discuss later. However, it is by no means certain whether it is possible to extrapolate from the acute response to the administration of 3-mercaptopicolinate to the response to prolonged carbohydrate deprivation. First, Conaglen *et al.* [4] have indicated that total hepatic glucose production via gluconeogenesis is not diminished in starved *gsd/gsd* rats. Secondly, the hypoglycaemic response to 3-mercaptopicolinate observed in starved *gsd/gsd* rats is not insubstantial, and is, moreover, accompanied by a rise in blood lactate consistent with inhibition of gluconeogenesis [2]. Thirdly, and perhaps more importantly, a role for the lysosomal hydrolysis of glycogen in the maintenance of glycaemia in the starved *gsd/gsd* rat must be reconciled with the temporal pattern of glycogen loss seen after food withdrawal, with a rather greater decline in glycogen content during the first 24 h of starvation than in subsequent periods [3]. In order to draw firm conclusions regarding the role of lysosomal glycogen hydrolysis in the homeostatic response to starvation, it will be necessary to correlate rates of glycogen loss with increased autophagy and acid α -glucosidase activities, as well as with rates of glucose utilization. Nevertheless, a role for lysosomal glycogen hydrolysis in glucose production is not necessarily excluded.

On the basis of changes in autophagic lysosome density, it has been demonstrated that the quantity of glycogen included in autophagosomes varies in direct proportion to the glycogen content of the liver [5,6]. Consequently, sequestration of glycogen within such