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Differing patterns of carbohydrate metabolism in liver and muscle

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ABSTRACT

The effect of a period of starvation followed by refeeding on skeletal muscle glycogen was investigated by the use of double-labelled radioactive glucose precursors in rats. Skeletal muscle glycogen, which is not depleted to anything like the extent of liver glycogen, shows a remarkable stability with respect to its overall molecular size distribution during starvation and subsequent refeeding. The experiments also indicate that there is a control mechanism in muscle tissue enabling the synthesis of lysosomal glycogen to be switched off during the initial part of the refeeding process. The results emphasise the inadequacy of the Cori cycle and a modified version is proposed.

INTRODUCTION

Since the pioneering work of the Coris some 50 years ago[1,2], it has often been accepted that, at least in a technical sense, since it lacks glucose-6-phosphatase, muscle is a nongluconeogenic tissue while remaining a glycogenic tissue. However, several publications[3–7] have shown that muscle, like liver, stores a significant portion of its glycogen reserves in the lysosomal compartment from whence it can only be released as free glucose, via the action of α-D-glucosidase[8]. Consequently, modification of the Cori cycle has been suggested[9] in order to recognise the potential glucogenic capacity of muscle. Additionally, a recent publication[10] has shown that frog muscle releases physiologically significant amounts of free glucose when recovering from strenuous exercise, emphasising the importance of the hydrolytic, in addition to the phosphorolytic, breakdown of glycogen[9].

It has also been demonstrated that hepatic glycogen replacement initially favours the synthesis of low molecular weight (cytosolic) glycogen in animals refeeding after a period of starvation, but this is later converted into high molecular weight (lysosomal) material[11]. In addition, there is a large subsequent oversynthesis of low molecular weight glycogen. These studies emphasise the

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heterogeneity of glycogen metabolism in liver, particularily in the post-refeeding synthetic phase. No similar studies have been reported for muscle glycogen.

Although, in mammals, the highest concentration of glycogen is found in the liver, the greatest quantity is stored in muscle tissue because of the latter's extensive distribution. Therefore, it was clearly of great interest to study the effects of starvation followed by refeeding upon muscle glycogen metabolism. Starvation also mimics the effects on tissue carbohydrate metabolism of other "stress" situations, for example, extended exercise and some of the glycogen storage diseases. These results, taken in conjunction with the heterogeneity of *post mortem* degradation of glycogen in both liver[12] and skeletal muscle[13], and the established importance in these circumstances of lysosomal (hydrolytic) breakdown[13], should provide a comparative assessment of carbohydrate metabolism in the two tissues and an assessment of the applicability of the Cori cycle.

EXPERIMENTAL

Rats were of a random-bred Wistar strain and were maintained under standard animal house conditions (14 h light, 10 h dark). Rats were starved for 5 days with free access to water. Radioactively labelled D-glucose (U-^{14}C and/or 6-^{3}H, Amersham, UK) was administered by ip injection either immediately prior to *ad libitum* refeeding, or some fixed time after this refeeding had commenced. Rats were sacrificed by cervical dislocation up to 48 h later.

Glycogen was extracted from liver and from muscle as previously described[14,15]. Radioactivity in glycogen was measured following ethanol precipitation of glycogen solutions overnight at 4°C and subsequent filtration, under vacuum, onto glass fibre discs.

Fractionation of glycogen solutions and determination of molecular size was performed using sucrose density gradient analysis as previously described[11]. Approximate molecular weight values for the fractions were obtained by interpolation of the data in ref. 16 and are shown in Table I. Glycogen concentrations in the presence of sucrose were determined by the method of Krisman[17].

RESULTS AND DISCUSSION

Starvation rapidly depletes the glycogen stores of liver tissue and dramatically alters its molecular weight profile[18]. Likewise, upon refeeding, the different

TABLE I

Approximate molecular weights of glycogen fractions isolated by sucrose density gradients (values interpolated from the data in ref 16)

Fraction no.	1	2	3	4	5	6	7	8	9	10
Molecular weight ($\times 10^{-6}$)	10	30	60	140	250	380	530	670	860	1400

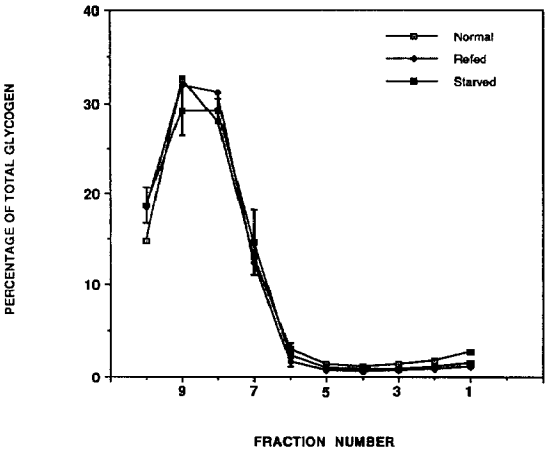


Fig. 1. The effect of starvation (24 h), and subsequent refeeding (12 h) on the molecular size distribution of rat skeletal muscle, as compared to the normal, well-fed situation. Standard deviations are indicated only for the starved sample in the interests of clarity, but were similar for the other samples. These results come from four independent experiments.

molecular sizes of glycogen are replaced at differing rates. However, as can be seen in Fig. 1, muscle glycogen maintains its molecular weight profile remarkably, even after prolonged starvation and subsequent refeeding. (After 5 days of starvation, muscle glycogen levels fell to 41% of their value in the well-fed animal. Liver glycogen levels fell to 15% after only 24 h. A previous report[19] showed a fall in muscle to 51% after 40 h.) Clearly, the two pools of glycogen, the cytosolic and the lysosomal, interact differently in the two tissues.

In confirmation of previous studies[11], Fig. 2 shows that the incorporation of radioactive glucose into liver glycogen, upon refeeding after prolonged starvation, occurs over the full molecular weight range with the incorporated label shifting into the higher molecular weight range after prolonged refeeding. It is interesting to note that there is no significant change between the pattern of incorporation of uniformly labelled glucose and that of glucose labelled at H-6. In hepatic experiments, therefore, we can ignore the complication of the three-carbon glycogen precursor suggested by McGarry and co-workers[20] on the basis of their double-isotope labelling experiments.

Fig. 3 shows the unexpected pattern of D-[U-^{14}C]glucose incorporation into skeletal muscle glycogen on refeeding after prolonged starvation. The radioactive incorporation increased very significantly between 6 and 25 h and occurred largely in the low molecular weight range of glycogen. Since high molecular weight glycogen, in both liver and muscle tissue, is located in the lysosomal compartment, this indicates that in muscle, unlike liver, this compartment is inactive in the refeeding situation. This is in obvious contrast to the corresponding situation in the liver (See Fig. 2 and ref. 11).

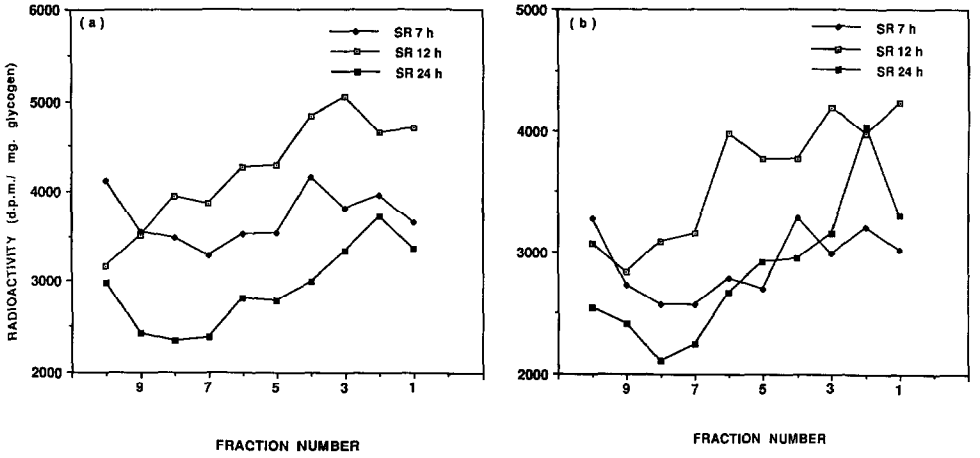


Fig. 2. The incorporation of radioisotope into liver glycogen with respect to molecular size, following ip injection of D-[U-^{14}C]glucose (a) or D-[6-^{3}H]glucose (b) into rats at the end of a period of starvation. The animals were then allowed to refeed *ad libitum* for 7 (SR7), 12 (SR12), or 24 h (SR24) prior to sacrifice. The results shown are from four independent experiments. Standard errors (not shown) averaged ±8% approximately.

In order to further investigate this unexpected finding, additional incorporation experiments, utilising two labels, were performed. One of the two kinds of labelled glucose was administered immediately prior to refeeding after prolonged starvation, the other 24 h into the refeeding period. This protocol was adopted to assess if there was any difference in muscle metabolism immediately post-starvation as compared to that significantly into the refeeding period. The experiments were

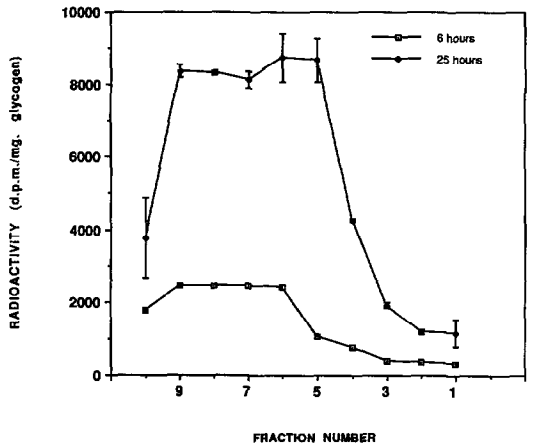


Fig. 3. The incorporation of radioisotope into skeletal muscle glycogen with respect to molecular size, following ip injection of D-[U-^{14}C]glucose into rats at the end of a period of starvation. The animals were allowed to refeed *ad libitum* for either 6 or 25 h prior to sacrifice. Standard deviations are indicated for the 25-h experiment.

repeated with the order of the administration of the isotopes reversed in order to eliminate any possible isotope effect, and to check on reproducibility. The results are shown in Figs. 4 and 5. (It is worthwhile noting the control experiment in Fig. 4b. Since there is some overlap of the windows used in the scintillation counter for the measurement of ^{14}C and ^{3}H radioactivity, measurement was made after 12 h of refeeding in the tritium window as well as the carbon-14 window, i.e., prior to the tritium injection. The results show that any overlap between the windows can be safely ignored.) From Fig. 4a, it would appear that the incorporation of radioactivity, immediately upon refeeding, from ^{14}C-labelled glucose is stable and not significantly variable over a 48-h period. This suggests that the injected glucose molecules are incorporated into the core of glycogen molecules located in the muscle cytosol. However, injection of the same isotopic precursor 24 h post the onset of refeeding (Fig. 5a) results in lower levels of incorporation which fluctuate markedly with time but which again are clearly targetted towards cytosolic glycogen. This implies that, after the initial refeeding phase is over, incorporation into glycogen is into the labile outer layers of the β-particles[21]. The incorporation of tritium from H-6-labelled glucose is more difficult to interpret (Figs. 4b and 5b). In confirmation of the ^{14}C experiments, glucose incorporation, whether immediately upon refeeding or later during the refeeding process, is into nonlysosomal glycogen. However, the levels of incorporation are low and, in Fig. 5b, clearly show a fluctuation over the period of refeeding, which parallels the ^{14}C incorporation in Fig. 5a. Since it was administered immediately prior to refeeding, it might be expected that incorporation of the tritiated glucose should exhibit the stable incorporation of isotope shown in Fig. 4a. Superficially, at least, this could lend credence to the postulate of a three-carbon intermediate between glucose and glycogen[20]. Certainly it is clear that, since the injected molecule is the same in both cases (glucose), only differing in being labelled uniformly (^{14}C) or asymmetrically (^{3}H), an indirect mode of incorporation of glucose into glycogen is a possibility (i.e., other than the "conventional" route: glucose–G6P–G1P–UDPG–glycogen). These results do suggest that an investigation of the distribution of isotope amongst the atoms of the radioactive glucoses incorporated into glycogen would be of interest, but this was beyond the scope of our current experiments.

Most importantly, however, these results have shown, by the lack of incorporation of radioactivity into lysosomal (high molecular weight) skeletal muscle glycogen, that there is a control system on the synthesis of lysosomal glycogen in muscle. A feed-back control mechanism on the uptake of glycogen into the liver lysosomes has already been reported[22]. It is clear also from these experiments that, as has been suggested previously[9], the Cori cycle requires modification to take account of the involvement of the lysosome in glycogen metabolism. The recent direct measurement of glucose release from isolated frog muscle[10], plus the extensive reports of the involvement of lysosomes in mammalian skeletal muscle, cannot be explained by the simple Cori model. Further, as can be seen from Table II, the ratio of lactate to glucose in the blood is very sensitive, in normal animals, to the

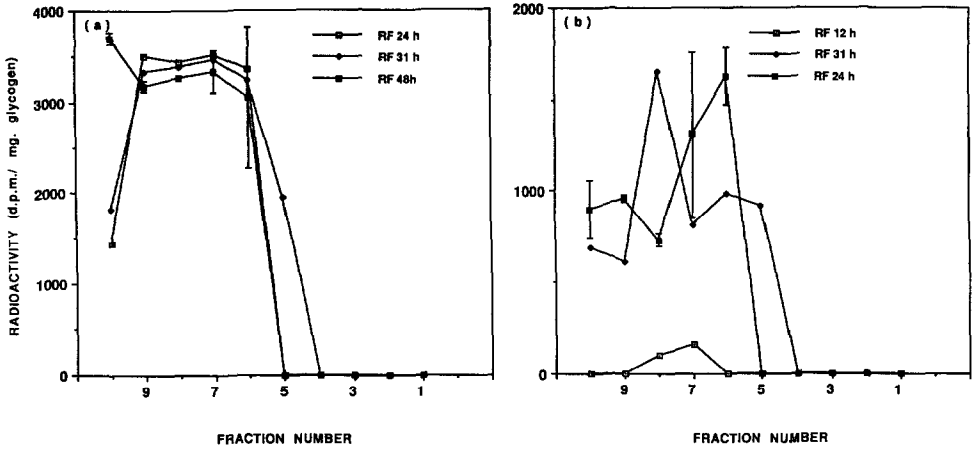


Fig. 4. The incorporation of radioisotope into skeletal muscle glycogen with respect to molecular size following ip injection of D-[U-^{14}C]glucose into rats after a period of starvation. The animals were allowed to feed *ad libitum* for periods of up to 48 h prior to sacrifice. An ip injection of D-[6-^{3}H]glucose was also administered 24 h into the refeeding period. Samples were measured for their ^{14}C (a) and ^{3}H (b) radioactivity. Standard deviations are indicated for only one sample in each figure in the interests of clarity, but were comparable in all cases.

switching off of hepatic lysosomal glycogen degradation by the (1 → 4)-α-D-glucosidase inhibitor, acarbose[23] which has no parallel effect on muscle tissue[8]. In animals deficient in hepatic phosphorylase kinase (GSD VIII), the ratio is lower than normal and appears to be unaffected by the subsequent action of acarbose. In conjunction with our experiments, these results indicate that blood glucose and lactate and liver and muscle glycogen are related in a complex manner. Accordingly, a modified version of the Cori cycle is shown in Fig. 6 which takes account of

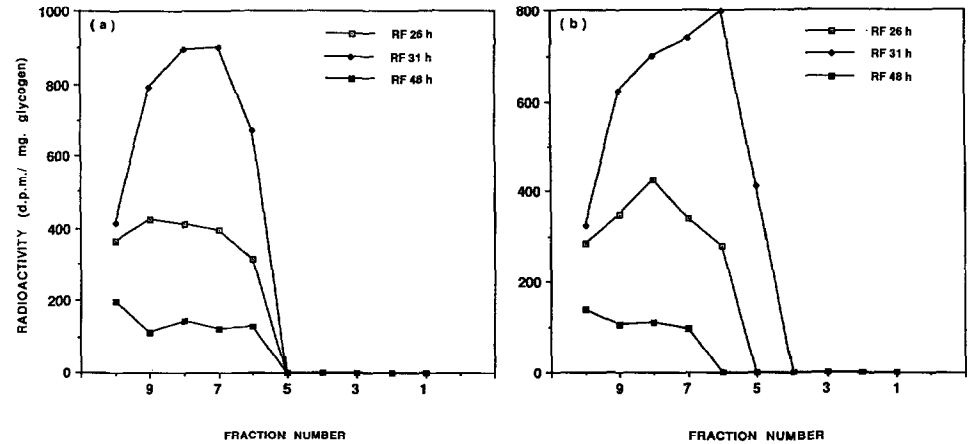


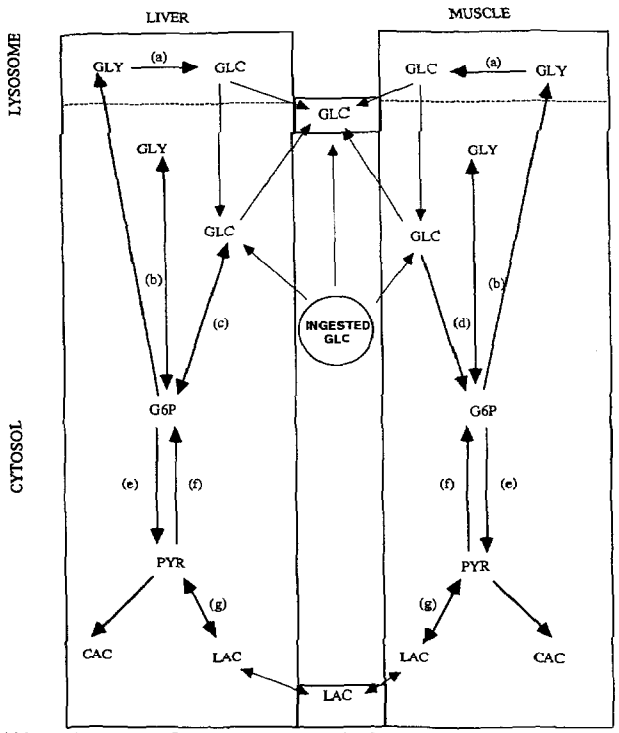
Fig. 5. As in Fig. 4, except that the order of administration of the radioisotopes was inverted (i.e., ^{3}H immediately prior to refeeding, ^{14}C 24 h into the refeeding period).

TABLE II

The ratio of blood lactate to blood glucose levels in well-fed and previously starved rats and GSD type VIII rats (genetic lack of hepatic phosphorylase kinase), and the effect of a hepatic (1 → 4)-α-D-glucosidase inhibitor (acarbose) [a]

Normal Time after treatment (days)	Lac/Glc	GSD VIII Time after treatment (days)	Lac/Glc
Well fed		Well fed	
N [b]	0.13	N	0.06
1	0.03	1	0.09
5	0.03	5	0.05
Starved/refed		Starved/refed	
N	0.15	N	0.05
1	0.08	1	0.07
5	0.07	5	0.03

[a] Data from Table IV of ref 23. [b] N, control (no acarbose treatment).



GLY = glycogen; GLC = glucose; G6P = glucose-6-phosphate;
PYR = pyruvate; LAC = lactate; CAC = citric acid cycle.
(a) (1→4)-α-D-glucosidase. (b) Phosphoglucomutase, pyrophosphorylase, etc.
(c) Hexokinase, glucose-6-phosphatase. (d) Hexokinase. (e) Glycolysis.
(f) Gluconeogenesis. (g) Lactate dehydrogenase.

Fig. 6. A modified version of the Cori cycle allowing the putative role of the lysosomal compartment in muscle and liver to be incorporated. Note that the release of free glucose into the cytosolic compartment (in both tissues) by the action of debranching enzyme[9] has been omitted in the interest of clarity.

lysosomal metabolism. It is clear that the whole balance of glycogen and glucose metabolism in the whole animal is complex and such factors as exercise, starvation/refeeding, and tissue pH (for example, the rapid lysosomal degradation *post mortem*[12,13]) make this a multiple parameter problem. However, while this model is a significant advance over the restrictions of the simple Cori model, it is now clear that many additional factors are involved in the delicate balance between tissue glycogens and glucose and lactate levels in both tissue and blood — for example, see the current, unresolved, debate over the relationship between cell volume and metabolism[24–27].

These results emphasise again the importance of proper consideration being given to lysosomal carbohydrate metabolism when the overall metabolism of glycogen in the tissues is being considered. The facts that up to 20% of glycogen may be located in the lysosomal compartment[14] and that this glycogen is of very high molecular weight and is further distinguished from cytosolic glycogen by its protein content are well established[4–7,9,11,14,15,28–31]. Also, the involvement of the lysosome in relatively abnormal circumstances, such as the starvation/refeeding phase or *post mortem*, is clearly demonstrated above and elsewhere[11–13]. Further experiments are required to establish properly the role of lysosomes in "normal" circumstances but could be aided by the proposed modified Cori cycle. The modified version, for example, allows for the uptake of lactate by muscle (which has been postulated to depend on the lactate concentration gradient[32]) and for the supply of glucose to muscle cells by other muscle cells. This concept is potentially of great importance in exercise physiology and sports science in general. (For example, the carbohydrate loading regime used by many marathon runners should be analysed in terms of the modified Cori cycle.)

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