Skeletal-muscle glycogen synthesis during the starved-to-fed transition in the rat

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The pattern of glycogen deposition in skeletal muscles of varying fibre composition was examined in rats during the starved-to-fed transition. In all the muscles studied, glycogen concentrations steadily increased during the first 8 h after chow re-feeding, and the fed value was exceeded. Rates of glycogen deposition varied, not with muscle fibre composition, but with the extent of glycogen depletion during starvation. There was no evidence for skeletal-muscle glycogen breakdown during the period of hepatic glycogenesis, making it unlikely that recycling of carbon from muscle glycogen to lactate is quantitatively important for the provision of glycogenic precursors to the liver, but moderate glycogen loss was observed from 8 to 24 h after re-feeding, when the liver is in the lipogenic mode. The factors influencing glucose disposal by skeletal muscle after re-feeding are discussed.

INTRODUCTION

In the liver, glucose 6-phosphate can be formed by the phosphorylation of glucose, from C_3 intermediates via gluconeogenesis, or via glycogen breakdown. One of the characteristics of the hepatic response to carbohydrate re-feeding after starvation is the suppression of net glucose output, with redirection of gluconeogenically produced glucose 6-phosphate from glucose output towards glycogen formation (Shikama & Ui, 1978; Newgard *et al.*, 1984; Holness & Sugden, 1987). This switch may assume quantitative importance for the replenishment of hepatic glycogen stores, since, under these conditions, net glucose phosphorylation in liver may be limited either by the level of glycaemia or by adaptive changes in the relative activities of glucokinase and glucose-6-phosphatase (reviewed by McGarry *et al.*, 1987). Thus studies in both experimental animals and man have shown that the indirect (gluconeogenic) pathway of glycogen synthesis predominates during the first few hours of re-feeding, although, at high (> 15 mM) glucose concentrations or as glycogen stores approach repletion, the relative contribution of the direct pathway increases (see, e.g., Hellerstein *et al.*, 1986; Huang & Veech, 1988; Landau & Wahren, 1988).

The indirect pathway of hepatic glycogenesis requires that glucose is initially metabolized to lactate (and related C_3 intermediates) before its utilization for hepatic glycogen synthesis. The major site(s) of glucose metabolism remains controversial. One proposal has been that lactate, formed from glucose and released into the hepatic vein by perivenous hepatocytes, is converted into glycogen in the periportal cells (Bartels *et al.*, 1987). However, consistent with diminished glucose phosphorylation capacity, balance studies have shown that the uptake of glucose across the liver is much less than glycogen formation on a mass basis. These studies have been reviewed elsewhere (McGarry *et al.*, 1987; Landau & Wahren, 1988). Alternative proposals have included the production of lactate from glucose during its

absorption from the intestine (Abumrad *et al.*, 1982; Bjorkman *et al.*, 1984). More recently, Cox & Palmer (1987) have suggested that recycling of carbon from carcass glycogen to lactate (and related C_3 intermediates) may also be quantitatively important for the provision of glycogenic precursors for the liver. This suggestion was based on the finding that in the mouse, after an intragastric glucose load, an initial phase of carcass glycogen synthesis is followed almost immediately (within 30 min) by mobilization of carcass glycogen, and presumably, as most extrahepatic tissues lack glucose-6-phosphatase, glycolytic flux to lactate and pyruvate. The decline in carcass glycogen was paralleled by an increase in hepatic glycogen, with no change in blood lactate (Cox & Palmer, 1987).

Skeletal muscle comprises approx. 40% of carcass weight (Young, 1970), and therefore glycogen in skeletal muscle might be expected to make a significant contribution to total carcass glycogen turnover. However, in a preliminary series of experiments with the adult rat (Holness & Sugden, 1988), glycogen concentrations in hind-limb skeletal muscle continued to rise for the first 2 h after the administration of an intragastric glucose load, by which time substantial hepatic glycogen repletion has already occurred (see, e.g., Holness & Sugden, 1987). The aim of the present experiments was to determine whether a temporal relationship exists between glycogen deposition/turnover in skeletal muscle and hepatic glycogen synthesis after re-feeding under physiological conditions where a complete diet is provided *ad libitum*. We analysed the pattern of glycogen repletion over a 24 h re-feeding period. Since it has been noted in other studies that skeletal muscles containing predominantly oxidative or glycolytic fibres may respond differently to changes in nutritional status (Goodman *et al.*, 1982; Preedy & Garlick, 1983), a comparison was made of the responses of skeletal muscles of varying fibre composition. A comparison was also made between the response of skeletal muscle and that of the heart, in which the glycogen concentration significantly increases,

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rather than decreases, after starvation (Zorzano *et al.*, 1985).

MATERIALS AND METHODS

Sources of biochemicals and standard laboratory chow were as described in Holness & Sugden (1987).

Female albino Wistar rats (180–220 g) were maintained on a 12 h-light/12 h-day cycle (light from 08:30 h), and were starved for 48 h in grid-bottomed cages before use. Experiments were started between 08:30 h and 09:00 h, when the previously starved rats were provided with laboratory chow [containing (w/w) 52% carbohydrate, 16% protein, 2% lipid, 30% non-digestible residue]. Chow was provided *ad libitum*, and intake was monitored at 2 h intervals during the 24 h re-feeding period. Intakes did not differ significantly from those shown in Holness & Sugden (1987). Rats were sampled at 2–24 h after the provision of chow. Tissues were freeze-clamped while the rats were under sodium pentobarbital anaesthesia (5 min; 6 mg/100 g body wt.). The mean heart and liver weights (expressed as g/100 g body wt.) in the starved state and at 2, 4, 6, 8 and 24 h after re-feeding were respectively as follows: heart, 0.374 ± 0.006 (14), 0.374 ± 0.011 (7), 0.383 ± 0.022 (5), 0.387 ± 0.022 (6), 0.357 ± 0.023 (8), 0.370 ± 0.005 (9); liver, 3.123 ± 0.067 (12), 3.228 ± 0.090 (11), 3.784 ± 0.130 (8), 4.272 ± 0.143 (9), 4.121 ± 0.121 (10), 5.073 ± 0.268 (10). Glycogen concentrations were measured in extracts of freeze-clamped hearts, livers and skeletal muscle as described previously (Holness & Sugden, 1987).

The results are presented as means $\pm$ S.E.M. for the numbers of rats specified. The statistical significance of differences was assessed by Student's unpaired *t* test.

RESULTS AND DISCUSSION

Skeletal-muscle glycogen in starvation

Issad *et al.* (1987) compared the rates of glucose utilization in a variety of skeletal muscles in resting post-absorptive or 48 h-fasted rats. In the post-absorptive

state, glucose utilization was higher in working muscles, such as the soleus, than in non-working muscles, such as the extensor digitorum longus and tibialis anterior. Decreases in glucose utilization in response to 48 h starvation were considerable (approx. 60–90%) in working muscles, but not significant in non-working muscles.

In the present experiments, the soleus muscle showed a marked decline in glycogen concentration in response to starvation (Table 1). This suggests that the glycogen-sparing effect of an elevated fatty acid concentration, which has been demonstrated to occur in soleus muscle during acute exercise (Rennie *et al.*, 1976), is insufficient to maintain the glycogen concentration in the face of a greatly decreased rate of glucose uptake/phosphorylation (Issad *et al.*, 1987), although it is not excluded that depletion of soleus glycogen may have preceded starvation-induced increases in lipolysis. Although glucose uptake/phosphorylation in the extensor digitorum longus and tibialis anterior muscles is little affected by starvation (Issad *et al.*, 1987), nonetheless glycogen depletion was observed. There was no obvious relationship between the fibre composition of the Type II muscles and their glycogenolytic responses to starvation (Table 1).

The time courses of the glycogenic responses after re-feeding were qualitatively remarkably similar in all the skeletal muscles studied, with an initial repletive phase of up to 8 h, followed at later times by moderate glycogen depletion (Fig. 1a). However, despite the overall similarity in glycogen deposition patterns, rates of glycogen deposition over the initial 0–4 h re-feeding period varied almost 3-fold, with the lowest rate in gastrocnemius and the highest rate in extensor digitorum longus (Table 1). This variation was not related to muscle fibre type, as might have been expected, but did bear a close relationship to the degree of glycogen depletion achieved during starvation. Thus more rapid repletion of glycogen was observed in those muscles where glycogen depletion was more marked, i.e. extensor digitorum longus (43% decrease) and soleus (34% decrease), compared with those muscles where depletion was more modest (plantaris, 25%; tibialis anterior, 23%; gastrocnemius,

Table 1. Skeletal-muscle glycogen concentrations in starvation and after re-feeding

Fibre compositions are taken from Ariano *et al.* (1973), and the percentage of oxidative fibres refers to the sum of Type I (slow-twitch oxidative) and Type IIa (fast-twitch oxidative) fibres. The peak glycogen concentration refers to the highest concentration observed during the 24 h re-feeding period. This normally occurred at 8 h (see Fig. 1a). The rate of glycogen deposition was calculated over the 0–4 h period of re-feeding. Significance of differences: fed versus starved, *** $P < 0.001$, * $P < 0.05$; starved versus re-fed, ††† $P < 0.001$, †† $P < 0.01$; fed versus re-fed, §§§ $P < 0.001$, §§ $P < 0.01$, § $P < 0.05$.

Skeletal muscle	Content of oxidative fibres (%)	Glycogen concentration ($\mu\text{g}/\text{mg}$ wet wt.)			Rate of glycogen deposition after re-feeding (ng/min per mg wt wt.)
		Fed	48 h-starved	Peak value after re-feeding	
Type I					
Soleus	100	6.24 ± 0.34 (7)	4.11 ± 0.36 (10)***	11.08 ± 0.69 (6)†††§§§	22.1
Type II					
Extensor digitorum longus	62	7.62 ± 0.59 (5)	4.38 ± 0.44 (10)***	12.63 ± 0.87 (6)†††§§	24.8
Plantaris	59	7.17 ± 0.52 (5)	5.35 ± 0.47 (10)*	9.91 ± 0.54 (5)†††§§	9.8
Tibialis anterior	68	7.36 ± 0.36 (5)	5.66 ± 0.52 (5)*	9.86 ± 0.99 (5)††§	13.3
Gastrocnemius	42	7.71 ± 0.53 (6)	6.27 ± 0.39 (8)*	11.66 ± 0.62 (17)†††§§§	9.1

Fig. 1. Muscle and liver glycogen concentrations after chow re-feeding of 48 h-starved rats

Glycogen concentrations in soleus (●), extensor digitorum longus (▲), plantaris (○), tibialis anterior (△) and gastrocnemius (□) muscles are shown in (a), and those in heart (▲) and liver (●) in (b). Error bars have been omitted for clarity in (a), but S.E.M. values did not exceed 10% of the mean in any instance.

19%). A similar relationship between the rate of glycogenesis and the extent of glycogen depletion appears to hold after exercise (Terjung *et al.*, 1974; Conlee *et al.*, 1978).

Skeletal-muscle glycogen turnover and the provision of precursors for hepatic glycogenesis

As hepatic and skeletal-muscle glycogen concentrations increase concurrently during the first 8 h after chow re-feeding (Figs. 1a and 1b), it is unlikely that the net mobilization of skeletal-muscle glycogen is quantitatively important as a source of C_3 precursors for hepatic glycogen synthesis via the indirect pathway in resting rats under physiological re-feeding conditions. By the time at

which any appreciable mobilization of skeletal-muscle glycogen is observed (8–24 h after re-feeding), hepatic glycogen repletion is complete (Fig. 1b). A significant decrease in cardiac glycogen concentration is observed within 2 h of the provision of chow, but concentrations continue to exceed the fed value throughout the 24 h re-feeding period (Fig. 1b). Although this pattern is consistent with limited glycolysis from glycogen in the heart, it would appear that cardiac glycogenolysis is quantitatively unimportant for the supply of glyconeogenic precursors for the liver, since only approx. 1.4 mg of cardiac glycogen/100 g body wt. is mobilized during the first 4 h of re-feeding. This could account for, at most, 1.2% of the liver glycogen deposited during this period (approx. 112.4 mg/100 g body wt.).

As the present findings differ from those of Cox & Palmer (1987), it remains possible that the five hind-limb muscles that were examined in the present paper are not representative of the whole carcass, which in the study of Cox & Palmer (1987) included the skin (T. N. Palmer, personal communication). However, although the skin may make a quantitatively important contribution to whole-body glucose disposal via its conversion into lactate (Cox & Palmer, 1988), it is not an important site of glycogen storage (Cox & Palmer, 1988). Furthermore, recently, in conjunction with ourselves, those authors have failed to observe a biphasic response of carcass glycogen to glucose re-feeding in the 24 h-starved rat (Cox *et al.*, 1989). The physiological significance of the response of carcass glycogen to intragastric glucose in the mouse must be questioned in view of profound yet transient post-prandial hyperglycaemia (a > 4.5-fold increase in glycaemia within 0.5 h; Cox & Palmer, 1987), which may dramatically affect glucose uptake and utilization not only in muscle but also in liver.

In the present experiments, the mean rate of hepatic glycogenesis during the initial 4 h of re-feeding was 0.47 mg/min per 100 g body wt. (see Fig. 1b and the Materials and methods section). If it is assumed that 60% of hepatic glycogen synthesis occurs via the indirect pathway (McGarry *et al.*, 1987), a minimum rate of glucose recycling of 0.28 mg/min per 100 g body wt. would be necessary for adequate provision of glyconeogenic precursor. During the initial phase of re-feeding, pyruvate oxidation remains largely suppressed, as demonstrated by the hypolactaemic effect of dichloroacetate, which leads to accelerated pyruvate dehydrogenase re-activation (Holness *et al.*, 1986). As the whole-body glucose-turnover rate even in the starved state is approx. 1.1 mg/min per 100 g body wt. (Issad *et al.*, 1987; see also Shikama & Ui, 1978), and hepatic glucose output is suppressed (Shikama & Ui, 1978; Newgard *et al.*, 1984; Holness *et al.*, 1988), an increase in glucose recycling is not a prerequisite for hepatic glycogen synthesis via the indirect pathway (although it may be observed as a consequence of hyperglycaemia). The accumulation of lactate which occurs after re-feeding (Holness *et al.*, 1988) demonstrates that lactate production exceeds lactate utilization.

A proportion of the pyruvate generated via glycolysis from glycogen in skeletal muscle during the period from 8 to 24 h after re-feeding may escape oxidation and enter the blood-stream. It is proposed that such re-cycling may provide precursors not for hepatic glycogenesis, but for hepatic lipogenesis, which is particularly active during this period (Holness *et al.*, 1988).

Glucose utilization in skeletal muscle after re-feeding

The rate of glycogen deposition gives a minimum estimate of skeletal-muscle glucose uptake/phosphorylation after re-feeding, and can be compared with the rate of glucose uptake/phosphorylation found under steady-state conditions *in vivo* in the post-absorptive and starved states (Issad *et al.*, 1987*; see also Pénicaud *et al.*, 1987). In soleus muscle, glucose utilization decreased from approx. 44 ng/min per mg to 8.6 ng/min per mg after 48 h starvation, whereas in the two Type II muscles studied by both Issad *et al.* (1987) and ourselves, namely the extensor digitorum longus and tibialis anterior, respective rates of glucose utilization were 5.3 and 5.9 ng/min per mg in the fed state and 3.9 and 4.1 ng/min per mg in the starved state. In all three muscles, rates of glycogen deposition observed from 0 to 4 h after re-feeding greatly exceeded the rates of glucose utilization observed in the fasted state (Table 1). As the indirect pathway for glycogen synthesis cannot be utilized in skeletal muscle (McGarry *et al.*, 1987), it can be concluded that there is an immediate and substantial increase in glucose uptake/phosphorylation in these skeletal muscles (cf. liver). In both of the non-working muscles (extensor digitorum longus and tibialis anterior), the minimum rate of glucose utilization actually exceeded the total rate of glucose utilization observed in the post-absorptive resting state [compare the values shown in Table 1 with those of Issad *et al.* (1987) and Pénicaud *et al.* (1987) given above]. No firm conclusions can be made as to whether the high rates of glycogen synthesis in such muscles are secondary to increased glucose uptake/phosphorylation, or whether, as after exercise (Fell *et al.*, 1982), stimulation of glycogen synthesis permits an accelerated rate of glucose 6-phosphate production.

In soleus muscle, the rate of glycogen repletion during the first 4 h after re-feeding was only 50% of the total rate of glucose utilization in the post-absorptive state, despite evidence obtained *in vitro* that the sensitivity of this muscle to insulin actually increases during fasting (Le Marchand-Brustel & Freychet, 1979; Brady *et al.*, 1981; Stirewalt *et al.*, 1985). Therefore, either glucose uptake/phosphorylation is incompletely restored during re-feeding, or glycogen deposition is accompanied by rapid glycolytic flux from glucose. The total rate of glucose utilization was not measured in the present study, but the concentration of fructose 2,6-bisphosphate [which generally varies with the rate of glycolysis (reviewed by Hue & Rider, 1987)] is maintained at the low values found in starvation during the first 8 h of re-feeding (French *et al.*, 1988). In contrast, the phase of the starved-to-fed transition associated with a decline in soleus-muscle glycogen (8–24 h; Fig. 1a) is characterized by the restoration of the concentration of fructose 2,6-bisphosphate (French *et al.*, 1988). On the basis of this temporal association, it is tempting to propose an initial restriction of glycolytic flux at the level of 6-phosphofructo-1-kinase. This may facilitate the attainment of glycogen concentrations exceeding those found in the fed state (Table 1; see also Fig. 1a) and may also be linked to a partial restraint on glucose uptake. Precisely such a relationship is observed in the heart in starvation

as a consequence of increased oxidation of lipid-derived fuels. Thus one might further speculate that the putative restriction on glucose uptake/glycolysis in soleus after re-feeding is linked to the preferential utilization of endogenous triacylglycerol as oxidative substrate, with net glycogenolysis following triacylglycerol exhaustion (at approx. 8 h). We therefore propose a role for the glucose/fatty-acid cycle after re-feeding, such that endogenous lipid fuels (or exogenous lipoproteins) continue to spare carbohydrate in order to facilitate the replenishment of depleted muscle glycogen stores.

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* In Issad *et al.* (1987) glucose utilization indices have been erroneously given as mg/min per g rather than mg/min per kg (see, e.g., Pénicaud *et al.*, 1987). Corrected values have been used for the discussion of the present paper.

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