Glucose utilization and disposal in cardiothoracic and skeletal muscles during the starved-to-fed transition in the rat

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Glucose utilization indices (GUI) increased to fed values in diaphragm and oxidative skeletal muscles and exceeded fed values in non-oxidative muscles within 2 h of re-feeding chow to 48 h-starved rats. Cardiac GUI reached fed values only after 7 h. Glycogen deposition accounted for most of the glucose phosphorylated in skeletal muscle over the first 2 h in oxidative muscles and over the first 4 h in non-oxidative muscles. In oxidative muscles, the contribution of glycogen deposition to total glucose 6-phosphate disposal diminished as re-feeding was extended from 2 to 6 h.

INTRODUCTION

Although skeletal muscle is collectively the most important quantitative site for glucose disposal in the body (Ferré *et al.*, 1985), heterogeneity exists between individual muscles both in terms of their capacities for glucose utilization in the fed state and in their responses to changes in carbohydrate supply. Higher rates of glucose uptake and phosphorylation are observed in the fed state in heart, diaphragm and postural skeletal muscles (which contain a relatively high percentage of oxidative fibres) than in non-oxidative skeletal muscles, which are not working in the resting state (Issad *et al.*, 1987; Holness & Sugden, 1990). Starvation is characterized by more marked suppression of glucose utilization (uptake/phosphorylation) in oxidative muscles (Issad *et al.*, 1987). Furthermore, the capacity for glucose oxidation, as assessed by the activity of the active form of the pyruvate dehydrogenase complex (PDH_a), decreases more precipitously in response to starvation (Holness *et al.*, 1989) and is restored less rapidly after re-feeding (Sugden & Holness, 1989) in oxidative than in non-oxidative muscles.

Starvation depletes endogenous glycogen in both oxidative and non-oxidative skeletal muscles (Holness *et al.*, 1988). Rates of glycogen deposition in individual muscles after re-feeding are determined primarily by the extent of glycogen depletion elicited by starvation (Holness *et al.*, 1988), and therefore are not necessarily predetermined by oxidative capacity. In the present study we define the changes in glucose utilization index (GUI) elicited by chow re-feeding after prolonged starvation in individual skeletal muscles in relation to the repletion of glycogen stores and carbohydrate degradation.

MATERIALS AND METHODS

Materials

Sources of materials were as in Holness *et al.* (1988) and Holness & Sugden (1990). Wako Insulin B-test kits and Wako NEFA C-test kits were from Alpha Laboratories, Eastleigh, Hants., U.K.

Animals

Female albino Wistar rats (200–250 g) on a 12 h-light/12 h-dark cycle (light from 08:00 h) were either fed *ad libitum* on a standard rodent diet (supplied by E. Dixon and Sons, Ware,

Herts., U.K.; 52 % digestible carbohydrate, 16 % protein, 2 % lipid and 30 % non-digestible residue, all by weight), starved for 48 h, or starved for 48 h and then re-fed on standard rodent diet *ad libitum*. Water was provided *ad libitum*. Chow intakes are shown in Table 1.

Measurement of tissue GUI *in vivo*

The study of changes in muscle GUI *in vivo* was conducted in conscious unrestrained rats each fitted with an indwelling cannula (for details, see Ferré *et al.*, 1985; Issad *et al.*, 1987). The fibre profiles (fast oxidative glycolytic: fast glycolytic: slow oxidative) of the hind-limb muscles selected for study are as follows (Ariano *et al.*, 1973): adductor longus, 12:0:88; soleus, 0:0:100; tibialis anterior, 66:32:2; extensor digitorum longus, 59:38:3; gastrocnemius, 37:58:5. Rats were sampled at 5–7 days after cannulation to permit recovery from surgery. In brief, a tracer dose of 2-deoxy[³H]glucose (30 µCi) was injected through the indwelling cannula. Arterial blood (100 µl) was collected at 1, 3, 5, 10, 20, 40 and 60 min after injection of 2-deoxy[³H]glucose for determination of blood glucose concentrations and 2-deoxy[³H]glucose radioactivity. Rats were killed at 1 h after the administration of radiolabel by injection of sodium pentobarbital (60 mg/kg body wt.) via the indwelling cannula. Blood and muscles (heart ventricle, diaphragm and hind-limb skeletal muscles) were treated as described in Ferré *et al.* (1985). GUI values were calculated as described by Issad *et al.* (1987) by dividing the radioactivity (d.p.m.) of the 2-deoxy[³H]glucose 6-phosphate in the tissue by the calculated integral of blood 2-deoxy[³H]glucose/[glucose]. As in Issad *et al.* (1987), values were not corrected for the discrimination factor for deoxyglucose in glucose metabolic pathways.

GUI values in individual muscles were measured over 1 h periods. Experiments in fed rats (with continuous access to food *ad libitum*) and 48 h-starved rats were completed before 11:00 h. Starved rats subsequently provided with chow *ad libitum* were sampled at intervals during re-feeding. Times of re-feeding given in Tables 1, 2 and 4 refer to the time of injection of radiolabel: the muscles were sampled after a further 1 h (see above). Glucose was measured by using glucose oxidase in the serial blood samples obtained via the indwelling cannula during the 2-deoxy[³H]glucose experiments as described by Ferré *et al.* (1985). Mean glucose concentrations over each of the 1 h periods are shown in Table 1. Blood glucose concentrations were significantly

Abbreviations used: GUI, glucose utilization index; PDH_a, the active form of the pyruvate dehydrogenase complex; NEFA, non-esterified fatty acids; EDL, extensor digitorum longus.

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Table 1. Chow intakes and blood glucose concentrations after the provision of chow *ad libitum* to 48 h-starved/chow-re-fed rats

Full details are given in the Materials and methods section. The period of re-feeding specified refers to the time of injection of 2-deoxy[³H]glucose; the blood glucose concentrations shown are the means of samples taken at 1, 3, 5, 10, 20, 40 and 60 min after this time. Results are means $\pm$ S.E.M. for five to seven rats. Statistically significant effects of re-feeding are indicated: ** $P < 0.01$; *** $P < 0.001$. Blood glucose concentrations after re-feeding were not significantly different from each other.

Period of re-feeding (h)	Chow intake (g)	Blood glucose concn. (mM)	Coefficient of variance (%)
0	—	3.69 $\pm$ 0.34	11.5 $\pm$ 1.5
1.9 $\pm$ 0.1	4.6 $\pm$ 0.7	5.84 $\pm$ 0.53**	9.4 $\pm$ 1.3
3.1 $\pm$ 0.1	5.4 $\pm$ 0.4	6.28 $\pm$ 0.41***	11.5 $\pm$ 1.2
5.0 $\pm$ 0.3	7.2 $\pm$ 0.7	6.30 $\pm$ 0.29***	12.0 $\pm$ 1.2
6.8 $\pm$ 0.1	7.6 $\pm$ 1.0	5.70 $\pm$ 0.48**	11.7 $\pm$ 1.3
8.4 $\pm$ 0.4	8.3 $\pm$ 1.2	6.44 $\pm$ 0.34***	15.2 $\pm$ 1.7
Fed	—	6.54 $\pm$ 0.23	11.6 $\pm$ 1.3

increased by chow re-feeding, but from 1.9 h to 8.4 h after re-feeding blood glucose concentrations did not fluctuate significantly (Table 1). The period from 1.9 to 8.4 h after re-feeding is therefore nearly steady state with respect to blood glucose. Mean coefficients of variance of blood glucose concentrations over each 1 h period of sampling are shown in Table 1.

Hormone and metabolite assays

At the end of the 2-deoxy[³H]glucose experiments, a 500 μ l blood sample was taken for the measurement of hormone and metabolite concentrations. Plasma insulin concentrations were measured by enzyme-immunoassay (Wako Insulin B-test kit). Plasma non-esterified fatty acids (NEFA) were measured by coupling the acylation of CoA by fatty acids to the formation of a coloured adduct by using a Wako NEFA C-test kit. A 250 μ l portion of whole blood was deproteinized in HClO₄, and ketone-body concentrations were measured in KOH-neutralized HClO₄ extracts of whole blood (Williamson *et al.*, 1962).

For measurements of glycogen concentrations, heart ventricle, diaphragm and hind-limb skeletal muscles were excised and freeze-clamped while rats were under sodium pentobarbital

anaesthesia (intraperitoneal injection of 60 mg/kg body wt.; 5 min exposure; see Holness *et al.*, 1988). Glycogen concentrations were determined, without prior precipitation with ethanol, as glucose after hydrolysis with amyloglucosidase (Keppler & Decker, 1974), in extracts of freeze-clamped tissue.

Statistics

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

RESULTS AND DISCUSSION

GUI values in diaphragm and skeletal muscle after re-feeding

Significant increases in GUI were observed after chow re-feeding in all the muscles studied (Table 2). GUI values measured over the period from 1.9 to 2.9 h after re-feeding were increased relative to the corresponding starved values by 11.5-fold in the diaphragm, 3.8-fold in the soleus and 9.0-fold in the adductor longus [oxidative (working) muscles], and by 7.4-fold in the gastrocnemius, 10.8-fold in the extensor digitorum longus (EDL) and 11.8-fold in tibialis anterior [non-oxidative (non-working) muscles]. GUI values of each of the muscles remained higher than the corresponding starved values throughout the re-feeding period (Table 2).

Oxidative muscles. Absolute GUI values after re-feeding were approx. 2-fold higher in oxidative skeletal muscles and diaphragm than in non-oxidative skeletal muscles (Table 2). In oxidative skeletal muscles, GUI values throughout the re-feeding period either were comparable with or slightly exceeded (by up to 60 %; diaphragm, 3.1 h) those observed in continually fed rats (Table 2). The highest values tended to be observed over the period from 3 to 6 h after the provision of chow.

Non-oxidative muscles. In each of the non-oxidative muscles, GUI values in the re-fed state greatly exceeded those observed in the fed state (Table 2). For example, in the tibialis anterior, GUI values after re-feeding for 1.9 h were 605 % of the values observed in continually fed rats. As a consequence, GUI values in non-oxidative muscles during the starved-to-fed transition are much more comparable with those observed in oxidative muscles than is the case in rats provided with continuous access to food. It is therefore clear that during the initial phase of the starved-to-fed transition not only oxidative but also non-oxidative muscles can

Table 2. GUI values of muscles of continually fed, 48 h-starved or 48 h-starved/chow-re-fed rats

Full details are given in the Materials and methods section. The period of re-feeding specified refers to the time of injection of 2-deoxy[³H]glucose. Results are means $\pm$ S.E.M. for five to seven rats. Statistically significant effects of re-feeding are indicated: ** $P < 0.01$; *** $P < 0.001$.

Period of re-feeding (h)	Glucose utilization index (ng of glucose/min per mg of tissue)						
	Heart	Diaphragm	Soleus	Adductor longus	Tibialis anterior	EDL	Gastrocnemius
0	2.5 $\pm$ 0.8	4.1 $\pm$ 1.1	9.0 $\pm$ 2.5	4.3 $\pm$ 0.9	2.2 $\pm$ 0.4	2.0 $\pm$ 0.8	1.1 $\pm$ 0.4
1.9 $\pm$ 0.1	37.9 $\pm$ 7.2***	47.2 $\pm$ 9.1***	34.3 $\pm$ 4.9***	38.8 $\pm$ 6.4***	26.0 $\pm$ 5.9**	21.6 $\pm$ 4.3**	8.1 $\pm$ 2.1**
3.1 $\pm$ 0.1	56.5 $\pm$ 3.5***	64.5 $\pm$ 7.9***	50.3 $\pm$ 6.8***	42.3 $\pm$ 7.9***	19.2 $\pm$ 2.9***	21.5 $\pm$ 3.1***	10.7 $\pm$ 1.8***
5.0 $\pm$ 0.3	48.8 $\pm$ 5.7***	44.3 $\pm$ 7.9***	51.2 $\pm$ 6.9***	45.0 $\pm$ 6.0***	16.8 $\pm$ 2.6***	15.7 $\pm$ 2.1***	10.3 $\pm$ 1.3***
6.8 $\pm$ 0.1	87.5 $\pm$ 12.4***	51.8 $\pm$ 5.9***	45.7 $\pm$ 7.7***	43.9 $\pm$ 9.1**	16.3 $\pm$ 3.0***	13.5 $\pm$ 3.2**	7.3 $\pm$ 1.4**
8.4 $\pm$ 0.4	102.2 $\pm$ 15.1***	43.8 $\pm$ 7.8***	39.7 $\pm$ 3.6***	41.2 $\pm$ 2.4***	9.0 $\pm$ 0.8***	10.0 $\pm$ 1.2***	6.3 $\pm$ 0.8***
Fed	74.4 $\pm$ 7.0	40.5 $\pm$ 3.6	37.9 $\pm$ 8.4	30.0 $\pm$ 4.5	4.3 $\pm$ 0.5	4.4 $\pm$ 0.4	3.6 $\pm$ 0.3

Table 3. Muscle glycogen concentrations in continually fed, 48 h-starved or 48 h-starved/chow-re-fed rats

Full details are given in the Materials and methods section. Glycogen concentrations were measured at 2 h intervals after re-feeding. The period of re-feeding specified refers to the time of sampling. Results are means $\pm$ S.E.M. for five to seven rats. Statistically significant effects of re-feeding are indicated: * $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Period of re-feeding (h)	Glycogen concn. (mg/g wet wt.)						
	Heart	Diaphragm	Soleus	Adductor longus	Tibialis anterior	EDL	Gastrocnemius
0	6.7 $\pm$ 0.7	1.5 $\pm$ 0.4	2.8 $\pm$ 0.3	2.6 $\pm$ 0.3	5.5 $\pm$ 0.4	4.5 $\pm$ 0.3	6.5 $\pm$ 0.4
2	4.8 $\pm$ 0.4*	5.8 $\pm$ 0.7***	7.3 $\pm$ 0.2***	5.8 $\pm$ 0.3***	7.9 $\pm$ 0.3***	6.8 $\pm$ 0.3***	7.3 $\pm$ 0.2
4	4.6 $\pm$ 0.4*	8.2 $\pm$ 0.9***	8.9 $\pm$ 0.3***	7.5 $\pm$ 0.4***	10.2 $\pm$ 0.5***	9.5 $\pm$ 0.5***	8.0 $\pm$ 0.3*
6	5.0 $\pm$ 0.6	9.1 $\pm$ 1.3***	9.6 $\pm$ 0.4***	7.6 $\pm$ 0.5***	10.8 $\pm$ 0.4***	8.6 $\pm$ 0.4***	8.8 $\pm$ 0.2***
Fed	5.7 $\pm$ 0.5	8.9 $\pm$ 0.5	5.0 $\pm$ 0.3	3.4 $\pm$ 0.3	7.2 $\pm$ 0.3	7.5 $\pm$ 0.3	8.9 $\pm$ 0.3

make a major contribution to whole-body glucose utilization. This contrasts with the situation in the fed state, where glucose utilization by oxidative muscles is considered to be of much greater importance as a determinant of whole-body glucose turnover than that by non-oxidative muscles (Pénicaud *et al.*, 1987).

Whereas in oxidative muscles GUI values remain high and relatively constant throughout the period of re-feeding, those in non-oxidative muscles responded in a biphasic manner (Table 2). The highest values were observed during the first 4 h after the initiation of re-feeding; a subsequent steady decline in GUI was observed, such that after approx. 8 h of re-feeding GUI values in non-oxidative muscles were only 35–59% of the highest GUI values observed. The pattern of response suggests a transient large increase in glucose disposal during the initial phase of the starved-to-fed transition.

Muscle glycogen synthesis after re-feeding

Glycogen synthesis in skeletal muscle mainly utilizes glucose 6-phosphate derived from circulating glucose as precursor. Glycogen concentrations in diaphragm and individual skeletal muscles were significantly increased in response to re-feeding (Table 3). The most rapid rates of net glycogen deposition were generally observed over the initial 2 h period of re-feeding rather than during the later phase (see Table 3). Consequently glycogen deposition during the initial 2 h of re-feeding accounted for between 35% (gastrocnemius) to 66% (soleus) of the total glycogen deposited over the entire 6 h period. There was a clear inverse relationship between this initial rate of glycogen deposition and the fasting glycogen concentration (correlation coefficient $r = 0.91$; see also Holness *et al.*, 1988). Glycogen concentrations were restored to fed values within 4–6 h of re-feeding (Table 3).

Oxidative muscles. The initial rate of glycogen deposition in the soleus (a mean rate of 37.5 ng glucose equivalents/min per mg of muscle measured over the first 2 h; see Fig. 1a) is of the same order as the observed GUI (Fig. 1a; see also Table 2). Similarly, initial rates of glycogen deposition in diaphragm and adductor longus (estimated rates of 35.8 and 26.7 ng of glucose equivalents/min per mg of muscle respectively; Fig. 1a) correspond to approx. two-thirds of the measured GUI (Fig. 1a; Table 2). In the absence of direct measurement of the discrimination factors for deoxyglucose in glucose metabolic pathways, direct comparison between GUI values and rates of glycogen deposition is precluded. However, it is tempting to speculate that during the initial period of re-feeding the pre-

dominant fate of hexose phosphate in these muscles may be glycogen synthesis. Since GUI values are subsequently maintained (Fig. 1a; Table 2), whereas mean rates of net glycogen deposition (measured during successive 2 h periods) markedly decline (Fig. 1a), glycogen synthesis must make a less significant contribution to total hexose phosphate disposal as the duration of re-feeding is extended. Therefore, by inference, the proportion of hexose phosphate available for entry into glycolysis must progressively increase.

We have demonstrated previously (Sugden & Holness, 1989) that PDH_a activities in oxidative muscles remain considerably lower than the corresponding fed values during the first 6 h of re-feeding after starvation for 48 h. The starved-to-fed transition is therefore characterized by a gradual switch from glucose storage (as glycogen) to glucose degradation in oxidative skeletal muscles.

Non-oxidative muscles. Estimated mean rates of glycogen deposition (measured from 0 to 2 h) in tibialis anterior, EDL and gastrocnemius were 20.0, 19.2 and 6.7 ng of glucose equivalents/min per mg of muscle respectively (Fig. 1b; Table 2). As is the case for oxidative muscles, glycogen deposition over this period would appear to account for a major proportion of the GUI in each muscle. High GUI values and high mean rates of glycogen deposition are maintained for a further 2 h (Fig. 1b). In tibialis anterior and EDL net rates of glycogen deposition subsequently rapidly declined, whereas GUI values were maintained (Fig. 1b). In gastrocnemius, the pattern of changes in GUI closely resembles that of glycogen deposition (Fig. 1b).

Cardiac GUI values after re-feeding

The heart, as an oxidative working muscle, is characterized by a high GUI in the fed state (Issad *et al.*, 1987; Holness & Sugden, 1990), and responds relatively rapidly to starvation with a decline in GUI (Holness & Sugden, 1990). However, cardiac glycogen concentrations increase, rather than decrease, in response to starvation (Table 3; Cruikshank, 1913; McLeod & Prendergast, 1921; French *et al.*, 1988). Re-feeding after 48 h starvation led to rapid and substantial (15-fold) increases in cardiac GUI (Table 2). However, in contrast with the response in skeletal muscle, GUI values after 2 h of re-feeding were substantially less (by 49%) than those observed in the continually fed state (Table 2). Cardiac GUI values continued to increase steadily as the duration of re-feeding was extended, and after 7 h of re-feeding were not significantly different from those found in continually fed rats (Table 2). Cardiac glycogen concentrations decreased after re-feeding (Table 3; see also Holness *et al.*, 1988).

Although complete restoration of the GUI in the heart is not

Fig. 1. Comparison of GUI values and glycogen deposition rates in oxidative (a) and non-oxidative (b) skeletal muscles at intervals after chow re-feeding of 48 h-starved rats

Full details are given in the Materials and methods section and the legends to Tables 2 and 3. GUI values are taken from Table 2; mean rates of glycogen deposition over successive 2 h periods have been estimated from the data shown in Table 3. GUI values ($\blacktriangle$, $\bullet$, $\blacklozenge$) and glycogen deposition rates ($\triangle$, $\circ$, $\diamond$) for diaphragm ($\blacktriangle$, $\triangle$), soleus ($\bullet$, $\circ$) and adductor longus ($\blacklozenge$, $\diamond$) are shown in (a), and for tibialis anterior ($\blacktriangle$, $\triangle$), EDL ($\bullet$, $\circ$) and gastrocnemius ($\blacklozenge$, $\diamond$) in (b).

immediate, the response is rapid in relation to that of PDH_a activity, which requires up to 24 h of re-feeding for complete re-activation after starvation for 48 h (Kerby & Randle, 1982; Sugden & Holness, 1989). Furthermore, since cardiac glycogen concentrations decline, the predominant fate of the additional glucose 6-phosphate generated by increased glucose uptake and phosphorylation must be conversion into pyruvate (via gly-

colysis) and release as pyruvate, lactate or alanine into the circulation. Release of C₃ derivatives of glucose into the circulation by the heart (and possibly other oxidative muscles) during the early (0–4 h) period after re-feeding may make a significant contribution to the provision of precursors for the indirect (gluconeogenic) pathway of hepatic glycogen synthesis (reviewed by Sugden *et al.*, 1989).

Factors regulating GUI during the starved-to-fed transition

Oxidative muscles. A role for a decline in the expression of the two glucose transporters (GLUT1 and GLUT4) present in skeletal muscle in the suppression of glucose uptake and phosphorylation by oxidative muscles during prolonged starvation can be excluded: expression of both GLUT1 and GLUT4 (the insulin-sensitive glucose transporter) substantially increases (by 2–3-fold) in starvation (Charron & Kahn, 1990). It has been proposed that the function of the glucose transporters may be impaired by the increased oxidation of NEFA and ketone bodies (Issad *et al.*, 1987; Holness & Sugden, 1990). Consistent with this, the increases in values of GUI observed in oxidative muscles after re-feeding occur concomitantly with a decline in the concentrations of NEFA and ketone bodies (Table 4).

The capacity of muscle for glucose uptake and phosphorylation is obviously limited by the expression of GLUT1 and GLUT4. Despite the increases in the expression of GLUT1 and GLUT4 observed in response to prolonged starvation and the fact that use of hexose phosphate for glycogen synthesis is greatly increased during the initial period of the starved-to-fed transition, values of GUI in oxidative skeletal muscles during this re-feeding period do not greatly exceed those observed in continually fed rats. Either values of GUI are maximal under the former conditions, owing to a restraint imposed at the level of glucose transport (possibly because of rapid reversion of the expression of the glucose transporters to fed values), or glucose transport is not rate-limiting and the value of the GUI is determined by the rate of glucose 6-phosphate removal (by glycogen synthesis and/or glycolysis). If the latter is correct, then the preferential and predominant use of available hexose phosphate for glycogen synthesis during the first 2 h of re-feeding implies a strong pull mechanism by glycogen synthesis and a restraint on the utilization of hexose phosphate via glycolysis.

Non-oxidative muscles. The time course and pattern of changes in GUI values in non-oxidative muscles during the first 4 h of re-

Table 4. Plasma insulin and NEFA concentrations and blood ketone-body (3-hydroxybutyrate and acetoacetate) concentrations in continually fed, 48 h-starved or 48 h-starved/chow-re-fed rats

Blood samples were taken from the rats used for GUI measurement (shown in Table 2) at the time of tissue sampling (see the Materials and methods section). Results are means $\pm$ S.E.M. for five to seven rats. Statistically significant effects of re-feeding are indicated: ** $P < 0.01$; *** $P < 0.001$.

Period of re-feeding (h)	Insulin (μ -units/ml)	Plasma NEFA (mM)	Blood ketone bodies (mM)
0	7.4 $\pm$ 1.4	0.82 $\pm$ 0.04	1.27 $\pm$ 0.07
1.9 $\pm$ 0.1	18.0 $\pm$ 2.6**	0.24 $\pm$ 0.04***	0.12 $\pm$ 0.02***
3.1 $\pm$ 0.1	42.3 $\pm$ 8.2**	0.25 $\pm$ 0.03***	0.17 $\pm$ 0.04***
5.0 $\pm$ 0.3	42.2 $\pm$ 8.1**	0.13 $\pm$ 0.04***	0.14 $\pm$ 0.05***
6.8 $\pm$ 0.1	50.1 $\pm$ 10.7**	0.10 $\pm$ 0.02***	0.15 $\pm$ 0.03***
8.4 $\pm$ 0.4	38.3 $\pm$ 8.5**	0.18 $\pm$ 0.04***	0.12 $\pm$ 0.03***
Fed	38.7 $\pm$ 6.3	0.11 $\pm$ 0.01	0.14 $\pm$ 0.02

feeding parallel those in net glycogen deposition. Therefore a major proportion of glucose 6-phosphate generated from blood glucose would appear to be utilized for glycogen deposition rather than degradation in these muscles even after 4 h of re-feeding, and declines only after 6 h of re-feeding. Although the insulin concentrations are not fully restored to the values found in continually fed rats during the early (0–3 h) phase of re-feeding, rates of glucose uptake and phosphorylation clearly exceed those observed in the fed state (Table 4). This may reflect a higher capacity for glucose transport (expression of GLUT1 and GLUT4). Alternatively, glucose transporters may be less critical in determining glucose uptake and phosphorylation than the rate of disposal of intracellular glucose 6-phosphate: potentiated rates of glucose phosphorylation may be achieved after re-feeding as a consequence of the increased demand for hexose phosphate for glycogen repletion. It is of interest that, during the later (6–8 h) period of re-feeding, insulin concentrations are higher but GUI values have declined (Table 4).

Concluding remarks

It is of interest to compare the response to re-feeding after prolonged starvation with the response to exogenous insulin during euglycaemic/hyperinsulinaemic clamp. In rats which have been subjected to starvation for only 5 h, oxidative muscles are more responsive to insulin (in terms of glucose uptake/phosphorylation and glycogen synthesis) than are non-oxidative muscles (James *et al.*, 1985). This was taken to indicate that insulin-induced increases in total peripheral glucose disposal occur predominantly in muscles containing a high proportion of oxidative fibres (James *et al.*, 1985). The present study indicates that, when physiological increases in insulin concentrations are induced by feeding a mixed diet after prolonged starvation, conditions where circulating concentrations of glucose as well as insulin are increased, non-oxidative as well as oxidative muscle

makes a significant contribution to glucose disposal, predominantly owing to glycogen repletion.

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