Glucose utilization and disposition by skeletal muscle during unrestricted feeding

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We measured glucose utilization index (GUI) values in individual skeletal muscles of conscious rats during the light (quiescent) and dark (feeding/activity) phases. There was a 2–3-fold variation in muscle GUI values, with peak values observed at the end of the dark phase and minimum values observed at 6–9 h into the light phase. GUI values in working muscles (soleus and adductor longus) were consistently higher than in non-working muscles (tibialis anterior and extensor digitorum longus), indicating that working muscles make the major contribution of the total skeletal muscle mass to glucose disposal during unrestricted feeding. There was a clear overall increase in muscle glycogen deposition during the first 9 h of the dark phase; this was concomitant with an increase in food consumption. Peak glycogen concentrations were reached after 9 h of darkness, but subsequently declined. The pattern of changes in muscle GUI values during the light and dark phases is discussed in relation to the role of insulin in facilitating glucose clearance.

INTRODUCTION

There is clear evidence that the concentration of glycogen in muscle can exert a feedback regulatory role in determining its own synthesis. Prior glycogen depletion increases muscle glycogen synthesis when carbohydrate is provided (reviewed by Felber, 1989); conversely, sparing of muscle glycogen by lipid infusion limits the non-oxidative disposal of glucose (as glycogen) during a hyperinsulinaemic clamp (see Felber, 1989). We have demonstrated that in certain types of skeletal muscle [notably those muscles which are not working in the resting state, such as tibialis anterior and extensor digitorum longus (EDL)], rates of glucose uptake and phosphorylation are tightly coupled to the use of glucose for glycogen synthesis. This is also the case in working muscles; however, the energy demands associated with contraction necessitate relatively high rates of glucose utilization even in the absence of any changes in net glycogen deposition (Holness & Sugden, 1991). We have demonstrated a convincing correlation between increases in rates of glucose utilization and net glycogen deposition in non-working muscles (Sugden *et al.*, 1990); furthermore, when the initial glycogen concentration was manipulated by starvation for various periods, an inverse relation existed between the initial glycogen concentration and the stimulation of glucose utilization elicited by chow re-feeding (Holness & Sugden, 1991).

One conclusion from these studies was that the contribution of non-working muscles to total glucose utilization by the muscle mass was highly variable and critically dependent on the precise nutritional status. As proposed by Issad *et al.* (1987), glucose uptake and phosphorylation by non-working muscles (in the resting state) makes only a relatively minor contribution to total glucose utilization by the muscle mass in the immediately post-absorptive state and after prolonged starvation; this is also the case on re-feeding after short-term (6 h) starvation, since high glycogen concentrations are retained (Holness & Sugden, 1991). In contrast, the quantitative contribution of non-working muscle to total glucose disposal is highly significant on re-feeding after prolonged (24–48 h) starvation (see, e.g. Sugden *et al.*, 1990; Holness & Sugden, 1991), and presumably would be equally important after other manipulations associated with profound glycogen depletion (such as severe exercise).

Changes in glucose tolerance are observed during the diurnal cycle in man (Wojtczak-Jaroszwowa, 1977) and in the rat (Hara & Saito, 1980; Pénicaud *et al.*, 1983; Leighton *et al.*, 1988). In general, it appears that glucose tolerance is decreased by previous food intake. Food intake might be expected to increase storage of glucose as muscle glycogen. In the present experiments, we have measured rates of glucose utilization directly in a range of skeletal muscles (both working and non-working) throughout the light (quiescent) and dark (feeding/activity) phases in the rat and compared them with changes in glycogen concentrations. The first aim was to examine whether changes in glucose tolerance associated with the normal feeding pattern might be related to changes in the contribution of non-working muscles to glucose disposal. The study was undertaken in part to examine whether the dramatic fluctuations in glucose utilization observed in non-working muscles during re-alimentation after food withdrawal might be of physiological relevance within the context of an unrestricted feeding cycle. The second aim was to establish the precise relationship between rates of glucose uptake and phosphorylation and glycogen concentrations in skeletal muscle during unrestricted feeding.

MATERIALS AND METHODS

Materials

Sources of materials were as in Holness *et al.* (1988) and Holness & Sugden (1990). Kits for measurement of plasma insulin concentrations were purchased from Alpha Laboratories, Eastleigh, Hants., U.K.

Animals

Mature (180–250 g) female albino Wistar rats were maintained on 12 h-light/12 h-dark cycles, with light either from 10:00 h or 20:00 h. Rats were permitted free access to a standard rodent diet, comprising 52 % carbohydrate, 15 % protein, 3 % fat and 30 % non-digestible residue (all by wt.) purchased from Special Diets Service (Witham, Essex, U.K.). The rats were sampled at 10:00, 13:00, 16:00 and 19:00 h (i.e. after 0, 3, 6, 9 or 12 h of either light or darkness) as indicated.

Abbreviations used: GUI, glucose utilization index; EDL, extensor digitorum longus.

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Measurement of tissue glucose utilization index (GUI) *in vivo*

Each rat was fitted with an indwelling cannula before the experiment using Hypnorm [fentanyl citrate (0.315 mg/ml)/fluanisone (10 mg/ml); 1 ml/kg intraperitoneal] and Diazepam (5 mg/ml; 1 ml/kg intraperitoneal) anaesthesia. GUI values were measured in conscious unrestrained rats after complete recovery from surgery (5–7 days after cannulation). Full details of the experimental protocol used for measurement of GUI are given in Sugden *et al.* (1990). Each GUI measurement was initiated by injection of a tracer dose of 2-deoxy[^3H]glucose (30 μCi) via the cannula. Times of sampling given in the Figures refer to the times of injection of radiolabelled 2-deoxyglucose, as most of the radiolabel was cleared from the blood within the first 20 min after its injection (results not shown; see also Sokoloff *et al.*, 1977; James *et al.*, 1985). Blood (100 μl) was collected at 1, 3, 5, 10, 20, 40 and 60 min after injection of the radiolabel. An additional 500 μl blood sample was taken at 60 min for the measurement of plasma insulin concentrations. The procedure was terminated after 60 min by the intravenous injection of sodium pentobarbital (60 mg/kg body wt.) through the cannula. Blood and muscles were extracted and treated as described by Ferré *et al.* (1985). Results have not been corrected for the possible discrimination between glucose and deoxyglucose in terms of tissue uptake and phosphorylation.

Glycogen measurements

Measurements of muscle glycogen concentrations were undertaken in a separate group of rats. Muscles were freeze-clamped in liquid N_2 while rats were under anaesthesia such that locomotor activity had ceased (sodium pentobarbital, 60 mg/kg body weight, intraperitoneal; 5 min) at the times indicated. Glycogen concentrations were determined, without prior precipitation with ethanol, as glucose after hydrolysis with amyloglucosidase (Keppler & Decker, 1974).

Statistics

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

RESULTS AND DISCUSSION

Blood glucose and plasma insulin concentrations in relation to chow intake

The pattern of chow intake observed during the light and dark periods is shown in Fig. 1. In confirmation of previous studies with rats fed *ad libitum* (see, e.g., Bruckdorfer *et al.*, 1974; Kimura *et al.*, 1970; Conlee *et al.*, 1976; Munday & Williamson, 1983), most (86 %) of the daily food intake was consumed during the dark rather than during the light phase. There was evidence for the bimodal feeding pattern reported previously by others to occur in female rats during the feeding period (Tachi *et al.*, 1981; Munday & Williamson, 1983), with a small but significant drop in food intake during the period from 6 to 9 h of the dark phase (Fig. 1).

Despite the marked differences in food intake during the dark and light periods, glycaemia (measured in samples of blood obtained via the indwelling cannula) remained remarkably constant, with only a modest decline during the light phase (Fig. 1; see also Leighton *et al.*, 1988). However, the reduction in chow intake observed during the light phase was associated with decreased plasma insulin concentrations and, furthermore, in-

creases in chow intake and plasma insulin concentrations occurred concomitantly during the dark phase (Fig. 1).

Muscle GUIs

GUI values for four individual skeletal muscles measured at 3 h intervals during the light and dark phases are shown in Fig. 2. There was a consistent and notable distinction between the relative rates of glucose uptake and phosphorylation by working muscles (represented by two postural muscles, the soleus and adductor longus) and non-working muscles (tibialis anterior and EDL): irrespective of the chow intake or plasma insulin concentration, rates of glucose utilization by the working muscles were always quite considerably higher than those of the non-working muscles.

Working muscles. There were clear changes in rates of glucose uptake and phosphorylation which were related to light or darkness (Fig. 2). There was a progressive decline in GUI values in both soleus and adductor longus during the light phase, corresponding closely with the decline in plasma insulin concentration. The lowest GUI values were observed at 9 h into the light phase; these were approx. 35 % of the highest values (observed at the end of the dark phase).

A small increase in GUI values was observed at the end of the light phase (Fig. 2), although the circulating insulin concentration remained low (Fig. 1). The onset of the dark phase was associated with significant increases in GUI values (Fig. 2); this response was associated with an increase in food intake and a rise in plasma insulin concentrations (Fig. 1). GUI values of both soleus and adductor longus were maintained at high values until 6 h into the dark phase, when the bimodal pattern of food intake (Fig. 1) was closely paralleled by a decline in GUI values

Fig. 1. Chow intakes, blood glucose and plasma insulin concentrations during unrestricted feeding

Rats were provided with free access to standard laboratory chow and water and sampled at 3 h intervals during either the light or the dark phase. Chow intakes (◆) are expressed as g of diet consumed during the preceding 3 h period. Blood glucose concentrations (▲) were measured by using glucose oxidase in the serial blood samples obtained via the indwelling cannula during measurements of GUI. Insulin concentrations (●) were measured in plasma from the terminal blood sample. Results are means $\pm$ S.E.M. for five to eight rats. * indicates statistically significant ($P < 0.05$) decreases from the 0 h light value; † indicates statistically significant ($P < 0.05$) increases from the lowest (9 h light) value.

Fig. 2. Skeletal muscle GUI values during unrestricted feeding

GUI values of soleus (▲), adductor longus (●), tibialis anterior (△) and EDL (○) were measured at 3 h intervals during the light or dark phases. Further experimental details are given in the Materials and methods section. Results are means $\pm$ S.E.M. for five to eight rats. * indicates statistically significant ($P < 0.05$) decreases from the 0 h light value; † indicates statistically significant ($P < 0.05$) increases from the lowest (9 h light) value.

between 6 h and 9 h (Fig. 2). GUI values in both muscles then subsequently increased to reach peak values at the end of the dark phase.

Non-working muscles. Although GUI values were relatively low in non-working muscles throughout the light/dark cycle, there was nevertheless, significant variation between the light and dark phases (Fig. 2). In general, values of GUI during the dark phase were approx. 2-fold higher than in the light phase. The peak values of GUI were observed at the end of the dark phase but were not maintained after the onset of the light phase, declining significantly within 3 h.

Glycogen concentrations

Leighton *et al.* (1988) measured muscle glycogen concentrations during the diurnal cycle. In those experiments, glycogen concentrations in two non-working muscles (EDL and gastrocnemius/plantaris) measured at 7 h into the dark (feeding) phase was 1.3–1.5-fold higher than those measured after 1 h of darkness. In the present experiments, glycogen concentrations in both working and non-working muscles were maintained for the first 6 h of the light phase, but then declined slightly during the last 3–6 h (Fig. 3).

We have demonstrated previously that a close relationship exists between glucose uptake and phosphorylation and net glycogen deposition during re-feeding after starvation in non-working muscles (Sugden *et al.*, 1990; Holness & Sugden, 1991). Since, in the present experiments, GUI values in non-working muscles were 2-fold higher during the dark phase than during the light phase, we investigated whether increased glycogen deposition paralleled this rise. In addition, we examined to what extent the increases in glucose utilization observed in working muscles during the dark phase might be related to changes in glycogen deposition.

Glycogen concentrations in the two non-working muscles selected for the present study (tibialis anterior and EDL) were

Fig. 3. Skeletal muscle glycogen concentrations during unrestricted feeding

Glycogen concentrations in soleus (▲), adductor longus (●), tibialis anterior (△) and EDL (○) were measured at 3 h intervals during the light or dark phase. Further experimental details are given in the Materials and methods section. Results are means $\pm$ S.E.M. for five to eight rats. * indicates statistically significant ($P < 0.05$) decreases from the 0 h light value; † indicates statistically significant ($P < 0.05$) increases from the lowest (9 h light) value.

approx. 40% higher than those in the working muscles (soleus and adductor longus) at the start of the dark phase (Fig. 3). A similar distinction exists between working and non-working muscles after 6–48 h of starvation, with lower glycogen concentrations in the working muscles (Holness *et al.*, 1988; Holness & Sugden, 1991). Glycogen concentrations in the non-working muscles gradually increased during the dark phase, attaining peak values after 9 h of darkness (Fig. 3). Thus, as in the study of Leighton *et al.* (1988), glycogen concentrations in non-working muscles measured in the middle of the dark (feeding) phase are higher than at the start of the dark phase. However, the increment in glycogen concentration over the entire 9 h period of darkness was only comparable with that which can be achieved within 2 h of re-feeding after 48 h of starvation (Sugden *et al.*, 1990). Significant glycogen deposition was also observed in the working muscles during the dark phase, with peak values observed after 9 h of darkness (Fig. 3). These values were more than 2-fold higher than the lowest values, observed towards the end of the light phase, but again the increment in glycogen concentration was only comparable with that observed in response to 2 h of re-feeding after 48 h of starvation (Sugden *et al.*, 1990).

The increases in GUI values observed in EDL and tibialis anterior during the dark phase (Fig. 2) are quantitatively very comparable to the mean rates of net glycogen deposition in these muscles (approx. 4.0 and 4.7 ng of glucose equivalents/min per mg wet wt. in tibialis anterior and EDL respectively). In contrast, the increases in values of GUI observed in working muscles during the dark phase greatly exceeded (by approx. 3.5-fold) those required to accommodate the observed mean rates of glycogen deposition. This suggests that the increase in GUI values (and implied increase in glucose 6-phosphate production) observed in working muscles during the dark phase is not solely a consequence of stimulation of use of glucose 6-phosphate for glycogen deposition. The increase in glucose 6-phosphate production over and above that required for glycogen synthesis

suggests a change either in the proportion of glucose oxidized or in the fuel utilized.

Increased net glycogen deposition during the first 9 h of the dark phase is likely to be a consequence of stimulation of glycogen synthase by insulin (Fig. 1). In contrast, net glycogen loss was observed in all four muscles over the last 3 h of the dark phase (Fig. 3), although insulin concentrations remained high (Fig. 1). In working muscles, the decline in GUI values (approx. 8 to 12 ng glucose/min per mg) observed between 6 and 9 h of darkness may be a consequence of the cessation of net glycogen deposition through feedback inhibition. Alternatively, net glycogenolysis may compensate for decreased glucose uptake and phosphorylation. Net glycogen loss between 9 and 12 h of darkness in all four muscles may facilitate the significant increases in GUI values observed at the end of the dark phase and the early light phase by alleviating end-product inhibition of glycogen synthase.

Concluding remarks

The present study has demonstrated considerable variation in rates of glucose uptake and phosphorylation by skeletal muscle during the light and dark phases. There was an overall increase in skeletal muscle glucose utilization during the dark (feeding/activity) phase; this was associated with increased carbohydrate input via food consumption and may perform an important homeostatic function as glycaemia remains relatively constant.

A striking finding of the present study is the considerable (almost 3-fold) variation in rates of glucose uptake and phosphorylation by working muscles between the light and dark phases. Overall, the pattern of change in GUI values closely parallels that in voluntary food intake. However, even when the rates of glucose uptake and phosphorylation by working muscles are at their lowest, working muscles still continue to make the major contribution to glucose disposal when access to food is unrestricted. This contrasts to the first few hours of re-feeding after prolonged starvation (Sugden *et al.*, 1990), where GUI values in working and non-working muscles are more comparable. The increased contribution of non-working muscles to glucose clearance on re-feeding after starvation has been attributed to their increased capacity to deposit glycogen after prior glycogen depletion. The failure to observe comparably high GUI values in non-working muscles during unrestricted feeding presumably reflects the overall relative stability of glycogen concentrations.

An important distinction between unrestricted and intermittent feeding demonstrated in the present study is the relative importance of insulin-dependent glycogen synthesis as a component of total glucose utilization. The present results indicate that, when the food supply is uninterrupted, the major pathway of glucose 6-phosphate disposal is catabolic, rather than anabolic (glycogen synthesis), even though quite dramatic fluctuations in circulating insulin concentrations are observed.

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