

# Glucose utilization by interscapular brown adipose tissue *in vivo* during nutritional transitions in the rat

Mark J. HOLNESS,\* Yong-Ling LIU,\* John S. BEECH† and Mary C. SUGDEN\*‡

\*Department of Biochemistry, Faculty of Basic Medical Sciences, Queen Mary and Westfield College, Mile End Road, London E1 4NS, U.K., and †Cellular Mechanisms Research Group, Medical Unit, London Hospital, Whitechapel, London E1 1BB, U.K.

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Glucose utilization indices (GUI) of interscapular brown adipose tissue (IBAT) declined by 84% after 48 h starvation. Two-thirds of the overall response was observed within 6 h, correlating with decreased insulin concentrations. Re-feeding 48 h-starved rats restored insulin concentrations and evoked a rapid 15-fold increase in IBAT GUI. GUI values after re-feeding were markedly higher than those observed at equivalent insulin concentrations in control post-absorptive rats.

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## INTRODUCTION

The conservation of carbohydrate in response to food withdrawal is achieved by suppression of tissue glucose utilization. Although depletion of hepatic glycogen occurs within the first 6 h of starvation (Holness & Sugden, 1989), the major suppression of glucose utilization by skeletal muscle (the predominant site of glucose utilization by the body) occurs relatively late, after prolonged exposure to an increased lipid (fatty acid and ketone body) supply (Holness & Sugden, 1990b). A decline in the circulating concentration of insulin is observed before the period over which the most marked increases in fatty acid and ketone-body concentrations occur (McGarry *et al.*, 1973). Rapid suppression of glucose utilization in certain insulin-sensitive tissues in response to this decrease may be of physiological importance for whole-body glucose conservation during acute starvation before significant suppression of glucose utilization by the oxidation of lipid-derived fuels is observed. Brown adipose tissue has an exceptionally high capacity for glucose utilization (Cooney & Newsholme, 1982). Furthermore, its rate of glucose uptake and phosphorylation at high insulin concentrations can approach a value (approx. 500 ng/min per mg of tissue) which, if it were to be maintained in the post-absorptive state, could account for almost 20% of whole-body glucose turnover (Ferré *et al.*, 1986). In the present study we have assessed the potential importance of changes in brown-fat glucose utilization in whole-body glucose balance. Glucose utilization indices (GUIs) of interscapular brown adipose tissue (IBAT) were measured under conditions of varying carbohydrate supply, namely in the fed state, in the post-absorptive state, during progressive starvation and during the absorptive phase of feeding after starvation.

## MATERIALS AND METHODS

### Materials

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

### Rats and experimental procedures

The study of changes in GUI values in IBAT *in vivo* was conducted in conscious unrestrained female albino Wistar rats (200–250 g) on a 12 h-light/12 h-dark cycle (light from 08:00 h) housed in a room maintained at  $22 \pm 2$  °C. Each rat was fitted with an indwelling cannula [see Ferré *et al.* (1985) and Issad *et al.*

(1987) for details] at least 5 days before the experiment to permit full recovery from surgery. After this period, food intakes and weight gains were comparable with those of rats not subjected to surgery. The rats were either fed *ad libitum* on a standard rodent diet [supplied by E. Dixon & Sons (Ware) Ltd.; 52% digestible carbohydrate, 16% protein, 2% lipid and 30% non-digestible residue, all by weight], starved for periods of up to 48 h (food removal at the end of the dark phase of the light/dark cycle (08:00 h), or starved for 48 h and then re-fed on standard rodent diet *ad libitum*. Experiments in rats with continuous access to food *ad libitum* (controls) were completed before 11:00 h. The study of the effects of re-feeding was conducted in conjunction with measurement of values of GUI in skeletal muscles, the results of which have been published separately (Sugden *et al.*, 1990b). Chow intakes during re-feeding after starvation for 48 h are given in Sugden *et al.* (1990b). Details of the experimental procedures involved in the measurements of GUI values, blood glucose concentrations and plasma insulin concentrations are also given in Sugden *et al.* (1990b). Blood and IBAT were treated as described in Ferré *et al.* (1985). The GUI values were calculated as described by Issad *et al.* (1987) by dividing the radioactivity (d.p.m.) of the 2-deoxy[<sup>3</sup>H]glucose 6-phosphate in IBAT by the calculated integral of blood 2-deoxy[<sup>3</sup>H]glucose/[glucose]. Mean coefficients of variance of blood glucose concentrations over each 1 h period of sampling were < 15% in each instance (see Sugden *et al.* 1990b). As in Issad *et al.* (1987), values were not corrected for the discrimination factor (lumped constant) for deoxyglucose in glucose metabolic pathways (Ferré *et al.*, 1986). Results have been expressed on a wet-weight basis. Times of starvation and re-feeding given in relation to measurement of values of GUI refer to the time of injection of radiolabel rather than to the time of tissue sampling, since the uptake of radiolabel occurs predominantly over the first 20 min after injection (Sokoloff *et al.*, 1977; James *et al.*, 1985).

### 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 during the fed-to-starved transition

Values obtained for IBAT GUI in the fed state and during

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Abbreviations used: IBAT, interscapular brown adipose tissue; GUI, glucose utilization index; PDH, pyruvate dehydrogenase complex; PDH<sub>a</sub>, active form of PDH.

‡ To whom correspondence and reprint requests should be addressed.

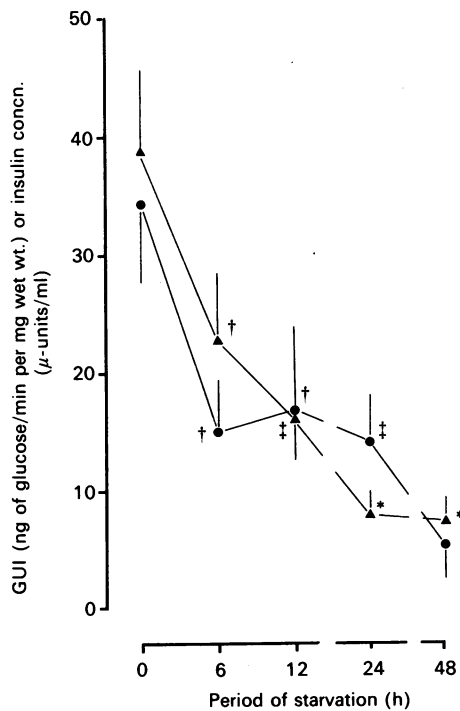


Fig. 1. Values of IBAT GUI (●) and insulin concentrations (▲) during progressive starvation

Full details of the experimental procedure are given in the Materials and methods section. Results are means  $\pm$  S.E.M. of 4–8 experiments. Statistically significant effects of starvation are indicated by: † $P < 0.05$ ; ‡ $P < 0.01$ ; \* $P < 0.001$ .

progressive starvation are shown in Fig. 1. The value of IBAT GUI in control rats (conscious rats with continual access to food *ad libitum*) of approx. 34 ng of glucose/min per mg is higher than that in anaesthetized post-absorptive (2 h-starved) rats (approx. 6 ng of glucose/min per mg; Ferré *et al.*, 1986), but similar to that in conscious rats previously fed on a high (69%) carbohydrate diet but starved for 5–7 h before sampling (Storlien *et al.*, 1986). The value of IBAT GUI observed in the control state is high and comparable with GUI values of diaphragm and working (postural) skeletal muscles (soleus and adductor longus) in resting rats under the identical nutritional condition, greatly exceeding the values of GUI in non-working muscles (see Holness & Sugden, 1990b; Sugden *et al.*, 1990b) and white adipose tissue (Issad *et al.*, 1987).

A marked decline in the value of GUI (to 44% of the initial control value) was observed in IBAT after starvation for 6 h (Fig. 1). IBAT GUI remained at this lower value for a further 18 h of starvation (Fig. 1). A subsequent modest decrease in the IBAT GUI was observed as the period of starvation was extended beyond 24 h, such that after 48 h starvation the value of GUI was only 16% of the initial control value (Fig. 1).

The decline in GUI observed during acute (6 h) starvation corresponds to 66% of the overall response to prolonged (48 h) starvation. This contrasts with the general pattern of the response to working skeletal muscles, which display only a minor proportion of the overall decrease in GUI elicited by prolonged starvation during the first 6 h of starvation (Holness & Sugden, 1990b). The marked and rapid restriction of glucose utilization in IBAT is consistent with the concept that a restraint on glucose utilization by brown fat may be essential for glucose conservation when carbohydrate is scarce. However, because of its small total mass (cf. skeletal muscle), brown fat is unlikely to make a major quantitative contribution to a decrease in whole-body glucose

utilization, in comparison with that of skeletal muscle. Assuming that the response of IBAT is typical of all the brown-adipose-tissue depots, total glucose utilization by all brown-fat depots in the control state is only 73  $\mu$ g/min (0.36 mg/min per kg body wt.), decreasing to 32  $\mu$ g/min (0.16 mg/min per kg body wt.) after 6 h starvation. (This calculation assumes the maximum reported total brown-adipose-tissue depot weight of 1.5 g in a 200 g rat and a conversion factor of deoxyglucose to glucose metabolic rate for brown fat of 0.7; see Ferré *et al.*, 1986). In the context of glucose-sparing, total differences in brown-fat glucose utilization between control and 6 h-starved rats can be calculated to be at most only approx. 0.2 mg/min per kg body wt., whereas whole-body glucose turnover in 3 h-fasted rats is approx. 18 mg/min per kg body wt. (Issad *et al.*, 1987).

Inactivation of muscle pyruvate dehydrogenase complex (PDH) occurs much more rapidly during starvation than does suppression of muscle glucose utilization. For example, cardiac PDH<sub>a</sub> inactivation precedes suppression of cardiac glucose utilization by up to 6 h (Sugden & Holness, 1990; Sugden *et al.*, 1990a); even longer transitional periods where high rates of glucose utilization are maintained, but the capacity for glucose oxidation is diminished, are observed in working skeletal muscles (Holness & Sugden, 1990b). The sequential suppression of pyruvate oxidation and glucose uptake and phosphorylation has been ascribed to the differential sensitivity of these pathways to increased lipid oxidation (Holness & Sugden, 1990b). In contrast, there is relatively co-ordinate suppression of the individual pathways of glucose utilization in IBAT during the initial 6 h of starvation: we have demonstrated previously that statistically significant effects of starvation on IBAT PDH<sub>a</sub> activities are first observed after starvation for 5 h, with inactivation of IBAT PDH<sub>a</sub> to 28% of fed value after 6 h (Holness & Sugden, 1990a).

#### IBAT GUI values after re-feeding

The provision of chow to rats previously starved for 48 h led to a dramatic increase in GUI in IBAT (Fig. 2). GUI values measured over the period from 1.9 to 2.9 h after re-feeding were

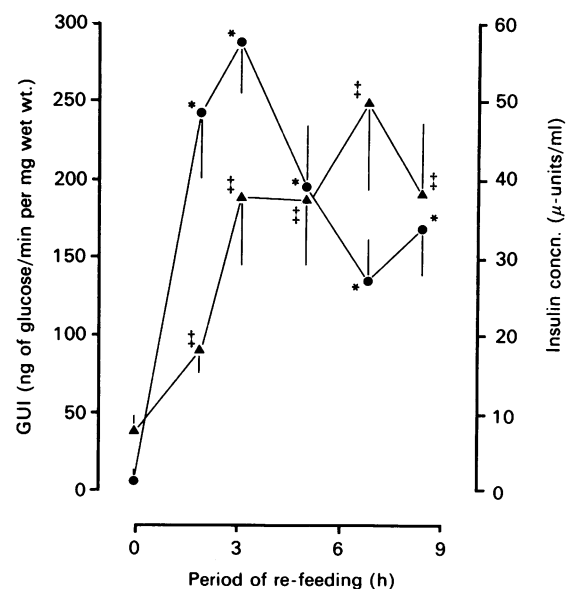


Fig. 2. Values of IBAT GUI (●) and insulin concentrations (▲) during re-feeding after 48 h starvation

Full details of the experimental procedure are given in the Materials and methods section. Note that the scale for GUI values is different from that in Fig. 1. Results are means  $\pm$  S.E.M. of 4–8 experiments. Statistically significant effects of re-feeding are indicated by: † $P < 0.05$ ; ‡ $P < 0.01$ ; \* $P < 0.001$ .

increased to approx. 250 ng/min per mg of tissue (Fig. 2), representing a 14.6-fold increase in IBAT GUI relative to the starved value and a 7-fold increase relative to the initial control value (compare Figs. 1 and 2). Very high IBAT GUI values continued to be observed over the period from 3.1 h to 8.5 h after re-feeding (Fig. 2). Although there was a 30–52% decline from peak values as the duration of re-feeding was extended, GUI values were still considerably higher than in the control (fed *ad libitum*) state even after re-feeding for more than 8 h (Fig. 2), and highest on a wet-weight basis of any tissue studied to date during this period (see Sugden *et al.*, 1990b). IBAT glucose utilization during the starved-to-fed transition may therefore be of more significance to whole-body glucose homeostasis during the starved-to-fed transition than during progressive starvation.

We have previously suggested that, contrary to the situation in the post-absorptive state, non-working muscles may assume a quantitatively significant role in glucose disposal during the initial phase of re-feeding (Sugden *et al.*, 1990b). This role becomes less significant as the period of re-feeding is extended and glycogen stores are repleted (Sugden *et al.*, 1990b). The pattern of the response of IBAT GUI resembles that of non-working skeletal muscles, but, because values of IBAT GUI are approx. 10-fold greater than those of non-working muscles (for details see Sugden *et al.*, 1990b), IBAT continues to contribute significantly to whole-body glucose disposal over the period from 5 to 9 h of re-feeding, when GUI values remain 4–6-fold greater than in the continually fed state.

#### GUI in relation to insulin concentrations during the fed-to-starved transition and after re-feeding

The initial major (44%) decline in GUI and IBAT over the first 6 h of starvation bears a good correlation with a 41% fall in the insulin concentration (Fig. 1). The results suggest that a fluctuation in the plasma insulin concentration may be a primary regulator of IBAT glucose uptake during the acute phase of starvation. If this is so, it may be inferred that IBAT glucose uptake and phosphorylation is sensitive to physiologically induced changes in insulin concentrations over a range of approx. 20–40  $\mu$ -units/ml (see Fig. 1). Whereas the insulin concentration falls to approx. 20% of the fed value within 24 h of the onset of starvation, IBAT GUI values after 6 h and 24 h of starvation are not significantly different from each other (Fig. 1). Furthermore, starvation for between 24 h and 48 h is associated with a significant decrease in IBAT GUI without any change in insulin concentration (Fig. 1). Consequently, the starvation-induced decrease in the value of IBAT GUI after 6 h bears little relation to changes in insulin concentrations.

Glucose utilization by IBAT can be suppressed by a period of exposure to a high-fat diet in the absence of a change in the insulin concentration (Storlien *et al.*, 1986). This effect has been attributed to the increased oxidation of lipid-derived fuels (Storlien *et al.*, 1986). It is therefore plausible to suggest that the increased availability and oxidation of fatty acids and ketone bodies may underlie the additional suppression of IBAT GUI

which is observed over the period from 24 h to 48 h after food withdrawal but which cannot be explained by a further decrease in circulating insulin.

The value of GUI in IBAT after re-feeding (Fig. 2) is comparable with that of post-absorptive (2 h-starved) rats after insulin-clamp (see Ferré *et al.*, 1986). However, although re-feeding was associated with increases in circulating insulin concentrations, insulin concentrations remained significantly lower than the fed value after re-feeding for at least 3 h (Fig. 2). The value of GUI after re-feeding for any given insulin concentration was as a consequence markedly higher than at a similar insulin concentration in the control or post-absorptive state. Although an elevation in insulin concentration may be permissive for any increase in IBAT GUI to be observed, these findings suggest that the exceptionally high values of GUI observed during the initial phase of re-feeding cannot be attributed directly to an increase in insulin alone. Mechanisms may involve meal-induced increases in blood flow (Glick *et al.*, 1984) or sympathetic stimulation (Rothwell *et al.*, 1982; Glick & Raum, 1986).

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