Glucose disposal by skeletal muscle in response to re-feeding after progressive starvation

Mark J. HOLNESS 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.

We investigated the extent to which increases in glucose utilization indices (GUIs) in individual skeletal muscles during chow re-feeding after 6 h, 24 h or 48 h starvation are related to the antecedent duration of starvation. Chow re-feeding after either acute or prolonged starvation led to an increase in glucose disposal by the muscle mass. Glucose intolerance after prolonged starvation was not associated with lower values of GUI in skeletal muscle. In both working and non-working muscles, the increment in GUI during the first 2 h of re-feeding was less after acute than after prolonged starvation. In non-working muscles the differential responses to re-feeding were due to higher GUI values after re-feeding rather than lower pre-prandial GUI values. Therefore the contribution of non-working muscles to glucose clearance is higher as the antecedent period of starvation is extended. Rates of glycogen deposition in non-working muscles after re-feeding were similar to absolute values of GUI, and a strong relationship existed between measured GUI values and rates of glycogen deposition.

INTRODUCTION

It is generally acknowledged that skeletal muscles collectively make a major contribution to insulin-stimulated glucose disposal within the body. However, rates of glucose disposal by individual muscles show considerable variation, depending both on their workload and on the precise nutritional condition. The cardio-thoracic and postural muscles exhibit high rates of glucose uptake and phosphorylation because they are constantly working, even in the resting (non-exercising) state (Issad *et al.*, 1987; Holness & Sugden, 1990): rates of glucose uptake and phosphorylation are low in these muscles only under conditions where lipid-derived fuels substitute for glucose as the primary energy substrate (Holness & Sugden, 1990; see also Issad *et al.*, 1987). Rates of glucose uptake and phosphorylation in non-working skeletal muscles are low in the fed state, even though fatty acids and ketone bodies are not available as alternative substrates (Issad *et al.*, 1987; Holness & Sugden, 1990). On the basis of this, it has been suggested that the contribution of non-working muscles to whole-body glucose utilization is relatively modest compared with that of working muscles (Ferré *et al.*, 1985; Pénicaud *et al.*, 1987; see also Issad *et al.*, 1987).

We have demonstrated recently that the relative contribution of non-working muscles to whole-body glucose utilization is significantly increased during the initial (2–4 h) period of re-feeding after prolonged starvation (Sugden *et al.*, 1990). Rates of glycogen deposition in individual muscles were similar to their rates of glucose uptake and phosphorylation (Sugden *et al.*, 1990). We therefore suggested that the predominant fate of glucose within these muscles might be storage as glycogen rather than glycolytic degradation (Sugden *et al.*, 1990). In the present study, we have investigated directly the extent to which initial increases in rates of glucose utilization in individual skeletal muscle are related to glycogen repletion by measuring glucose utilization indices (GUIs) during chow re-feeding after prior depletion of endogenous glycogen to varying extents by progressive starvation. The results provide insight into the factors governing insulin-stimulated glucose disposal by skeletal muscle

and the effect of the duration of starvation on the contribution of skeletal muscle to whole-body glucose utilization during re-feeding.

MATERIALS AND METHODS

Sources of materials were as in Holness & Sugden (1990). Wako Insulin B-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 08:30 h) were maintained on a diet of standard rodent chow (52% digestible carbohydrate, 16% protein, 2% lipid, 30% non-digestible residue; all by weight). Experiments using rats allowed continuous access to food *ad libitum* (termed fed) were completed before 11:00 h. Additional rats were starved for 6, 24 or 48 h, starting at 08:30 h (the end of the dark phase). Groups of starved rats were re-fed on standard rodent diet *ad libitum* for the periods specified. The chow intakes during re-feeding are shown in Table 1. Less food was consumed by the rats previously starved for only 6 h (see also Holness & Sugden, 1989). Water was provided *ad libitum* throughout.

Measurement of GUIs *in vivo*

Each rat was fitted with an indwelling cannula at 5–7 days before sampling (see Holness & Sugden, 1990, for details). The rats were conscious and unrestrained throughout the experiments. A tracer dose of 2-deoxy[³H]glucose (30 µCi) was injected through the indwelling cannula, and arterial blood (100 µl) was sampled 1, 3, 5, 10, 20, 40 and 60 min later for determination of blood glucose concentrations (with glucose oxidase) and 2-deoxy[³H]glucose radioactivity. Mean glucose concentrations and coefficients of variance of blood glucose concentration over each 1 h period of sampling are shown in Table 1. Rats were not sampled during the first 2 h of re-feeding, since steady-state conditions are not observed (see also Sugden *et al.*, 1990). However, since glucose is nearly at steady state during the period

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

* To whom correspondence and reprint requests should be addressed.

Table 1. Chow intakes and blood glucose concentrations during re-feeding after starvation for 6, 24 or 48 h

Details are given in the Materials and methods section. The blood glucose concentrations shown are the means of samples taken at 1, 3, 5, 10, 20, 40 and 60 min after injection of 2-deoxy[^3H]glucose. Mean coefficients of variance (%) of blood glucose are shown in parentheses. Results are means $\pm$ S.E.M. for 6–12 rats. Statistically significant effects of re-feeding are shown by: * $P < 0.05$; *** $P < 0.001$.

Starvation period (h)	Period of re-feeding (h)...	Chow intake (g)			
		0	2.0 $\pm$ 0.1	3.0 $\pm$ 0.1	5.0 $\pm$ 0.1
6	–	–	1.6 $\pm$ 0.1	2.8 $\pm$ 0.2	2.7 $\pm$ 0.5
24	–	–	4.4 $\pm$ 0.6	4.9 $\pm$ 0.5	8.6 $\pm$ 1.8
48	–	–	5.1 $\pm$ 0.7	5.0 $\pm$ 0.6	7.2 $\pm$ 0.7
Starvation period (h)	Period of re-feeding (h)...	Blood glucose concn. (mM)			
		0	2.0 $\pm$ 0.1	3.0 $\pm$ 0.1	5.0 $\pm$ 0.1
6	–	5.8 $\pm$ 0.3 (10.3)	5.3 $\pm$ 0.2 (8.9)	5.1 $\pm$ 0.2 (9.5)	5.0 $\pm$ 0.3 (10.5)
24	–	4.5 $\pm$ 0.2 (10.9)	5.6 $\pm$ 0.4* (14.5)	5.6 $\pm$ 0.5 (9.6)	5.8 $\pm$ 0.7 (12.5)
48	–	3.7 $\pm$ 0.3 (11.5)	5.8 $\pm$ 0.6* (11.1)	6.3 $\pm$ 0.4*** (15.5)	6.3 $\pm$ 0.3*** (12.0)

from 2 to 6 h after re-feeding (coefficients of variance of blood glucose concentrations over each 1 h period are less than approx. 15%; Table 1), measurements of GUI over this period are valid (see also Sugden *et al.*, 1990).

At the termination of each experiment (60 min after injection of radiolabel), sodium pentobarbital (60 mg/kg body wt.) was injected via the indwelling cannula. An additional 500 μl blood sample was taken for the measurement of plasma insulin concentrations (Wako Insulin B-test kit), and muscles were excised and freeze-clamped after 5 min exposure to sodium pentobarbital (see Holness *et al.*, 1988). The fibre profiles of the skeletal muscles selected for study are given in Holness & Sugden (1990). Blood and muscles were treated as described in Ferré *et al.* (1985), and rates of glucose uptake and phosphorylation are calculated as GUIs, no attempt being made to correct for possible discrimination between glucose and deoxyglucose in terms of tissue uptake and phosphorylation (Sugden *et al.*, 1990; see also Issad *et al.*, 1987).

Assay of glycogen

Glycogen was extracted and estimated 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 the numbers of rats specified.

RESULTS AND DISCUSSION

Insulin and glucose concentrations after re-feeding

Plasma insulin concentrations were decreased from the fed value (38.7 $\mu\text{-units/ml}$) after even very-short-term (6 h) starvation (Fig. 1; see also Holness *et al.*, 1991). Insulin concentrations were significantly increased by re-feeding with chow *ad libitum*, but only re-attained the values for animals fed *ad libitum* after 4 h of re-feeding (Fig. 1). Importantly, plasma insulin concentrations during the period from 2 to 6 h after the provision of chow were not significantly affected by the antecedent period of starvation (Fig. 1). Therefore, although chow intakes during re-feeding

were lower after 6 h starvation, and glycaemia was higher if the starvation period preceding re-feeding was extended (Table 1), it is possible to make direct comparisons between the groups in terms of changes in glucose utilization elicited by a physiological elevation of plasma insulin.

Response of skeletal-muscle GUI to re-feeding after progressive starvation

GUI values in individual skeletal muscles are shown in Tables 2 (working muscles) and 3 (non-working muscles). There was a progressive effect of starvation to decrease GUI values (see also Holness & Sugden, 1990).

GUI values in representative working (postural) skeletal muscles were restored to those for animals fed *ad libitum* within 2 h of increases in plasma insulin concentrations elicited physiologically by chow re-feeding (Table 2). Increments in values of

Fig. 1. Plasma insulin concentrations during chow re-feeding after starvation for 6 h (▲), 24 h (●) or 48 h (◆)

Details are given in the Materials and methods section. Results are means $\pm$ S.E.M. for 6–12 rats. Statistically significant effects of re-feeding are shown by: † $P < 0.05$; ‡ $P < 0.01$.

Table 2. GUI values measured in soleus and adductor longus during chow re-feeding after starvation for 6, 24 or 48 h

Details are given in the Materials and methods section. GUI values in soleus and adductor longus for fed animals were 38.8 ± 6.7 (14) and 30.3 ± 4.1 (14) ng of glucose/min per mg respectively. Other results are means $\pm$ S.E.M. for 6–12 rats. Statistically significant effects of starvation are shown by: $\dagger P < 0.05$; $\dagger\dagger P < 0.01$; $\dagger\dagger\dagger P < 0.001$. Statistically significant effects of re-feeding are shown by: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Muscle	Starvation period (h)	Period of re-feeding (h)...	GUI (ng of glucose/min per mg wet wt.)			
			0	2.0 $\pm$ 0.1	3.0 $\pm$ 0.1	5.0 $\pm$ 0.1
Soleus	6		27.9 $\pm$ 5.3	39.3 $\pm$ 3.4	39.3 $\pm$ 3.6	43.2 $\pm$ 7.9
	24		21.3 $\pm$ 2.1 $\dagger$	37.9 $\pm$ 3.4**	34.9 $\pm$ 3.4**	33.9 $\pm$ 6.1
	48		9.0 $\pm$ 3.1 $\dagger\dagger$	33.9 $\pm$ 4.5***	48.4 $\pm$ 5.8***	49.7 $\pm$ 5.4***
Adductor longus	6		25.4 $\pm$ 3.9	31.0 $\pm$ 2.5	35.1 $\pm$ 2.5*	37.8 $\pm$ 4.2*
	24		11.4 $\pm$ 2.1 $\dagger\dagger$	33.8 $\pm$ 3.2***	26.7 $\pm$ 3.1**	31.1 $\pm$ 4.5**
	48		4.3 $\pm$ 1.2 $\dagger\dagger\dagger$	34.0 $\pm$ 5.7***	39.1 $\pm$ 5.8***	43.2 $\pm$ 5.4***

Table 3. GUI values measured in tibialis anterior, EDL and gastrocnemius during chow re-feeding after starvation for 6, 24 or 48 h

Details are given in the Materials and methods section. GUI values in tibialis anterior, EDL and gastrocnemius for fed animals were 4.3 ± 0.7 (14), 4.4 ± 0.5 (14) and 3.6 ± 0.4 (14) ng of glucose/min per mg respectively. Other results are means $\pm$ S.E.M. for 6–12 rats. Statistically significant effects of starvation are shown by: $\dagger\dagger P < 0.01$. Statistically significant effects of re-feeding are shown by: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Muscle	Starvation period (h)	Period of re-feeding (h)...	GUI (ng of glucose/min per mg wet wt.)			
			0	2.0 $\pm$ 0.1	3.0 $\pm$ 0.1	5.0 $\pm$ 0.1
Tibialis anterior	6		5.4 $\pm$ 0.9	7.0 $\pm$ 1.2	7.2 $\pm$ 0.6	5.2 $\pm$ 0.8
	24		4.5 $\pm$ 1.0	13.2 $\pm$ 1.9**	15.2 $\pm$ 2.4**	8.7 $\pm$ 1.1*
	48		2.4 $\pm$ 0.7	23.0 $\pm$ 3.8**	19.2 $\pm$ 2.9***	16.3 $\pm$ 2.0***
EDL	6		4.3 $\pm$ 0.7	8.7 $\pm$ 1.3*	7.3 $\pm$ 0.8*	5.3 $\pm$ 0.5
	24		3.7 $\pm$ 0.6	16.8 $\pm$ 2.6***	17.7 $\pm$ 2.5***	9.1 $\pm$ 1.5**
	48		2.0 $\pm$ 1.3	22.5 $\pm$ 2.9**	21.5 $\pm$ 2.9***	15.6 $\pm$ 1.6***
Gastrocnemius	6		3.0 $\pm$ 0.6	6.4 $\pm$ 1.1*	5.5 $\pm$ 0.7*	6.5 $\pm$ 1.1*
	24		2.4 $\pm$ 0.6	6.2 $\pm$ 1.2*	7.5 $\pm$ 0.9***	8.8 $\pm$ 1.6**
	48		1.1 $\pm$ 0.4 $\dagger\dagger$	8.1 $\pm$ 1.3**	10.8 $\pm$ 1.3***	9.9 $\pm$ 0.9***

GUI observed during the first 2 h of re-feeding were rather less after short-term than long-term starvation (Table 2), mainly because of differences in basal (pre-prandial) values of GUI (Table 2). GUI values were maintained at approximately those for animals fed *ad libitum* throughout the remainder of the re-feeding period (Table 2).

GUI values in representative non-working muscles were also significantly increased in response to chow re-feeding (Table 3). Increments in GUI values observed after 2 h of chow re-feeding were, however, dramatically increased if the starvation period preceding re-feeding was prolonged (Table 3): GUI was increased 1.3–2.1-fold in rats previously starved for 6 h, compared with 2.6–4.5-fold in 24 h-starved rats and 7.4–11.3-fold in 48 h-starved rats. The effects of prior starvation on the initial responses of GUI in non-working muscles to re-feeding predominantly arise because of major differences between the absolute values of GUI obtained after re-feeding (rather than to the minor differences existing between pre-prandial values).

In contrast with the situation in working muscles, absolute GUI values in non-working muscles greatly exceeded corresponding values for animals fed *ad libitum* if the starvation period preceding re-feeding was prolonged (Table 3). The effect of starvation was particularly dramatic in tibialis anterior and extensor digitorum longus (EDL), where GUI values for animals fed *ad libitum* were exceeded by 1.6–2.0-fold in rats previously starved for 6 h, 3.1–3.8-fold in rats previously starved for 24 h and 5.2–5.3-fold in rats previously starved for 48 h. GUI values gradually declined towards those for animals fed *ad libitum* as

the period of re-feeding was extended (Table 3). An essentially similar pattern to that observed in tibialis and EDL was observed in gastrocnemius, except that the magnitude of the effect of starvation on the response to re-feeding was somewhat less (Table 3).

The results indicate that the absolute contribution of working muscles to whole-body glucose utilization during the period from 2 to 6 h after re-feeding is largely independent of the preceding period of starvation. Consequently, under conditions where insulin concentrations are relatively high, the contribution of working muscles to whole-body glucose turnover does not vary greatly. In contrast, glucose utilization in non-working muscles after re-feeding is critically dependent on the preceding period of starvation, with a significantly increased absolute and relative contribution of non-working muscles to glucose clearance if the starvation period preceding re-feeding is extended.

The highly variable responses of GUI values in non-working muscles to re-feeding after starvation for different periods cannot be ascribed to different circulating insulin concentrations (Fig. 1). Since energy requirements of non-working muscles are constant, the variable response cannot be ascribed to an altered energy demand. We have previously commented on the apparent correlation between GUI values and initial rates of glycogen deposition in non-working muscles on re-feeding after prolonged (48 h) starvation (Sugden *et al.*, 1990). We therefore more closely evaluated the relationship between the pre-prandial glycogen concentration, the initial rate of glycogen deposition and the rate of glucose utilization in individual muscles.

Table 4. Skeletal-muscle glycogen concentrations during starvation and after re-feeding

Experimental details are given in the Materials and methods section. Results are means $\pm$ S.E.M. for 6–12 rats. Statistically significant effects of starvation are shown by: $\dagger P < 0.05$; $\ddagger P < 0.01$; $\ddagger\ddagger P < 0.001$. Statistically significant effects of re-feeding are shown by: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Rats	Glycogen concn. (mg/g wet wt.)				
	Soleus	Adductor longus	Tibialis anterior	EDL	Gastrocnemius
Fed	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
6 h starved	5.3 $\pm$ 0.4	3.6 $\pm$ 0.3	6.3 $\pm$ 0.2 $\dagger$	5.7 $\pm$ 0.2 $\ddagger\ddagger$	7.2 $\pm$ 0.3 $\ddagger\ddagger$
6 h starved/2 h re-fed	5.6 $\pm$ 0.2	5.8 $\pm$ 0.8*	6.9 $\pm$ 0.4	6.4 $\pm$ 0.2*	6.5 $\pm$ 0.9
24 h starved	3.0 $\pm$ 0.2 $\ddagger\ddagger\ddagger$	2.1 $\pm$ 0.3 $\ddagger\ddagger$	4.2 $\pm$ 0.4 $\ddagger\ddagger\ddagger$	3.7 $\pm$ 0.4 $\ddagger\ddagger\ddagger$	6.7 $\pm$ 0.3 $\ddagger\ddagger\ddagger$
24 h starved/2 h re-fed	6.5 $\pm$ 0.8**	4.9 $\pm$ 0.4***	6.0 $\pm$ 0.6*	5.8 $\pm$ 0.6*	7.6 $\pm$ 0.4
48 h starved	2.8 $\pm$ 0.3 $\ddagger\ddagger\ddagger$	2.6 $\pm$ 0.3 $\dagger$	5.5 $\pm$ 0.4 $\ddagger\ddagger$	4.5 $\pm$ 0.3 $\ddagger\ddagger\ddagger$	6.5 $\pm$ 0.4 $\ddagger\ddagger\ddagger$
48 h starved/2 h re-fed	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

Fig. 2. Glycogen deposition rates during the first 2 h of chow re-feeding after starvation for 6 h ($\blacktriangle$, $\triangle$), 24 h ($\bullet$, $\circ$) or 48 h ($\blacklozenge$, $\lozenge$)

Details are given in the Materials and methods section and in the text. Mean rates of net glycogen deposition are calculated from the data shown in Table 4 for working muscles (soleus and adductor longus) as black symbols and for non-working muscles (tibialis anterior, EDL and gastrocnemius) as white symbols. The points lie on a line ($y = -5.67x + 41.94$), with $r = 0.86$.

Regulatory role of muscle glycogen in insulin-stimulated glucose disposal in skeletal muscle

The depletion of skeletal-muscle glycogen elicited by starvation for 6, 24 or 48 h is shown in Table 4. Glycogen concentrations in soleus and adductor longus were 56% and 76% of the fed value respectively after starvation for 48 h (Table 4). The major glycogen losses occurred between 6 and 24 h of starvation (Table 4). Significant glycogen loss from non-working muscles occurred within 6 h of starvation, but again the major decreases in glycogen concentrations occurred between 6 and 24 h (Table 4).

The relationship between the pre-prandial glycogen concentration and the estimated mean net rate of glycogen deposition over an initial 2 h period of chow re-feeding (calculated from the data shown in Table 4) is shown for working and non-working skeletal muscles in Fig. 2. Overall, there is an inverse relationship between the estimated initial rate of glycogen deposition and the fasting glycogen concentration. This may be a consequence of

the regulatory effect of the prevailing glycogen concentration on glycogen synthase activity (Stalmans & Hers, 1973).

Relationship between initial rates of glycogen deposition and GUI values during re-feeding

Initial rates of glycogen deposition in non-working muscles were comparable with absolute values of GUI in rats starved for 24 or 48 h before re-feeding (equivalent to 104–121% of GUI in 24 h-starved/re-fed rats and 83–87% of GUI in 48 h-starved/re-fed rats). In rats starved for only 6 h before re-feeding, a considerably lower percentage of total GUI could be accounted for by glycogen deposition. These findings suggest that the increased capacity for glucose uptake and phosphorylation by non-working muscles in response to re-feeding after more prolonged starvation is determined primarily by their increased capacities for insulin-stimulated glycogen synthesis.

In contrast with the situation in non-working muscles, initial rates of glycogen deposition in working muscles accounted for a very variable proportion of absolute values of GUI. The fate of hexose phosphate is therefore correspondingly variable, and is not necessarily glycogen deposition. However, when rates of glycogen deposition are compared with the increase in GUI values elicited by re-feeding, it can be calculated that (except for the soleus at 6 h, where glycogen deposition accounts for only 17% of the increment in GUI) at least 76% of the additional glucose that is phosphorylated is converted into glycogen.

General discussion

The physiological elevation of the plasma insulin concentration elicited by chow re-feeding after either acute or prolonged starvation leads to an increase in glucose disposal by the muscle mass. There is no indication that the glucose intolerance observed on re-feeding after 48 h starvation (Table 1) is associated with lower values of GUI in skeletal muscle. Glucose intolerance after prolonged starvation cannot therefore be attributed to peripheral insulin resistance at the level of skeletal-muscle glucose uptake and phosphorylation or glycogen synthesis. The ability of non-working skeletal muscles to facilitate peripheral glucose clearance through increased rates of glucose uptake and phosphorylation is greatly facilitated if a prolonged period of starvation precedes re-feeding. The starvation-dependent variation in the contribution of non-working muscles to whole-body glucose turnover appears to be related to the capacity for use of hexose phosphate as a precursor for glycogen synthesis. However, the increase in glycogen deposition rate evoked by extending the antecedent period of starvation beyond 24 h cannot be explained by more extensive prior glycogen depletion (see Table 4). It is therefore

implied that additional regulatory mechanisms operate to facilitate glycogen deposition during re-feeding. Such mechanisms may include starvation-induced increases in muscle insulin sensitivity (Le Marchand-Brustel & Freychet, 1979; Brady *et al.*, 1981; Stirewalt *et al.*, 1985) or the effects of hyperglycaemia to promote muscle glycogen deposition (Felber *et al.*, 1988).

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REFERENCES

- Brady, L. J., Goodman, M. N., Kalish, F. N. & Ruderman, N. B. (1981) *Am. J. Physiol.* **240**, E184–E190
- Felber, J. P., Golay, A., Felley, C. & Jequier, E. (1988) *Diabetes/Metab. Rev.* **4**, 691–700
- Ferré, P., Leturque, A., Burnol, A.-F., Pénicaud, L. & Girard, J. (1985) *Biochem. J.* **228**, 103–110
- Holness, M. J. & Sugden, M. C. (1989) *Biochem. J.* **258**, 529–533
- Holness, M. J. & Sugden, M. C. (1990) *Biochem. J.* **270**, 245–249
- Holness, M. J., Schuster-Bruce, M. J. L. & Sugden, M. C. (1988) *Biochem. J.* **254**, 855–859
- Holness, M. J., Liu, Y.-L., Beech, J. S. & Sugden, M. C. (1991) *Biochem. J.* **273**, 233–235
- Issad, T., Pénicaud, L., Ferré, P., Kandé, J., Baudon, M.-A. & Girard, J. (1987) *Biochem. J.* **246**, 241–244
- Keppler, D. & Decker, K. (1974) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1127–1131, Academic Press, New York and London
- Le Marchand-Brustel, Y. & Freychet, P. (1979) *J. Clin. Invest.* **64**, 1505–1515
- Pénicaud, L., Ferré, P., Kandé, A., Leturque, A., Issad, T. & Girard, J. (1987) *Am. J. Physiol.* **252**, E365–E369
- Stalmans, W. & Hers, H. G. (1973) in *The Enzymes* (Boyer, P. D., ed.), pp. 309–361, Academic Press, New York
- Stirewalt, W. S., Low, R. B. & Slaiby, J. M. (1985) *Biochem. J.* **227**, 355–362
- Sugden, M. C., Liu, Y.-L. & Holness, M. J. (1990) *Biochem. J.* **272**, 133–137

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