Changes in rates of glucose utilization and regulation of glucose disposal by fast-twitch skeletal muscles in late pregnancy

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Glucose utilization indices (GUI) were measured *in vivo* in conjunction with active pyruvate dehydrogenase complex (PDH_a) and glycogen synthase (GS) activities in fast-twitch skeletal muscles [extensor digitorum longus (EDL), tibialis anterior and gastrocnemius] of late-pregnant rats and age-matched virgin control rats in the fed state, after 24 h starvation and at 2 h after re-feeding with standard laboratory chow *ad libitum* after 24 h starvation. As demonstrated previously [Holness and Sugden (1990) Biochem. J **277**, 429–433], GUI values of fast-twitch skeletal muscles of virgin rats were low in the fed *ad libitum* and the 24 h-starved states, but dramatically increased after subsequent chow re-feeding. GUI values of fast-twitch skeletal muscles of late-pregnant rats were also low in the fed and starved states and were increased by re-feeding, but the increase in GUI values elicited by re-feeding was greatly attenuated. PDH_a activities in EDL, tibialis anterior and gastrocnemius in the fed state were unaffected by late pregnancy, and skeletal-muscle PDH_a activities were decreased after 24 h of starvation in both groups. Whereas re-feeding of virgin rats with standard diet for 2 h restored PDH_a activities in fast-twitch skeletal muscles to values for rats continuously fed *ad libitum*, PDH_a activities in fast-twitch skeletal muscles of late-pregnant rats, although increased in response to re-feeding, remained considerably less than the corresponding fed *ad libitum* values after 2 h of re-feeding. In contrast, neither skeletal-muscle GS re-activation nor rates of skeletal-muscle glycogen deposition after re-feeding were markedly affected by late pregnancy. The results are discussed in relation to the specific targeting of individual pathways of glucose disposal in fast-twitch skeletal muscles during re-feeding in late pregnancy.

INTRODUCTION

Although fast-twitch skeletal muscles account for approx. 60% of the total skeletal muscle mass (Pénicaud et al., 1987), these muscles exhibit relatively low rates of glucose uptake/phosphorylation (and therefore make a relatively minor contribution to whole-body glucose turnover) in the continuously fed state (Ferré et al., 1985; Issad et al., 1987; Holness and Sugden, 1990). Under these conditions the major contribution to whole-body glucose turnover rate is made by slow-twitch muscles (Issad et al., 1987; Holness and Sugden, 1990). In contrast, fast-twitch muscles can make a dramatically enhanced contribution to whole-body glucose utilization during re-feeding after starvation, when their rates of glucose utilization are high and comparable with those of slow-twitch muscles (Sugden et al., 1990; Holness and Sugden, 1991).

The extent to which glucose uptake/phosphorylation by individual fast-twitch skeletal muscles is enhanced during re-feeding after starvation shows a positive correlation with rates of net glycogen deposition (Holness and Sugden, 1991). In view of a clear inverse relationship between initial rates of glycogen deposition in individual fast-twitch skeletal muscles during chow re-feeding *ad libitum* and the extent of skeletal-muscle glycogen depletion during the preceding period of starvation (Holness and Sugden, 1991), we proposed that the enhanced capacity of fast-twitch skeletal muscles for glucose uptake/phosphorylation during re-feeding after extended starvation reflects the increased potential for insulin-stimulated glycogen deposition secondary to the greater degree of prior glycogen depletion. Other studies have demonstrated that retention or sparing of skeletal-muscle glycogen leads to insulin resistance at the level of glucose clearance (uptake/phosphorylation and non-oxidative disposal) by skeletal muscle during hyperinsulinaemic–euglycaemic clamp (reviewed by Felber, 1989; Sugden and Holness, 1990).

Insulin resistance is encountered in late pregnancy both in man and in experimental animals (Leturque et al., 1984; Hauguel et al., 1987). Studies in the unconscious (anaesthetized) rat have shown that an important component of insulin resistance in late pregnancy is impaired stimulation of glucose uptake/phosphorylation in fast-twitch skeletal muscles (Leturque et al., 1986; Hauguel et al., 1987). The molecular basis for gestational insulin resistance in relation to skeletal-muscle glycogen storage during feeding and fasting has not been elucidated. However, there is evidence from studies *in vitro* with isolated muscle preparations (Leturque et al., 1981) that insulin resistance at the level of glucose uptake/phosphorylation in peripheral tissues *in vivo* in late pregnancy may involve circulating factors (absent from experiments *in vitro*) that modulate insulin action downstream from receptor binding and signalling. Since the increased availability and oxidation of lipids can spare skeletal-muscle glycogen (Rennie et al., 1976; see also Felber, 1989), and the circulating concentrations of lipid-derived fuels are elevated in late pregnancy (Scow et al., 1964; Otway and Robinson, 1968; Knopp et al., 1970; Girard et al., 1977; Holness et al., 1991), a causal relationship between lipid-fuel utilization, skeletal-muscle glycogen sparing and peripheral insulin resistance in late pregnancy would appear to be a strong possibility worthy of investigation.

In the present experiments, we have investigated the regulation of glucose uptake/phosphorylation during late (19 days) pregnancy in fast-twitch skeletal muscles *in vivo*. Glucose utilization index (GUI) values were determined in muscles from conscious

Abbreviations used: EDL, extensor digitorum longus; GS, glycogen synthase; GS_a, glucose 6-phosphate-independent GS; GS_t, total GS; GUI, glucose utilization index; NEFA, non-esterified fatty acids, PDH, the pyruvate dehydrogenase complex; PDH_a, the active form of PDH.

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unrestrained rats sampled in the fed *ad libitum* state, after 24 h starvation and during re-feeding with chow *ad libitum* after 24 h starvation. These three nutritional conditions were selected as they are associated with nutrient-induced fluctuations in plasma insulin concentrations and do not involve artificial manipulation of circulating factors (such as lipid fuels) that may regulate fast-twitch skeletal-muscle glucose utilization. Because of increasing evidence that control of skeletal-muscle glucose uptake/phosphorylation can be exerted by modulation of insulin action at post-transport sites, we examined the activity of the insulin-regulated enzyme glycogen synthase (GS) in relation to measurements of glycogen concentrations in fast-twitch skeletal muscles during late pregnancy. In addition, since glucose oxidation is stringently regulated at the level of the pyruvate dehydrogenase complex (PDH), which is sensitive to changes in rates of lipid-fuel oxidation (Fuller and Randle, 1984; French et al., 1988; Holness and Sugden, 1990), we also measured the active form of PDH (PDH_a) and assessed changes in fast-twitch skeletal-muscle PDH_a activities in relation to changes in the circulating concentrations of non-esterified fatty acids (NEFA) and ketone bodies.

EXPERIMENTAL

Materials

Sources of materials are as given in Holness and Sugden (1990). Enzymes, coenzymes and substrates and kits for measurements of blood glucose concentrations were purchased from Boehringer Mannheim, Lewes, East Sussex, U.K., or Sigma Chemical Co., Poole, Dorset, U.K. 2-Deoxy[1-^{3}H]glucose and UDP-D-[U-^{14}C]-glucose (UDP-[^{14}C]glucose) were purchased from Amersham International, Amersham, Bucks., U.K. Kits for measurements of plasma insulin and NEFA concentrations were purchased from Alpha Laboratories, Eastleigh, Hants., U.K. Standard rodent diet was purchased from Special Diets Services, Witham, Essex, U.K.

Animals

Age-matched female albino Wistar rats (from Charles River) maintained at 20 ± 2 °C on a 12 h-light/12 h-dark cycle (light from 10:00 h) were used for the study. Rats were studied at 19–20 days of pregnancy (term is 22–24 days). The duration of pregnancy was estimated as described by Lederman and Rosso (1981). The average number of foetuses per rat was 13 (range 10–19), with no statistically significant differences between the groups of rats sampled. Body weights of virgin and pregnant rats were: control, 226 ± 5 g (30); pregnant, 357 ± 3 g (36). Virgin and late-pregnant rats were permitted free access to standard rodent chow (52% carbohydrate, 15% protein, 3% fat and 30% non-digestible residue; all by weight), or starved for 24 h, or starved for 24 h before subsequent free access to standard rodent chow. Chow intakes over the first 2 h re-feeding period were 7.0 ± 1.2 g and 6.3 ± 1.0 g respectively in virgin and late-pregnant rats.

Measurement of skeletal-muscle GUI *in vivo*

Details of the experimental protocol used for measurement of GUI values are given in detail in Ferré et al. (1985), Issad et al. (1987) and Holness and Sugden (1990). Values of the correction factor for the discrimination against 2-deoxyglucose in glucose metabolic pathways in fast-twitch muscles are close to unity (Ferré et al., 1985; Pénicaud et al., 1987) and not significantly affected by late pregnancy (Leturque et al., 1986). GUI values in control and late-pregnant rats can thus be equated with rates of glucose uptake/phosphorylation and compared directly. Each rat was fitted with an indwelling cannula, under Hypnorm [fentanyl citrate (0.315 mg/ml)/fluanisone (10 mg/ml); 1 ml/kg intraperitoneally] and Diazepam (5 mg/ml; 1 ml/kg intraperitoneally) anaesthesia. Rates of 2-deoxy[^{3}H]glucose uptake/phosphorylation were measured in unrestrained conscious rats at 5–7 days after cannulation. Each measurement was initiated by the injection of a 30 μCi tracer dose of 2-deoxy[^{3}H]glucose via the indwelling cannula. Blood (100 μl) was collected via the cannula at 1, 3, 5, 10, 20, 40 and 60 min after injection of radiolabel. Mean glucose concentrations for blood sampled over the 1 h period of the experiment are shown in Figure 2. Coefficients of variance of blood glucose concentrations were less than 15% (results not shown). A terminal (500 μl) blood sample was taken after 1 h for measurement of the plasma insulin and NEFA concentrations and blood ketone-body (3-hydroxybutyrate plus acetoacetate) concentrations. The experiment was then terminated by injection of sodium pentobarbital (60 mg/kg body wt.) through the indwelling cannula. Blood and skeletal-muscle samples were treated as described by Ferré et al. (1985). GUI values in individual muscles were measured over 1 h periods. Since experiments using 2-deoxyglucose are heavily weighted towards effects occurring during the first 15 min after administration of the bolus of radiolabel, when blood 2-deoxyglucose specific radioactivity, and therefore rates of 2-deoxyglucose uptake/phosphorylation, are highest (Sokoloff et al., 1977; James et al., 1985), times of re-feeding given in Figure 1 refer to the times of injection of radiolabelled 2-deoxyglucose.

Enzyme activities

Enzyme activities were measured in extracts of freeze-clamped muscles obtained in a parallel series of experiments. Rats were sampled while under sodium pentobarbital anaesthesia (5 min; 60 mg/kg body wt.) such that locomotor activity had ceased. In these experiments the re-feeding period specified in Tables 1 and 2 refers to the time of sampling.

GS

GS activities were assayed at pH 6.8 in the absence and presence of glucose 6-phosphate as described by Nimmo et al. (1976), except that BSA (1 mg/l) and dithiothreitol (5 mM) were included in the enzyme diluent. One unit of enzyme activity is the amount of enzyme that incorporates 1 μmol of [^{14}C]glucose into glycogen/min. The GS activity ratio is defined as the activity in the absence of glucose 6-phosphate divided by that in its presence.

PDH_a

PDH complex (active form, PDH_a) and citrate synthase activities were measured in freeze-clamped muscle extracts as described previously (Caterson et al., 1982) except that Triton X-100 (2.5%, w/v) and protease inhibitors [benzamidine (1 mM), leupeptin (10 μM), tosyl-lysylchloromethane ('TLCK'; 0.3 mM)] were included in the extraction medium (see Stace et al., 1992). PDH_a activities are expressed relative to citrate synthase to correct for any differences in extraction efficiencies between groups. A unit of PDH_a or citrate synthase activity is defined as that which converts 1 μmol of substrate into product/min at 30 °C.

Metabolite measurements

Glycogen

Individual skeletal muscles were freeze-clamped in liquid N_2 while rats were under sodium pentobarbital anaesthesia (5 min; 60 mg/kg body wt., intraperitoneally) such that locomotor activity had ceased. Glycogen concentrations were determined, without prior precipitation with ethanol, as glucose after hydrolysis with amyloglucosidase (Keppler and Decker, 1974).

Circulating metabolites

Blood glucose concentrations were measured in samples obtained during the GUI measurements by a glucose oxidase kit. Total ketone-body concentrations were measured in KOH-neutralized $HClO_4$ extracts of the terminal blood samples (Williamson et al., 1962). NEFA and insulin were estimated in plasma obtained from the terminal blood samples by using kits.

Statistics

Statistical significance of differences was assessed by Student's unpaired t test, and results are given as means ± S.E.M. for the numbers of rats specified.

RESULTS

The fast-twitch skeletal muscles selected for the present study were EDL, tibialis anterior and gastrocnemius. The first two muscles contain approx. 66% and 59% fast-twitch-oxidative fibres and 32% and 38% fast-twitch-glycolytic fibres respectively, whereas gastrocnemius contains 37% fast-twitch-oxidative fibres and 58% fast-twitch-glycolytic fibres (Ariano et al., 1973).

GUI values in fast-twitch skeletal muscles in late pregnancy

GUI values in EDL, tibialis anterior and gastrocnemius after 24 h starvation and at intervals after re-feeding with chow *ad libitum* are shown for virgin and late-pregnant rats in Figure 1. GUI values in skeletal muscles of virgin and late-pregnant rats permitted continuous access to food *ad libitum* and sampled within 3 h of the end of the dark period (termed fed *ad libitum*) are shown for comparison. As demonstrated previously (Holness et al., 1991), late pregnancy was associated with significant suppression of GUI values in fast-twitch muscles in the fed state (Figure 1).

As demonstrated previously (Ferré et al., 1985; Pénicaud et al., 1987; Holness and Sugden, 1990), GUI values in fast-twitch skeletal muscles of virgin rats were relatively low in both the fed *ad libitum* and the 24 h-starved states. There was a dramatic enhancement of GUI values in all three fast-twitch skeletal muscles in response to chow re-feeding *ad libitum* after 24 h starvation in the virgin controls. The highest GUI values were observed over the first 2–4 h of chow re-feeding. GUI values were increased by 5.9-fold ($P < 0.001$), 5.1-fold ($P < 0.001$) and 3.4-fold ($P < 0.01$) within 2 h of food administration in EDL, tibialis anterior and gastrocnemius, representing increases in rates of glucose utilization of 15.2, 14.6, and 5.1 ng/min per mg wet wt. respectively. As the re-feeding period extended beyond 4 h, there was a gradual decline in GUI values towards those observed in the continuously fed state.

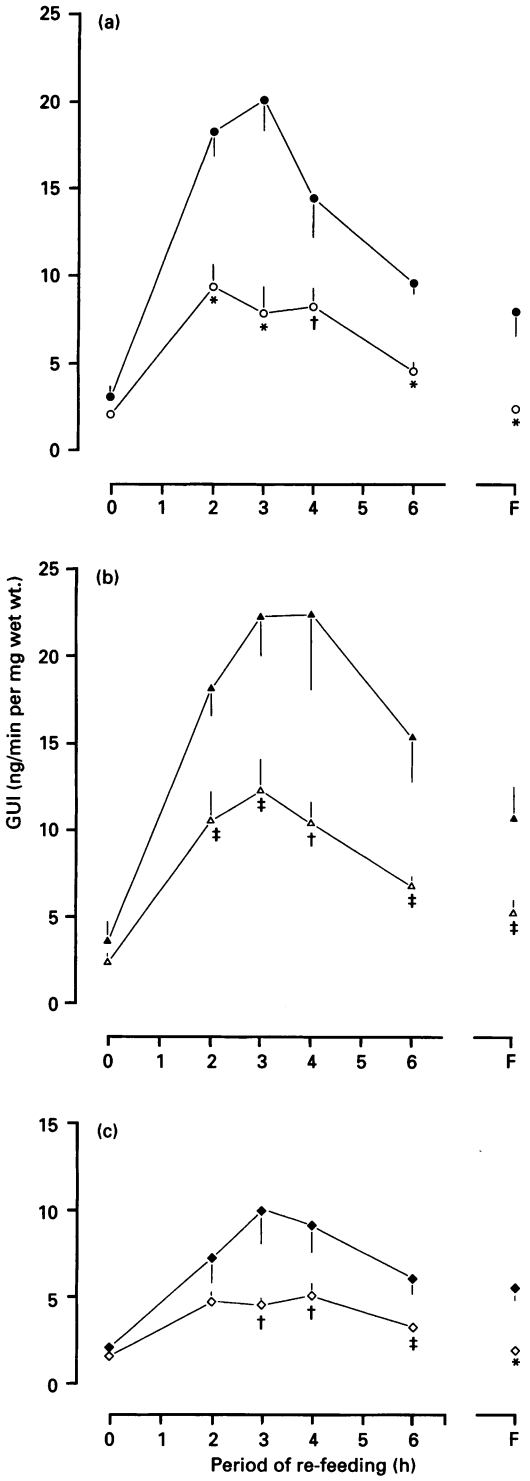

Figure 1 GUI values in EDL (a) tibialis anterior (b) and gastrocnemius (c) skeletal muscles of virgin or late-pregnant rats during chow re-feeding *ad libitum* after 24 h starvation

Full details are given in the Materials and methods section. GUI values are shown for EDL (●, ○), tibialis anterior (▲, △) and gastrocnemius (◆, ◇) skeletal muscles of virgin (●, ▲, ◆) or late-pregnant (○, △, ◇) rats sampled after 24 h starvation and at intervals during chow re-feeding *ad libitum* after 24 h starvation. GUI values in the skeletal muscles of rats sampled in the fed *ad libitum* state (F) are also shown. Results are means ± S.E.M. for three to nine rats. Statistically significant effects of chow re-feeding *ad libitum* on GUI values were observed in all three skeletal muscles in both virgin and late-pregnant rats at every time point studied ($P < 0.01$ in each case). Statistically significant effects of late pregnancy are shown: †$P < 0.05$; ‡$P < 0.01$; *$P < 0.001$.

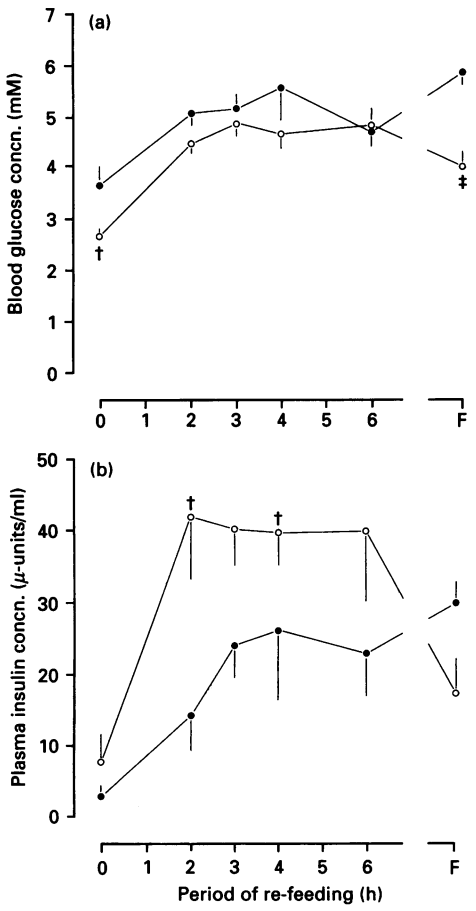

Figure 2 Blood glucose (a) and plasma insulin (b) concentrations of virgin or late-pregnant rats during chow re-feeding *ad libitum* after 24 h starvation

Full details are given in the Materials and methods section. Blood glucose and plasma insulin concentrations in virgin (●) or late-pregnant (○) rats in the 24 h-starved state, during chow re-feeding *ad libitum* after 24 h starvation and in the fed *ad libitum* state (F) are shown (**a**) and (**b**) respectively. Results are means ± S.E.M. for three to nine rats. Statistically significant effects of chow re-feeding *ad libitum* on blood glucose concentrations were observed at 2 and 3 h after re-feeding in virgin rats ($P < 0.05$) and at 2, 3, 4 and 6 h in late-pregnant rats ($P < 0.001$). Statistically significant effects of chow re-feeding *ad libitum* on plasma insulin concentrations were observed at 3 h ($P < 0.01$) and 6 h ($P < 0.05$) after re-feeding in virgin rats and at 2 h ($P < 0.01$), 3 h, 4 h (both $P < 0.001$) and 6 h ($P < 0.05$) in late-pregnant rats. Statistically significant effects of late pregnancy are shown: †$P < 0.05$; ‡$P < 0.01$.

GUI values in EDL, tibialis anterior and gastrocnemius muscles of late-pregnant rats in the 24 h-starved states were also low and were not significantly different from those measured in virgin rats (Figure 1). As for virgin rats, significant ($P < 0.001$) increases in GUI values were observed in all three fast-twitch skeletal muscles of late-pregnant rats within 2 h of chow re-feeding (Figure 1). Again, GUI values in fast-twitch skeletal muscles of late-pregnant rats during the first 3–4 h of re-feeding exceeded those observed in late-pregnant rats permitted continuous access to chow *ad libitum*. As in virgin rats, GUI values in fast-twitch skeletal muscles of re-fed late-pregnant rats subsequently declined (Figure 1).

Despite the marked and highly significant ($P < 0.01$) increases in GUI values observed at 2 h after chow re-feeding in late pregnancy in EDL and tibialis anterior, GUI values after 2 h of chow re-feeding were nevertheless significantly lower ($P < 0.01$) than those in EDL and tibialis anterior muscles of virgin controls (Figure 1). Consequently the increases in rates of glucose utilization in EDL and tibialis anterior of late-pregnant rats observed after 2 h of re-feeding were only 48% and 55% respectively of the corresponding increases observed in virgin rats. GUI values in gastrocnemius measured at 2 h after chow re-feeding were not significantly different in late-pregnant rats compared with virgin controls; however, statistically significant effects of pregnancy to suppress GUI values in gastrocnemius were observed at 3 h, 4 h and 6 h after re-feeding ($P < 0.05$). The effect of late pregnancy to attenuate stimulation of GUI values in fast-twitch skeletal muscles was not a consequence of any altered pattern of chow intake (results not shown), nor an altered response of GUI values to chow re-feeding (Figure 1).

In summary, although late pregnancy had no statistically significant effect on GUI values in EDL, tibialis anterior and gastrocnemius muscles measured after 24 h of starvation (when rates are relatively low), significant effects of late pregnancy to suppress GUI values in these fast-twitch skeletal muscles were revealed during chow re-feeding after 24 h starvation, i.e. over the period where GUI values are enhanced (Figure 1).

Blood glucose and plasma insulin concentrations after starvation and during subsequent re-feeding in late pregnancy

Increases in blood glucose and plasma insulin concentrations are important aspects of the regulation of rates of glucose uptake/phosphorylation by skeletal muscle during the first 2 h of chow re-feeding after starvation (see, e.g., Holness and Sugden, 1991; Sugden et al., 1990). In the present experiments, the provision of chow *ad libitum* to previously starved virgin rats led to a significant (39%; $P < 0.05$) increase in peripheral-blood glucose concentrations after 2 h (Figure 2a). Blood glucose concentrations after this re-feeding period in virgin rats were 13% lower ($P < 0.05$) than those observed in virgin rats permitted continuous access to food *ad libitum* (Figure 2a). After 4 h of re-feeding, glucose concentrations were not statistically significantly different from values for animals fed *ad libitum* (Figure 2a). The provision of chow *ad libitum* to previously starved late-pregnant rats also elicited a significant (68%; $P < 0.001$) increase in peripheral-blood glucose concentrations within 2 h of re-feeding (Figure 2a). Blood glucose concentrations after 2 h of re-feeding in pregnant rats were 12% higher ($P > 0.05$) than those observed in late-pregnant rats permitted continuous access to food *ad libitum* (Figure 2a). Elevated blood glucose concentrations were maintained over the entire 6 h re-feeding period (Figure 2a). Whereas peripheral-blood glucose concentrations in the fed state and after 24 h starvation were significantly lower in late-pregnant rats than in virgin rats (Figure 2a), blood glucose concentrations after re-feeding were not significantly different between virgin and late-pregnant rats (Figure 2a).

Peripheral-plasma insulin concentrations in 24 h-starved virgin rats were 10% of those found in virgin rats continuously fed *ad libitum* (Figure 2b). Re-feeding elicited a significant ($P < 0.005$) 4.9-fold increase in plasma insulin concentrations within 2 h (Figure 2b). Plasma insulin concentrations continued to rise as the re-feeding period was extended, and after 3–4 h of re-feeding reached values not statistically significantly different from those observed in rats fed *ad libitum* (Figure 2b; see also Holness and Sugden, 1991). Plasma insulin concentrations in 24 h-starved late-pregnant rats were 44% of those for pregnant rats fed *ad libitum* (Figure 2b). There was a dramatic ($P < 0.01$; 5.5-fold) increase in the plasma insulin concentration within 2 h of re-feeding in the late-pregnant group (Figure 2b). Furthermore, in contrast with the response of plasma insulin to chow re-feeding

Table 1 Skeletal-muscle glycogen metabolism after starvation and subsequent chow re-feeding in late-pregnant and virgin rats

Results are means ± S.E.M. for the numbers of rats shown in parentheses. Total GS activities were unaffected by nutritional status or by late pregnancy and were (means ± S.E.M., in units/g wet wt. for the numbers of rats in parentheses): EDL, control 0.74 ± 0.05 (14), pregnant 0.69 ± 0.05 (13); tibialis anterior, control 1.00 ± 0.07 (15), pregnant 0.93 ± 0.10 (14); gastrocnemius, control 0.73 ± 0.09 (15), pregnant 0.67 ± 0.05 (14). Statistically significant effects of late pregnancy are shown by †$P < 0.05$. Statistically significant effects of starvation are shown by ‡$P < 0.05$, ‡‡$P < 0.01$, ‡‡‡$P < 0.001$. Statistically significant effects of re-feeding are shown by *$P < 0.05$, **$P < 0.01$.

	Virgin			Pregnant		
Nutritional status...	Starved (24 h)	Re-fed (2 h)	Fed	Starved (24 h)	Re-fed (2 h)	Fed
GS						
EDL						
GS_a (unit/g wet wt.)	0.31 ± 0.03 (6)	0.41 ± 0.03* (5)	0.38 ± 0.06 (3)	0.25 ± 0.03 (5)	0.43 ± 0.06* (5)	0.34 ± 0.03 (3)
% GS_a	37.0 ± 7.2‡ (6)	58.9 ± 6.2* (5)	56.3 ± 2.4 (3)	40.5 ± 5.6 (5)	59.8 ± 5.8* (5)	52.8 ± 2.6 (3)
Tibialis anterior						
GS_a (unit/g wet wt.)	0.35 ± 0.03 (7)	0.40 ± 0.03 (5)	0.47 ± 0.06 (3)	0.36 ± 0.05 (6)	0.46 ± 0.05 (5)	0.40 ± 0.06 (3)
% GS_a	35.8 ± 4.3‡ (7)	44.5 ± 3.8 (5)	57.7 ± 5.5 (3)	38.3 ± 5.3 (6)	49.7 ± 3.6 (5)	50.9 ± 5.8 (3)
Gastrocnemius						
GS_a (unit/g wet wt.)	0.17 ± 0.02 (7)	0.26 ± 0.04 (5)	0.29 ± 0.08 (3)	0.17 ± 0.03‡‡ (6)	0.30 ± 0.04* (5)	0.43 ± 0.05 (3)
% GS_a	24.9 ± 3.3‡ (7)	38.7 ± 4.7* (5)	43.5 ± 6.3 (3)	30.1 ± 4.0‡‡‡ (6)	43.0 ± 3.9* (5)	54.9 ± 1.8 (3)
Glycogen concn. (mg/g)						
EDL	3.9 ± 0.4‡‡‡ (6)	5.4 ± 0.3** (10)	6.0 ± 0.2 (8)	4.2 ± 0.4‡‡‡ (10)	5.5 ± 0.4* (11)	7.6 ± 0.5† (18)
Tibialis anterior	4.4 ± 0.4‡‡ (6)	6.3 ± 0.3** (10)	6.9 ± 0.7 (8)	4.5 ± 0.4‡‡‡ (10)	6.0 ± 0.3* (14)	7.9 ± 0.4 (18)
Gastrocnemius	6.2 ± 0.6 (6)	6.6 ± 0.3 (10)	7.4 ± 0.4 (7)	5.6 ± 0.4‡‡‡ (10)	6.8 ± 0.4* (14)	8.2 ± 0.4 (18)

in virgin rats, plasma insulin concentrations in late-pregnant rats exceeded (by 2.5-fold; $P < 0.05$) the fed *ad libitum* value after the first 2 h of re-feeding (Figure 2b). Plasma insulin concentrations in late-pregnant rats were maintained over the subsequent 2–6 h of re-feeding. Whereas insulin concentrations in the fed state were significantly lower in late-pregnant than in virgin rats, insulin concentrations measured at 2–3 h after the provision of chow *ad libitum* after 24 h starvation were significantly ($P < 0.05$) higher in late-pregnant rats (Figure 2b), even though peripheral glucose concentrations in virgin and late-pregnant rats were similar (Figure 2a).

Skeletal-muscle glycogen metabolism after starvation and during subsequent re-feeding in late pregnancy

A good correlation between enhanced GUI values and net glycogen deposition has been observed in fast-twitch skeletal muscles of virgin rats over the initial phase of chow re-feeding after starvation (Sugden et al., 1990; Holness and Sugden, 1991). Since the magnitude of the rise in GUI values in fast-twitch skeletal muscles elicited by chow re-feeding was greatly attenuated in late-pregnant rats, it was of obvious importance to examine whether there was an associated impairment of skeletal-muscle glycogen synthesis and deposition. Glycogen synthase is a key regulatory enzyme for glycogen synthesis: glucose 6-phosphate-independent glycogen synthase (GS_a) and the percentages of total glycogen synthase (GS_t; measured in the presence of 10 mM glucose 6-phosphate) present as GS_a are shown for the three individual fast-twitch skeletal muscles in Table 1. Skeletal-muscle glycogen concentrations were also measured (Table 1).

GS_a activities and the percentages of GS_t present as GS_a (% GS_a) in fast-twitch skeletal muscles of 24 h-starved virgin rats (Table 1) were similar to those observed previously in quadriceps muscles of 24 h-starved (virgin) rats (Kruszynska and McCormack, 1989). In the present study, the percentages of GS_a in all three fast-twitch skeletal muscles of virgin rats were significantly (57–66%; $P < 0.05$) lower in the 24 h-starved than in the fed *ad libitum* state (Table 1). GS_t activities were unaffected by the nutritional status (Kruszynska and McCormack, 1989; see also the legend to Table 1). The glycogen contents of EDL and tibialis anterior muscles of virgin rats were depleted after 24 h of starvation to approx. 65% of those observed in the fed *ad libitum* state ($P < 0.01$; Table 1); the glycogen concentration in gastrocnemius did not decrease significantly in response to 24 h starvation (Table 1). There was a consistent effect of chow re-feeding *ad libitum* after 24 h starvation to increase GS_a activities and % GS_a in all three fast-twitch skeletal muscles (Table 1). In two of the three skeletal muscles (EDL and gastrocnemius) the effects of 24 h starvation on GS_a activity and % GS_a were reversed within 2 h of re-feeding ($P < 0.05$), and a trend towards reversal was observed in the third skeletal muscle (tibialis anterior) (Table 1). In addition, chow re-feeding *ad libitum* of 24 h-starved virgin rats for 2 h significantly ($P < 0.01$) increased glycogen concentrations in EDL and tibialis anterior, reversing the starvation-induced decreases in glycogen concentrations. Net rates of glycogen deposition in EDL, tibialis anterior and gastrocnemius muscles of virgin rats (estimated from the change in glycogen concentration observed after 2 h of re-feeding) were 12.5, 15.8 and 3.3 ng of glycosyl units/min per mg respectively.

GS_a activities and % GS_a in fast-twitch skeletal muscles of fed late-pregnant rats were not significantly different from those present in skeletal muscles of fed virgin rats (Table 1). Neither the decreases in skeletal-muscle GS_a activities evoked by 24 h starvation nor the increases in skeletal-muscle GS_a activities observed in response to 2 h re-feeding after 24 h starvation were significantly influenced by late pregnancy (Table 1). Skeletal-muscle glycogen concentrations in late-pregnant rats were significantly ($P < 0.001$) decreased by 24 h starvation to approx. 55–68% of the corresponding fed *ad libitum* values. Re-feeding chow *ad libitum* for 2 h increased ($P < 0.05$ versus starved) glycogen concentrations in these skeletal muscles to approx. 72–83% of their respective (late-pregnant) fed *ad libitum* values. Initial rates of net glycogen deposition in EDL and tibialis anterior were 10.8 and 12.5 ng of glucose equivalents/min per mg, approx. 14–21% lower than corresponding rates in the re-

Table 2 Skeletal-muscle PDH_a activities in relation to plasma NEFA and blood ketone-body concentrations after starvation and subsequent chow re-feeding in late-pregnant and virgin rats

Results are means ± S.E.M. for the numbers of rats shown in parentheses. Statistically significant effects of late pregnancy are shown by †$P < 0.05$, ††$P < 0.01$. Statistically significant effects of starvation are shown by ‡$P < 0.05$, ‡‡$P < 0.01$, ‡‡‡$P < 0.001$. Statistically significant effects of re-feeding are shown by *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Abbreviations: C.S., citrate synthase.

	Virgin			Pregnant		
Nutritional status...	Starved (24 h)	Re-fed (2 h)	Fed	Starved (24 h)	Re-fed (2 h)	Fed
Plasma NEFA (mM)	0.69 ± 0.10‡‡ (3)	0.12 ± 0.06** (4)	0.16 ± 0.04 (7)	0.76 ± 0.07‡‡‡ (7)	0.42 ± 0.11*† (7)	0.36 ± 0.06† (10)
Blood ketone bodies (mM)	1.11 ± 0.19‡‡ (3)	0.22 ± 0.08** (5)	0.11 ± 0.04 (5)	2.58 ± 0.26‡‡‡†† (7)	0.10 ± 0.03*** (6)	0.33 ± 0.09† (9)
PDH_a (m-units/unit of C.S.)						
EDL	3.6 ± 1.4‡‡ (4)	15.3 ± 3.0** (5)	15.1 ± 2.9 (5)	1.4 ± 0.1‡ (4)	3.5 ± 1.0†† (6)	15.7 ± 5.1 (8)
Tibialis anterior	2.8 ± 1.3‡‡ (4)	21.4 ± 3.7** (5)	16.8 ± 2.6 (5)	2.2 ± 0.7‡‡‡ (4)	4.5 ± 1.0‡‡ (6)	20.2 ± 2.9 (8)
Gastrocnemius	2.6 ± 1.3 (5)	7.2 ± 0.8* (4)	16.9 ± 3.3 (5)	2.4 ± 1.1‡‡‡ (4)	3.4 ± 1.0† (6)	22.9 ± 3.7 (8)
C.S. (units/g wet wt.)						
EDL	25.9 ± 2.4 (4)	25.4 ± 1.2 (5)	26.4 ± 0.8 (5)	22.7 ± 1.8 (4)	23.5 ± 1.8 (6)	25.1 ± 1.0 (7)
Tibialis anterior	31.0 ± 2.8 (4)	32.5 ± 4.0 (5)	33.5 ± 2.4 (5)	24.0 ± 3.6 (4)	27.7 ± 2.7 (6)	32.7 ± 2.2 (7)
Gastrocnemius	19.6 ± 3.7 (5)	25.4 ± 3.1 (4)	22.3 ± 1.8 (5)	20.3 ± 2.7 (4)	22.6 ± 2.2 (6)	20.3 ± 2.4 (7)

fed virgin controls. The initial rate of net glycogen deposition in gastrocnemius was approx. 10.0 ng of glucose equivalents/min per mg, 3-fold higher than in the re-fed virgin control. Skeletal-muscle glycogen concentrations in the fed *ad libitum* state tended to be higher in late-pregnant than in virgin rats, a statistically significant effect of late pregnancy to increase skeletal-muscle glycogen concentrations being observed in EDL (27% increase; $P < 0.05$).

In summary, rates of net glycogen deposition in EDL and tibialis anterior over the first 2 h of re-feeding were somewhat lower (by approx. 20%) in late-pregnant rats than in virgin rats. This decline is neither a consequence of the retention of a higher concentration of muscle glycogen after starvation [high concentrations of glycogen inhibit GS re-activation via dephosphorylation (Villar-Palasi and Larner, 1966; Stalmans and Hers, 1973; see also Danforth, 1965)] nor of impaired re-activation of GS. Rates of glycogen deposition in gastrocnemius were not decreased by pregnancy.

Fast-twitch skeletal-muscle PDH_a activities in relation to plasma NEFA and ketone-body concentrations in late pregnancy

In addition to glucose and insulin, lipid fuels are important regulators of skeletal-muscle glucose uptake/phosphorylation (French et al., 1988; Jenkins et al., 1988; Holness and Sugden, 1990), oxidation (Fuller and Randle, 1984; French et al., 1988; Holness and Sugden, 1990) and non-oxidative glucose disposal (Thiébaud et al., 1982). Suppression of glucose uptake/phosphorylation in skeletal muscle during starvation in virgin rats is, in part, secondary to the increased oxidation of NEFA and ketone bodies, the circulating concentrations of which are substantially increased (Holness et al., 1989; Holness and Sugden, 1990; see also Table 2). In addition, there is evidence that PDH_a inactivation in skeletal muscle during starvation is a direct consequence of enhanced rates of lipid oxidation (Caterson et al., 1982; Fuller and Randle, 1984; Holness et al., 1989; Holness and Sugden, 1990).

Plasma NEFA and blood ketone-body concentrations after 24 h starvation and at 2 h after re-feeding in virgin and late-pregnant rats are shown in Table 2. Late pregnancy was associated with significant (approx. 2–3-fold) increases in plasma NEFA and ketone-body concentrations in the fed state: in addition, blood ketone-body concentrations after 24 h starvation were 2.3-fold higher in late-pregnant than in virgin rats (Table 2). The effects of 24 h starvation to increase circulating NEFA and ketone-body concentrations were reversed within 2 h of re-feeding in virgin rats. Reversal of starvation-induced increases in NEFA and ketone-body concentrations was also evoked by re-feeding in late-pregnant rats, but, importantly, plasma NEFA concentrations were significantly higher in late-pregnant rats than in the virgin controls, not only in the fed state but also at 2 h after re-feeding, where insulin concentrations were substantially elevated (Table 2).

PDH_a activities in fast-twitch skeletal muscles of virgin and late-pregnant rats in the fed state, after 24 h of starvation and at 2 h after chow re-feeding after starvation, are shown in Table 2. PDH_a activities in EDL, tibialis anterior and gastrocnemius muscles of virgin rats in the fed state were similar to those reported previously (Hennig et al., 1975; Caterson et al., 1982; Holness et al., 1989). PDH_a activities in EDL, tibialis anterior and gastrocnemius muscles of virgin rats declined significantly (by 76%, 83% and 85% respectively; $P < 0.01$ in each case) in response to 24 h starvation. The effects of 24 h starvation on PDH_a activities were completely reversed within 2 h of re-feeding in EDL and tibialis anterior muscles of virgin rats; there was also significant ($P < 0.05$) enhancement of PDH_a activities in gastrocnemius muscles of virgin rats over the 2 h re-feeding period (Table 2).

PDH_a activities in EDL, tibialis anterior and gastrocnemius muscles were unaffected by late pregnancy in the fed *ad libitum* state (Table 2). As was the case in virgin rats, starvation for 24 h led to a significant (in this case approx. 90%; $P < 0.05$) decline in PDH_a activities in these three fast-twitch skeletal muscles. Only minor (not statistically significant) increases in skeletal-muscle PDH_a activities were observed after 2 h of re-feeding in late-pregnant rats (to 15–22% of corresponding fed *ad libitum* values; see Table 2). Thus, after 2 h of chow re-feeding, fast-twitch skeletal-muscle PDH_a activities in late-pregnant rats remained substantially lower than corresponding values for the 2 h-re-fed virgin controls (Table 2). The attenuated responses of fast-twitch skeletal-muscle PDH_a activities to acute re-feeding after 24 h starvation in late pregnancy could not be attributed to changes in the activities of citrate synthase (Table 2), a mitochondrial marker enzyme which is suitable for use as a reference

enzyme to assess the efficacy of mitochondrial extraction (Stace et al., 1990, 1992; Denyer et al., 1989).

DISCUSSION

We have examined the effects of late pregnancy on glucose uptake/phosphorylation and glucose disposal via glycogen biosynthesis (via GS) or oxidation (via PDH) in a selection of fast-twitch skeletal muscles. Emphasis has been placed on the response to chow re-feeding *ad libitum* after 24 h starvation, since it is under these conditions that fast-twitch skeletal muscles make their major contribution to glucose disposal (see the Introduction, and Sugden et al., 1990; Holness and Sugden, 1991).

Insulin resistance at the level of glucose uptake/phosphorylation in EDL and epitrochlearis (fast-twitch skeletal muscles) and white adipose tissue has been demonstrated previously in late pregnancy in the post-absorptive state under the conditions of constant intravenous insulin infusion and variable continuous intravenous glucose infusion, i.e. the hyperinsulinaemic–euglycaemic clamp (Leturque et al., 1986; Hauguel et al., 1987; Gilbert et al., 1991). The present experiments were performed under the more physiologically relevant condition of natural feeding of a mixed diet. The 2-fold higher plasma insulin concentrations observed after 2 h of chow re-feeding in late pregnancy were able to normalize both glycaemia and ketonaemia (Figure 2a and Table 2). In contrast, stimulation of glucose utilization (uptake/phosphorylation) by fast-twitch skeletal muscles was greatly impaired (Figure 1).

A decreased capacity to utilize glucose 6-phosphate for glycogen synthesis underlies the less pronounced stimulation of glucose utilization by fast-twitch skeletal muscles observed on re-feeding after acute compared with prolonged starvation (Holness and Sugden, 1991). Although modest suppression of glycogen deposition in EDL and tibialis anterior was observed over the first 2 h of re-feeding in late pregnancy (Table 1), this is quantitatively inadequate to account for the profound attenuation of stimulation of glucose utilization observed in these fast-twitch skeletal muscles. Even more strikingly, suppression of GUI values in gastrocnemius muscle during re-feeding is observed concomitantly with an enhanced estimated initial rate of glycogen deposition. Clearly, factors in addition to or other than any impairment of glycogen synthesis contribute to diminished glucose uptake/phosphorylation by fast-twitch skeletal muscles during re-feeding in late pregnancy. In addition, since glycogen deposition does not decline in parallel with the suppression of glucose uptake/phosphorylation, the metabolic changes associated with late pregnancy would appear to target individual sites of insulin action. Regulation of glucose disposal distal to glucose uptake/phosphorylation is clearly indicated in the present study, where pyruvate oxidation (but not glycogen deposition) is specifically suppressed in late pregnancy.

It has been demonstrated that lipid substrates (fatty acids derived from circulating or endogenous triacylglycerol; NEFA or ketone bodies) are oxidized in preference to glucose by muscle (the glucose/fatty acid cycle; see Randle et al., 1964). Furthermore, increased availability of circulating lipid substrates (Groop et al., 1991) or of endogenous triacylglycerol (Storlein et al., 1991) is associated with skeletal-muscle insulin resistance at the level of glucose utilization. The failure to observe insulin resistance at the level of glucose utilization with isolated muscle preparations from late-pregnant rats (Leturque et al., 1981) indicates that factors other than the increased accumulation of endogenous (muscle) triacylglycerol underlies skeletal-muscle insulin resistance in late pregnancy. Plasma NEFA (but not ketone-body) concentrations remained elevated at 2 h after re-feeding in late pregnancy (Table 2). An enhanced circulating NEFA supply can evoke suppression of glucose uptake/phosphorylation in cardiothoracic and skeletal muscles *in vivo* (Holness and Sugden, 1990). It is therefore plausible that enhanced oxidation of NEFA by fast-twitch skeletal muscles after re-feeding might underly the suppression of glucose utilization observed in late pregnancy: even though fast-twitch skeletal muscles are conventionally regarded as users of circulating glucose or endogenous glycogen as energy substrates, their oxidative capacities are not negligible (see Winder et al., 1974; Holloszy, 1973, 1975).

Fatty acid oxidation suppresses muscle PDH_a activity *in vitro* (Randle et al., 1964) and *in vivo* (Holness et al., 1989; Holness and Sugden, 1990). The failure to evoke rapid PDH_a re-activation in fast-twitch skeletal muscles by 2 h re-feeding in late pregnancy (Table 2) indicates that pyruvate is not the primary oxidative substrate under these conditions. It is plausible that increased utilization of NEFA as oxidative substrates is responsible for suppression both of glucose utilization and of PDH re-activation during re-feeding in late pregnancy.

We gratefully acknowledge financial support from the British Heart Foundation and the U.K. Medical Research Council. We thank Professor P. Cohen and Dr. P. J. Parker for helpful advice regarding the assay of glycogen synthase in muscle extracts. M.J.H. is a Drummond Research Fellow.

REFERENCES

Ariano, M. A., Armstrong, R. B. and Egerton, V. R. (1973) J. Histochem. Cytochem. **21**, 51–55
Caterson, I. D., Fuller, S. D. and Randle, P. J. (1982) Biochem. J. **208**, 53–60
Danforth, W. H. (1965) J. Biol. Chem. **240**, 588–593
Denyer, G. S., Lam, D., Cooney, G. J. and Caterson, I. D. (1989) FEBS Lett. **250**, 464–468
Felber, J. P. (1989) in Diabetes 1988 (Larkins, R., Zimmet, P. and Chisholm, D., eds.), pp. 171–174, Elsevier/North-Holland, Amsterdam
Ferré, P., Leturque, A., Burnol, A.-F., Pénicaud, L. and Girard, J. (1985) Biochem. J. **228**, 103–110
French, T. J., Goode, A. W., Holness, M. J., MacLennan, P. A. and Sugden, M. C. (1988) Biochem. J **256**, 935–939
Fuller, S. J. and Randle, P. J. (1984) Biochem. J. **219**, 635–646
Gilbert, M., Pere, M. C., Baudelin, A. and Battaglia, F. C. (1991) Am. J. Physiol. **260**, E938–E945
Girard, J., Ferré, P., Gilbert, M., Kervran, A., Assan, R. and Marliss, E. B. (1977) Am. J. Physiol. **232**, E456–E463
Groop, L. C., Saloranta, C., Shank, M., Bonadonna, R. C., Ferrannini, E. and DeFronzo, R. A. (1991) J. Clin. Endocrinol. Metab. **72**, 96–107
Hauguel, S., Gilbert, M. and Girard, J. (1987) Am. J. Physiol. **252**, E165–E169
Hennig, G., Loffler, G. and Wieland, O. H. (1975) FEBS Lett. **59**, 142–145
Holloszy, J. O. (1973) in Exercise and Sport Sciences Reviews (Wilmore, J., ed.), pp. 45–71, Academic Press, New York
Holloszy, J. O. (1975) Med. Sci. Sports **7**, 155–164
Holness, M. J. and Sugden, M. C. (1990) Biochem. J. **270**, 245–249
Holness, M. J. and Sugden, M. C. (1991) Biochem. J. **277**, 429–433
Holness, M. J., Liu, Y.-L. and Sugden, M. C. (1989) Biochem. J. **264**, 771–776
Holness, M. J., Changani, K. K. and Sugden, M. C. (1991) Biochem. J. **280**, 549–552
Issad, T., Pénicaud, L. Ferré, P., Kandé, J., Baudon, M.-A. and Girard, J. (1987) Biochem. J. **246**, 241–244
James, D. E., Kraegen, E. W. and Chisholm, D. J. (1985) Am. J. Physiol. **248**, E575–E580
Jenkins, A. B., Storlein, L. H., Chisholm, D. J. and Kraegen, E. W. (1988) J. Clin. Invest. **82**, 293–299
Keppler, D. and Decker, K. (1974) in Methods in Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1127–1131, Academic Press, New York and London
Knopp, R. H., Ruder, H. J., Herrera, E. and Freinkel, N. (1970) Acta Endocrinol. (Copenhagen) **65**, 352–360
Kruszynska, Y. T. and McCormack, J. G. (1989) Biochem. J. **258**, 699–707
Lederman, S. and Rosso, P. (1981) J. Nutr. **111**, 1823–1832
Leturque, A., Sabatin, P., Ferré, P. and Girard, J. (1981) Biochem. J. **200**, 181–184
Leturque, A., Burnol, A.-F., Ferré, P. and Girard, J. (1984) Am. J. Physiol. **246**, E25–E31
Leturque, A., Ferré, P., Burnol, A.-F., Kandé, J., Maulard, P. and Girard, J. (1986) Diabetes **35**, 172–177
Nimmo, H. G., Proud, C. G. and Cohen, P. (1976) Eur. J. Biochem. **68**, 31–44
Otway, S. and Robinson, D. S. (1968) Biochem. J. **106**, 677–682

Pénicaud, L., Ferré, P. Kandé, J., Leturque, A., Issad, T. and Girard, J. (1987) Am. J. Physiol. **252**, E365–E369
Randle, P. J., Newsholme, E. A. and Garland, P. B. (1964) Biochem. J. **93**, 652–665
Rennie, M. J., Winder, W. W. and Holoszy, J. O. (1976) Biochem. J. **156**, 647–655
Scow, R. O., Chernick, S. S. and Brinley, M. S. (1964) Am. J. Physiol. **206**, 796–804
Sokoloff, L., Reivic, M., Kennedy, C., Des Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O. and Shinohara, M. (1977) J. Neurochem. **28**, 897–916
Stace, P. B., Marchington, D. R., Kerbey, A. L. and Randle, P. J. (1990) FEBS Lett. **273**, 91–94
Stace, P. B., Fatania, H. R., Jackson, A., Kerbey, A. L. and Randle, P. J. (1992) Biochim. Biophys. Acta **1135**, 201–206
Stalmans, W. and Hers, H. G. (1973) in The Enzymes (Boyer, P. D., ed.), pp. 309–361, Academic Press, New York
Storlein, L. H., Jenkins, A. B., Chisholm, D. J., Pascoe, W. S., Khouri, S. and Kraegen, E. W. (1991) Diabetes **40**, 280–289
Sugden, M. C. and Holness, M. J. (1990) J. Endocrinol. **127**, 187–190
Sugden, M. C., Liu, Y.-L. and Holness, M. J. (1990) Biochem. J. **272**, 133–137
Thiébaud, D., DeFronzo, R. A., Jacot, E., Golay, A., Acheson, K., Maeder, E., Jéquier, E. and Felber, J. P. (1982) Metab. Clin. Exp. **31**, 1128–1136
Villar-Palasi, C. and Larner, J. (1966) Fed. Proc. Fed. Am. Soc. Exp. Biol. **25**, 583
Williamson, D. H., Mellanby, J. and Krebs, H. A. (1962) Biochem. J. **82**, 90–96
Winder, W. W., Baldwin, K. M and Holoszy, J. O. (1974) Eur. J. Biochem. **47**, 461–467

Received 20 November 1992/14 January 1993; accepted 28 January 1993