Progressive suppression of muscle glucose utilization during pregnancy

Mark J. HOLNESS, K. Kumar CHANGANI and Mary C. SUGDEN*

Department of Biochemistry, Basic Medical Sciences, Queen Mary & Westfield College, Mile End Road, London E1 4NS, U.K.

Glucose utilization indices (GUIs) were measured in heart and a range of skeletal muscles in conscious, unrestrained, virgin or pregnant rats in the absorptive and post-absorptive phases. A clear effect of pregnancy to diminish muscle GUIs was identified, the magnitude of which was greatest in late gestation in the absorptive phase. Differences in the time courses and magnitudes of the response to pregnancy were observed between individual muscles. The effects of pregnancy are discussed in relation to an increased availability of lipid fuels and to decreased insulin and glucose concentrations.

INTRODUCTION

The necessity to accommodate the developmental and fuel requirements of the conceptus confers on the pregnant mother a considerable nutritional burden. Evidence has accrued for substantial subversion of maternal metabolism in response to this challenge. Some of the metabolic adaptations described during late pregnancy include post-absorptive hypoglycaemia (Scow *et al.*, 1964; Herrera *et al.*, 1969; Knopp *et al.*, 1973), an increased rate of glucose turnover (Ogata *et al.*, 1981; Leturque *et al.*, 1981) and insulin resistance (Leturque *et al.*, 1984, 1987; Hauguel *et al.*, 1987). Studies in the rat have permitted the key peripheral target tissues in which insulin-stimulated glucose disposal is decreased to be investigated: these include liver, white adipose tissue and skeletal muscle (Leturque *et al.*, 1986).

Tissue glucose utilization in the post-absorptive state has been compared in anaesthetized 19-day-pregnant rats (term is 21 days) and anaesthetized age-matched controls (Leturque *et al.*, 1986). Despite significantly decreased blood glucose concentrations, late pregnancy failed to influence muscle glucose utilization, except in extensor digitorum longus (EDL) (which contains predominantly fast-twitch fibres; Ariano *et al.*, 1973). We have extended this study by systematic measurement of the progressive effects of pregnancy on glucose utilization by a range of muscles differing in their functions and fibre compositions. Since anaesthesia depresses glucose utilization in postural skeletal muscles (Pénicaud *et al.*, 1987), rats were conscious and unrestrained throughout the experiments. In addition, as late pregnancy accelerates the response to food withdrawal (Felig & Lynch, 1970; Freinkel, 1972; Girard *et al.*, 1977), glucose utilization indices (GUIs) were measured not only in the post-absorptive state but also during the absorptive phase.

MATERIALS AND METHODS

Materials

Sources of materials were as in Holness *et al.* (1988) and Holness & Sugden (1990). Kits for measurement of non-esterified fatty acids (NEFA), insulin and triacylglycerol were purchased from Alpha Laboratories, Eastleigh, Hants., U.K.

Animals

Age-matched female albino Wistar rats on a 12 h-light/12 h-dark cycle (light from 10:00 h) were used for the study. Pregnant rats were studied at 10, 15 and 20 days of pregnancy, the stage

of pregnancy being determined as described by Lederman & Rosso (1981). The average number of fetuses per rat was 13 (range 9–20), with no statistically significant differences between the groups of rats sampled at 10, 15 and 20 days of pregnancy. Mean weights of the pregnant rats at 10, 15 and 20 days of pregnancy were 246 ± 10 g, 278 ± 5 g and 339 ± 8 g respectively. Mean weights of the age-matched virgin rats, used as non-pregnant controls, were 245 ± 8 g. Some virgin and pregnant rats were permitted free access to a standard rodent diet (52% carbohydrate, 15% protein, 3% fat and 30% non-digestible residue) and sampled at 10:00 h precisely: others were sampled in the post-absorptive state at 16:00 h (after withdrawal of food for 6 h). Chow intakes in virgin and 20-day-pregnant rats were: virgin rats, 19.7 ± 2.2 g of chow during the dark phase and 3.7 ± 0.7 g during the light phase; pregnant rats, 23.6 ± 1.8 g during the dark phase and 5.1 ± 0.6 g during the light phase. Thus approx. 82% (pregnant rats) or 84% (virgin rats) of daily food consumption occurred during the dark phase. The pattern of food intake over the dark period was similar in the pregnant and virgin rats: in both cases approx. 32% of the total intake over the dark period was consumed during the 3 h immediately preceding sampling at 10:00 h (results not shown).

Measurement of tissue GUI *in vivo*

Details of the experimental protocol used for measurement of GUI are given in Sugden *et al.* (1990). Each rat was fitted with an indwelling cannula before the experiment, under Hypnorm [fentanyl citrate (0.315 mg/ml) / fluanisone (10 mg/ml); 1.0 ml/kg intraperitoneally] and Diazepam (5 mg/ml; 1 ml/kg intraperitoneally) anaesthesia. GUI values were measured in conscious unrestrained rats after complete recovery from surgery (5–7 days after cannulation). Each GUI measurement was initiated by injection of a tracer dose of 2-deoxy[3 H]glucose (30 μ Ci) via the cannula. Arterial blood (100 μ l) was collected at 1, 3, 5, 10, 20, 40 and 60 min after injection of radiolabel, by which time radioactivity remaining in the blood was negligible. The experiment was terminated by injection of sodium pentobarbital (60 mg/kg body wt.) through the cannula at 60 min. Blood and tissue samples were treated as described by Ferré *et al.* (1985). Glucose concentrations and coefficients of variance of blood glucose are shown in Tables 1 and 2. Total ketone bodies were measured in KOH-neutralized HClO₄-extracts of whole blood sampled at the end of the experiments (Williamson *et al.*, 1962).

Values of the correction factor for the discrimination of 2-

Abbreviations used: GUIs, glucose utilization indices; EDL, extensor digitorum longus; NEFA, non-esterified fatty acids.

* To whom correspondence should be addressed.

Table 1. Muscle GUI values in the post-absorptive state

Details are given in the Materials and methods section. Statistically significant effects of pregnancy are shown: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	GUI (ng of glucose/min per mg)			
	Virgin	Duration of pregnancy		
		10 days	15 days	20 days
Heart	85.9 ± 12.4	77.9 ± 9.3	59.4 ± 7.5	22.2 ± 1.8***
Diaphragm	31.5 ± 3.6	42.0 ± 7.0	28.2 ± 5.6	15.1 ± 5.4*
Soleus	20.8 ± 3.1	27.0 ± 4.7	12.3 ± 1.7*	12.5 ± 2.2*
Adductor longus	17.3 ± 2.6	17.7 ± 5.0	9.2 ± 1.1*	4.7 ± 0.6***
Tibialis anterior	5.0 ± 0.7	6.4 ± 1.8	3.1 ± 0.5*	2.0 ± 0.5**
EDL	3.3 ± 0.1	4.3 ± 0.8	2.7 ± 2.2*	1.6 ± 0.3***
Glucose concn. (mM)	4.68 ± 0.13	4.69 ± 0.19	4.03 ± 0.14**	3.37 ± 0.38**
Coeff. of variance (%)	13.1 ± 1.1	12.9 ± 1.6	13.9 ± 1.3	12.3 ± 3.5

deoxyglucose in glucose metabolic pathways have been determined previously for a range of muscles in virgin and late-pregnant rats and are not significantly affected by pregnancy (Leturque *et al.*, 1986). Consequently, GUI values in control and pregnant rats can be compared directly.

Statistics

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

RESULTS AND DISCUSSION

Effects of pregnancy on GUI values in the post-absorptive state in conscious rats

In a previous study (Leturque *et al.*, 1986), basal rates of glucose utilization by diaphragm and soleus muscles of anaesthetized rats in the immediately post-absorptive (3–6 h starved) state were similar in virgin and 19-day-pregnant rats. However, it was subsequently demonstrated by the same group that pentobarbital anaesthesia greatly depresses glucose utilization in these muscles (Pénicaud *et al.*, 1987). GUI values were therefore measured in conscious unrestrained rats. The range of muscles was extended to include heart (like diaphragm, a rhythmically contracting muscle) and a range of individual skeletal muscles differing in terms of their fibre composition and contractile activities (in the resting state). Pregnant rats were sampled during early (10 days) and late (15 and 20 days) gestation (term is 21 days).

Values of GUI in soleus and diaphragm muscles of post-absorptive virgin rats measured in the present study (Table 1, lines 2 and 3) were considerably higher than those obtained by Leturque *et al.* (1986). This difference is consistent with the effects of anaesthesia to suppress glucose utilization in these muscles noted previously (Pénicaud *et al.*, 1987). Glucose utilization by diaphragm was not significantly affected by 10 or 15 days of pregnancy in the post-absorptive state, even in the absence of anaesthesia (Table 1). However, it was possible to detect a significant effect of pregnancy to decrease GUI in diaphragm at 20 days of gestation, at which time the GUI value was decreased by 52% (Table 1). The value of GUI in diaphragm measured in 20-day-pregnant rats was similar to that obtained by Leturque *et al.* (1986) at 19 days of pregnancy [approx. 15 ng/min per mg (Table 1) versus approx. 23 ng/min per mg]. Soleus GUI

was suppressed by 41% relative to the virgin control at 15 days of gestation, and a low GUI value continued to be observed in soleus at 20 days of gestation (Table 1). The value of GUI measured in soleus muscles of conscious rats at 20 days of pregnancy was, as that in diaphragm, similar to that measured in anaesthetized rats by Leturque *et al.* (1986) (values of 13 ng/min per mg and 8 ng/min per mg respectively). Cardiac GUI was even more dramatically affected by 20 days of pregnancy than that of diaphragm, with a GUI value of only 26% of the post-absorptive virgin control (Table 1). Furthermore, statistically significant effects of pregnancy were observed at days 15 and 20 in the post-absorptive state in three further muscles: adductor longus (which, like soleus, is a working postural muscle), tibialis anterior and EDL. In adductor longus, the effect of pregnancy was progressive, with 46% suppression of glucose uptake and phosphorylation at 15 days and 73% suppression of glucose uptake and phosphorylation at 20 days of pregnancy (Table 1). Suppression of glucose utilization by the two non-working muscles (tibialis anterior and EDL) was also progressive, with up to 60% and 52% suppression of GUI respectively at 20 days (Table 1). The effect of pregnancy is therefore not restricted to muscles which are constantly contracting, nor is it exclusively observed in working postural muscles.

Effects of pregnancy on muscle GUI values during the absorptive phase

The effects of pregnancy to suppress muscle glucose utilization in the post-absorptive state may be viewed as part of a strategy to spare glucose for the conceptus when dietary carbohydrate is unavailable. However, to assess the physiological importance of the peripheral insulin-resistance observed during insulin clamps in late pregnancy (Leturque *et al.*, 1984, 1986, 1987; Hauguel *et al.*, 1987), it is important to establish whether muscle glucose utilization is significantly affected by pregnancy when dietary carbohydrate is available. A second series of experiments was therefore undertaken in rats in the absorptive phase at 10:00 h (immediately at the end of the dark phase). Coefficients of variance of blood glucose over the 1 h period of study were less than 15% in both virgin and pregnant rats (Table 2).

GUI values measured in muscles of virgin rats in the absorptive phase were generally higher than those observed in rats allowed free access to food but sampled at 2–3 h after the end of the dark phase (compare values in Table 2 with those given in Holness & Sugden, 1990) and, except for heart, than those measured in the

Table 2. Muscle GUI values in the absorptive state

Details are given in the Materials and methods section. Statistically significant effects of pregnancy are shown: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	GUI (ng of glucose/min per mg)			
	Virgin	Duration of pregnancy		
		10 days	15 days	20 days
Heart	110.0 ± 11.6	72.3 ± 11.9*	59.0 ± 10.7**	28.8 ± 6.4***
Diaphragm	64.5 ± 7.3	42.3 ± 5.8*	45.0 ± 6.6*	17.5 ± 4.5***
Soleus	58.9 ± 4.8	45.7 ± 5.6	43.2 ± 3.2*	24.2 ± 3.0***
Adductor longus	50.9 ± 5.7	36.2 ± 3.8*	32.1 ± 3.5*	21.6 ± 4.0**
Tibialis anterior	15.6 ± 2.6	7.4 ± 1.1*	6.1 ± 1.8*	3.8 ± 0.8**
EDL	11.6 ± 2.3	5.7 ± 1.4*	5.7 ± 2.0*	2.3 ± 0.2**
Glucose concn. (mM)	5.81 ± 0.23	5.00 ± 0.18*	3.68 ± 0.18***	3.37 ± 0.10***
Coeff. of variance (%)	11.6 ± 2.0	12.0 ± 2.6	13.8 ± 2.4	12.4 ± 3.7

present experiments in the post-absorptive state at 16:00 h (compare values in Tables 1 and 2). Whereas in the post-absorptive state significant effects of pregnancy to decrease cardiac GUI were only observed at 20 days, values of cardiac GUI in rats measured during the absorptive phase were consistently lower throughout pregnancy (Table 2). Cardiac GUI was decreased by 34 % after only 10 days of pregnancy, and even more marked effects of pregnancy were observed at 15 and 20 days of gestation, at which times cardiac GUI values were suppressed by 46 % and 74 % respectively (Table 2). The response of glucose utilization by the diaphragm to pregnancy was quantitatively and qualitatively very similar to that of the heart (Table 2). Significant (34 %) suppression of GUI was observed at 10 days of pregnancy (cf. at 20 days in the post-absorptive state) and, again, GUI values progressively decreased as the duration of pregnancy was extended, with very substantial (73 %) suppression of glucose utilization after 20 days of gestation.

Significant effects of pregnancy were revealed in the absorptive phase after 10 days in three of the four skeletal muscles selected for study (Table 2). By day 15, clear effects of pregnancy to suppress skeletal-muscle glucose utilization were observed in all four skeletal muscles (Table 2). The effects of 15 days gestation were more marked in the non-working muscles (tibialis anterior, EDL; 51–61 % suppression of GUI) than in the postural muscles (soleus, adductor longus; 27–37 % suppression of GUI). As in the cardiothoracic muscles, the most marked effects of pregnancy to suppress glucose utilization by skeletal muscles were observed at 20 days of gestation, at which time values of GUI were suppressed by 58 % in the oxidative working muscles and by 76–80 % in tibialis anterior and EDL.

It is notable that, in contrast with the findings in virgin rats, consistent differences between GUI values measured in the absorptive and post-absorptive states during pregnancy were observed only in soleus and adductor longus (Tables 1 and 2; $P < 0.01$ for differences at 15 and 20 days of pregnancy, $P < 0.05$ at 10 days of pregnancy). Similarly, whereas blood glucose concentrations were considerably higher in the absorptive phase than in the post-absorptive phase in virgin rats, this was not the case in pregnancy (Tables 1 and 2). The failure to observe consistent differences between GUI values measured in the absorptive and post-absorptive states during pregnancy may therefore reflect these differences in glycaemia, or alternatively decreased insulin-sensitivity/responsiveness of glucose transport and/or utilization.

Table 3. Insulin and lipid fuel concentrations in virgin and 20-day-pregnant rats in the absorptive state

Details are given in the Materials and methods section. Insulin, NEFA and triacylglycerol concentrations were measured in plasma, and ketone-body (3-hydroxybutyrate and acetoacetate) concentrations in KOH-neutralized extracts of whole blood. Statistically significant effects of pregnancy are shown: ** $P < 0.01$.

	Virgin	20 days pregnant
Insulin (μ -units/ml)	26.2 ± 2.2	14.1 ± 3.1**
NEFA (mM)	0.34 ± 0.08	0.80 ± 0.12**
Total ketone bodies (mM)	0.12 ± 0.04	0.70 ± 0.15**
Triacylglycerols (mM)	1.45 ± 0.23	1.70 ± 0.19

Concentrations of insulin and lipid fuels during late gestation

Leturque *et al.* (1986) have suggested that lower rates of glucose utilization observed in some tissues at 19 days of gestation in the post-absorptive state might reflect relative hypoglycaemia. In the present experiments, blood glucose concentrations in the post-absorptive state were 14 % and 28 % lower in pregnant than in virgin rats at days 15 and 20 of pregnancy respectively (Table 1). Despite free access to a high-carbohydrate diet and a significantly increased food intake during the dark phase (see the Materials and methods section), blood glucose concentrations in the absorptive phase were also significantly lower during pregnancy (Table 2). Hepatic glycogen concentrations measured in the absorptive phase were unaffected by pregnancy [values of 72.6 ± 8.2 ($n = 6$) mg/g, 65.7 ± 9.9 ($n = 4$) mg/g, 73.3 ± 5.7 ($n = 6$) and 58.8 ± 4.5 ($n = 11$) mg/g respectively in virgin and 10-, 15- and 20-day-pregnant rats; $P > 0.05$ in each case].

Plasma insulin concentrations in virgin and 20-day-pregnant rats in the absorptive phase are shown in Table 3. Commensurate with the lower level of glycaemia, the insulin concentration was decreased by 46 % in the pregnant rat. Both NEFA and ketone-body concentrations were significantly increased in 20-day-pregnant rats compared with virgin controls, but triacylglycerol concentrations were not significantly affected.

General discussion

The present study has identified a clear effect of pregnancy to diminish muscle glucose uptake and phosphorylation. The mag-

nitude of the effect of pregnancy to suppress muscle glucose utilization is dependent on both the duration of pregnancy and the maternal carbohydrate status. The considerable suppression of glucose utilization observed during late pregnancy can be ascribed both to increased availability of lipid fuels (which can substitute for carbohydrate as energy substrate in oxidative tissues) and to decreased insulin and glucose concentrations. Differences in the response to pregnancy were observed between individual skeletal muscles: in general, effects of pregnancy were observed earlier and were of greater magnitude in non-working skeletal muscles than in the postural muscles. It is presumed that, although the supply of fatty acids and ketone bodies is increased, the oxidation of lipid fuels alone is insufficient to fulfil totally the increased energy requirements of the postural muscles occasioned by the significant increase in body weight. This contrasts with the situation in the heart, where rates of glucose utilization during late pregnancy are as low as those seen after 24–30 h of starvation in virgin rats (Holness & Sugden, 1990), implying a predominant utilization of lipid as energy substrate.

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