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Glucose turnover during pregnancy in anaesthetized post-absorptive rats

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During pregnancy the decline in blood [glucose] does not result from the increased distribution space of glucose. The absolute rate of glucose turnover increases in late pregnancy in parallel with the rise in the mass of the conceptus. Nevertheless, glucose turnover per kg body wt. is not increased in late pregnancy, since the lower blood [glucose] decreases glucose utilization by maternal tissues.

During pregnancy the mother must provide a continuous supply of glucose for her own tissues and for the increasing demand of the growing conceptus. It has been reported that after an overnight fast (Silverstone *et al.*, 1961; Bleicher *et al.*, 1964; Victor, 1974) or in the post-absorptive state (Cousins *et al.*, 1980) the blood [glucose] was lower in pregnant than in non-pregnant women. Similarly, the blood [glucose] is lower in pregnant than in virgin rats in both the fed and unfed states (Scow *et al.*, 1964; Herrera *et al.*, 1969; Knopp *et al.*, 1973). The decline in blood [glucose] during pregnancy has been attributed to the inability of the mother to accelerate her glucose production in response to the increasing needs of the growing conceptus (Felig, 1973) or to an increase in the distribution space of glucose (Kalhan *et al.*, 1979). Very few studies have examined the impact of pregnancy on maternal glucose turnover. In fed twin-pregnant ewes near term, absolute and weight-related glucose turnover are increased by respectively 63 and 33% (Bergman, 1963; Bergman *et al.*, 1974). In contrast, glucose turnover per kg body wt. was not increased in overnight-fasted pregnant women at term (Kalhan *et al.*, 1979) and in fed pregnant guinea pigs (Gilbert *et al.*, 1981). In preliminary experiments it has been reported that absolute glucose turnover was increased by 50–80% in 24h-unfed pregnant rats as compared with non-pregnant rats (Ogata *et al.*, 1980). The aims of the present study were to answer two questions: (1) does the decline in blood [glucose] during pregnancy result from an increase in the distribution space of glucose?; (2) is glucose turnover altered during pregnancy in the post-absorptive state?

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Experimental

Animals

The rats were an albino Wistar strain bred in the laboratory and fed *ad libitum* on laboratory chow (carbohydrate 65%, protein 24%, fat 11%). They were housed at 24°C with light from 07:00h to 19:00h. The stage of pregnancy was determined as described previously (Girard *et al.*, 1973). The average number of foetuses in each pregnant rat was 10 ± 0.3 ($n = 56$), and pregnant rats with less than eight foetuses were not included in this study. Age-matched virgin rats were used as non-pregnant controls. Food was removed at 09:00h and the experiments were started at 14:00h, so virgin and pregnant rats were considered to be in the post-absorptive state.

Estimation of glucose distribution space

Glucose distribution space was determined after intravenous injection of glucose (5.5mmol/kg body wt.) in rats anaesthetized with pentobarbitone (30mg/kg body wt. intraperitoneally) and with a carotid artery catheterized for blood sampling. Blood samples (100μl) were obtained at 5, 10, 15, 20, 25 and 30min after glucose injection. Blood was deproteinized with 0.083M-$Ba(OH)_2$ and 0.087M-$ZnSO_4$, centrifuged at 16000*g* for 2min, and glucose in the supernatant was determined by the glucose oxidase method (Girard *et al.*, 1973). The theoretical blood [glucose] at zero time (G_0) was determined by extrapolating to $t = 0$ the slope of a graph of the log of blood [glucose] against time. The distribution space of glucose (D) in ml was calculated from the formula $D = G_0/Q$, where G_0 = [glucose] at $t = 0$ (μmol/ml), and Q = the amount of glucose injected (μmol).

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Measurement of glucose turnover rate

The rats were anaesthetized with pentobarbitone (30mg/kg body wt., intraperitoneally) and one carotid artery was catheterized for blood sampling. Body temperature was continuously recorded with a telethermometer (YSI, Yellow Spring, OH, U.S.A.) and maintained at 37°C with heating lamps. [6-^{3}H]Glucose was chosen because it appears that its detritiation by hepatic cycling is minimal and it gives an adequate approximation of an irreversible tracer of glucose (Katz *et al.*, 1976). [6-^{3}H]Glucose (The Radiochemical Centre, Amersham, Bucks., U.K.) was infused through the saphenous vein. A priming dose of 4μCi was injected in 2min and a continuous infusion was immediately started at a rate of 0.2μCi/min by using an infusion pump (Braun, Melsungen, Germany).

Preliminary experiments had shown that a steady-state specific radioactivity of glucose in blood was established by 30min and later (results not shown). Blood samples (200μl) were obtained at 30, 40, 50 and 60min after the start of [6-^{3}H]glucose infusion. Blood samples were deproteinized immediately with $Ba(OH)_2/ZnSO_4$, centrifuged at 16000*g* for 2min and samples of the supernatant used for determination of blood [glucose] by the glucose oxidase method. Other samples of the supernatant were evaporated to dryness at +70°C to remove ^3H_2O and the dry residue was redissolved in 0.2ml of water before addition of 10ml of Unisolve I (Koch–Light, Colnbrook, Bucks., U.K). All samples were counted for radioactivity in a Nuclear Chicago liquid-scintillation spectrometer. In preliminary experiments, samples of the supernatant were passed through ion-exchange resin columns (internal diam. = 5mm) containing 2.5cm of Dowex AG1 (X8; formate form) and 2.5cm of Dowex AG 50W (X8; H^+ form) to remove possible ^{3}H-containing metabolites other than glucose and water. The eluates from the columns were then evaporated to dryness and treated as above. The glucose specific radioactivity in the portion passed through the columns was found to be 98 ± 2% (±S.E.M.; $n = 10$) of that in the sample not passed through the column. The passage through ion-exchange resin columns was therefore discontinued.

Glucose-turnover rates at steady state were calculated from the formula $R = F/SA$, where R = the glucose turnover rate in blood (μmol/min), F = the infusion rate of [6-^{3}H]glucose (d.p.m./min) and SA = the specific radioactivity of glucose in blood (d.p.m./μmol). As pregnant and virgin rats had very different basal blood [glucose], we have calculated the metabolic clearance of glucose, since this parameter is an index of the ability of tissues to remove glucose from blood independently of blood [glucose] (Cherrington *et al.*, 1978). The metabolic clearance of glucose at steady state was calculated as the ratio of glucose turnover (μmol/min) and of blood glucose (μmol/ml) and expressed in ml/min.

Statistics

Results are expresssed as means ± S.E.M. of 8 to 24 determinations. Significance of differences was determined by using Student's *t* test.

Results and discussion

Pregnancy in the rat is associated with increased food intake (Scow *et al.*, 1964; Knopp *et al.*, 1975) and increased total absorptive capacity of the gut (Fell *et al.*, 1963). These changes could affect glucose turnover independently of gestation in the fed state. In order to measure endogenous glucose production and utilization in a situation where nutritional conditions were similar in both pregnant and virgin animals, we have used post-absorptive rats. Food was withdrawn 5h before glucose turnover measurement, and in these conditions the [glucose] difference between the carotid artery and the portal vein was slightly positive both in pregnant (0.13 ± 0.11mmol/l) and in virgin rats (0.20 ± 0.13mmol/l). Thus the gut was not adding glucose in portal circulation from previously ingested food, and the values of glucose turnover measured were representative of the post-absorptive state.

As previously reported (Scow *et al.*, 1964; Knopp *et al.*, 1973), blood [glucose] decreased slightly between 14 and 21 days of gestation (Table 1). The distribution space of glucose was not increased on day 14 of gestation, although blood [glucose] was already decreased (Table 1). On days 19 and 21 of gestation the distribution space of glucose was respectively 40 and 54% higher than in virgin rats (Table 1). However, despite this increase in the distribution space of glucose, there was only a 10% decrease in blood glucose between days 14 and 21 of gestation (Table 1). These data suggested that the decline in blood [glucose] during pregnancy did not result from an increased distribution space of glucose, as previously proposed by Kalhan *et al.* (1979).

On day 14 of gestation the glucose turnover rate was significantly lower than in virgin rats; it was decreased by 17% when expressed in absolute value and by 33% when expressed per kg body wt. (Table 1). As the metabolic clearance rate of glucose was similar in pregnant and virgin rats (Table 1), this suggested that the lower glucose turnover in 14-day-pregnant rats resulted essentially from the 24% decrease in their blood [glucose] (Table 1). On days 19 and 21 of pregnancy the absolute value of glucose turnover rate was increased by respectively 42 and 47% (Table 1). This increase could be related to the rapid growth of the total mass of the

Table 1. *Blood glucose, glucose distribution space and glucose kinetics in post-absorptive virgin and pregnant rats*
For details see the Experimental section. Values are means ± S.E.M., with the numbers of experiments indicated in parentheses. Significance of differences was determined by Student's *t* test. $*P < 0.05$, $**P < 0.001$ when compared with virgin rats; $\dagger P < 0.05$ when compared with 14-day-pregnant rats.

Physiological state	Body wt. (g)	Blood [glucose] (μmol/ml)	Glucose distribution space (ml/rat)	Glucose turnover (μmol/min)	Glucose turnover (μmol/min per kg)	Metabolic clearance of glucose (ml/min)	Metabolic clearance of glucose (ml/min per kg)
Virgin (24)	190 ± 8	5.5 ± 0.1	50 ± 4	10.2 ± 0.4	52 ± 2	1.8 ± 0.1	9.4 ± 0.3
Pregnant							
14 days (8)	243 ± 8**	4.2 ± 0.1**	59 ± 3	8.5 ± 0.4*	35 ± 2**	2.0 ± 0.1	8.5 ± 0.7
19 days (19)	276 ± 4**	4.0 ± 0.1**	70 ± 3*	14.5 ± 0.6**	52 ± 2	3.6 ± 0.4**	13.1 ± 0.4**
21 days (22)	304 ± 5**	3.8 ± 0.1**†	77 ± 4*	16.7 ± 0.9**	54 ± 3	4.4 ± 0.3**	14.2 ± 0.8**

conceptus (foetus + placenta) in late pregnancy. Indeed, the mass of the conceptus increased from approx. 4 g for a litter of ten at 14 days of gestation to approx. 55 g for the same litter size at 21 days of gestation. As during the same period the maternal weight increased by approx. 61 g (Table 1), this suggested that all the maternal weight gain was represented by foetal and placental tissues. Assuming that glucose utilization by maternal tissues remains unchanged between 14 and 21 days of gestation, one may estimate that the increase in glucose turnover during this period is entirely due to the growing mass of the conceptus. By dividing the increase in glucose turnover between days 14 and 21 of pregnancy (8.2 μmol/min) by the increase in the mass of conceptus (51 g), one may estimate that the rate of glucose utilization by the conceptus is 160 μmol/min per kg, a value 3-fold higher than the maternal glucose utilization (52 μmol/min per kg). The presence in the maternal organism of a site of high glucose utilization after 14 days of gestation is clearly indicated by the increase in absolute or weight-related rise in metabolic clearance of glucose on days 19 and 21 of gestation (Table 1). In keeping with this, it has been shown in the sheep that foetal and placental glucose-utilization rates were respectively 2- and 20-fold higher per kg tissue weight compared with the maternal glucose turnover per kg body weight (Meschia *et al.*, 1980). Despite the large increase in the mass of conceptus, which has a higher rate of glucose utilization than maternal tissues, the weight-related rates of glucose turnover of 19- and 21-day-pregnant rats are similar to those measured in virgin rats (Table 1). This unexpected finding is due to the fact that blood [glucose] is lower in pregnant rats and thus results in a decreased glucose utilization by maternal tissues.

In summary, these studies provide an answer to the two major questions addressed in the introduction. First, the decline in blood [glucose] during pregnancy does not result from the increase in the distribution space of glucose. Second, the absolute glucose turnover increases in late gestation in parallel with the rise in total mass of the conceptus. The glucose turnover per kg body weight is not increased during pregnancy, despite the presence of the conceptus, which is a site of very high glucose utilization, because the lower blood [glucose] slows down glucose utilization by maternal tissues.

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