Glucose Turnover Rate during Pregnancy in the Conscious Guinea Pig

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Summary

The glucose turnover rate (GTR) has been determined in conscious pregnant guinea pigs from 33-63 days of gestation. Eighteen animals were studied, using a constant infusion technique of trace amounts of 6-[³H]-glucose and U-[¹⁴C]-glucose. The GTR 6-[³H] (mg·min⁻¹) increased progressively with gestation. At term the value is approximately 2-fold higher than in the nonpregnant females. The weight specific GTR 6-[³H] (mg·min⁻¹·kg⁻¹) did not change significantly with gestation. The % of [¹⁴C] recycling increased from 0% at 40 days gestation to 20% at term.

As with GTR, the metabolic clearance of glucose (ml·min⁻¹) increased with gestational age and exceeded the clearance in nonpregnant animals after 50 days of pregnancy. Expressed per kg of body weight, the metabolic clearance of glucose was not significantly different in pregnant and nonpregnant animals.

Speculation

At term, in the pregnant guinea pig, the fetal mass can equal 50% of the maternal prepregnancy weight. Because glucose is an important substrate for the conceptus, the enormous fetal mass could be expected to have a significant impact upon maternal rate of glucose production. These studies suggest that this weight specific glucose utilization rate of the guinea pig conceptus is not substantially higher than that of the maternal tissues.

Rates of glucose turnover have been determined in several adult mammalian species using tracer glucose infusion. Because glucose provides a large portion of the exogenous calories consumed by the fetus (2), the growth of the fetal mass with advancing gestation might be expected to impact substantially on maternal glucose metabolism. However, few studies have examined the impact of advancing gestation on maternal glucose turnover rate (GTR). In the unstressed large mammal, studies have reported the GTR only in late gestation. For the pregnant ewe, which as a ruminant depends on a high rate of gluconeogenesis to meet the glucose requirements, a higher GTR has been reported for twin compared to singleton pregnancy (4). This suggests that the conceptus may be a substantial consumer of glucose in late gestation. Using stable isotopes, the GTR has also been measured in human pregnancy, and was found not to change significantly with pregnancy (5, 14). To our knowledge, there have been no studies of GTR in the small mammals during unstressed conditions, that is, with the animals free from operative and anesthetic stress.

The guinea pig is particularly suitable for studies of the impact of pregnancy upon maternal metabolism, since the total fetal mass produced in this species can equal 40-60% of the maternal prepregnancy weight (12). This enormous fetal mass could be expected to have a significant impact upon maternal rates of utilization of such substrates as glucose because glucose is an important substrate for the conceptus (2). There have been previous reports of GTRs in guinea pigs but those studies were carried out in fasted

nonpregnant animals (20-22). The current report presents data on the rates of glucose turnover in nonpregnant and pregnant guinea pigs studied under chronic steady-state conditions.

MATERIALS AND METHODS

Eighteen pregnant, tricolor, guinea pigs weighing from 600-1200 g were used for these studies. The gestations were dated within 1 day by the time of exposure to the male. Seven nonpregnant animals were also studied. These animals weighed 686 ± 30 g and had not been pregnant for at least 2 months. Animals were fed *ad libitum* a vitamin enriched solid guinea pig diet. The surgical procedures and anesthesia were carried out as previously described (19). Animals were anesthetized with ketamine-hydrochloride and xylazine hydrochloride. Polyvinyl catheters were implanted in the maternal femoral and carotid arteries, tunneled subcutaneously and exteriorized through the skin at the back of the neck. The catheters were kept in a closed cap and were flushed with a heparin solution (250 U/ml in 0.9 NaCl) every 3 days. Since previous studies had shown that the recovery of food intake and normalization of substrate concentrations after surgery and anesthesia required approximately 4 days (19), all of the experiments were performed at least 4 days postsurgery.

GTRs were determined by a constant infusion technique which provided 0.75 μCi of 6-[³H]-glucose and 0.35 μCi of U-[¹⁴C]-glucose per min. Blood samples were obtained at 0, 60, 75, 90 and 105 min after the start of the infusion. Preliminary experiments had confirmed that a steady-state specific activity of glucose in the plasma was established by 60 min (Fig. 1). Turnover rates at steady-state were calculated from the formula $R = F/S.A.$, where R is the turnover rate of glucose in the whole blood, F is the infusion rate of radio-labeled glucose in dpm/min, and S.A. is the whole blood specific activity of glucose in dpm per milligram at steady-state. The metabolic clearance of glucose was calculated as the ratio of $R/[GLC]$ where [GLC] is the whole blood glucose concentration. The % of [¹⁴C]-recycling was estimated from the difference between the GTRs simultaneously determined with [³H] and [¹⁴C]-glucose:

$$\% \text{ recycling} = \frac{([^3\text{H}]\text{-GTR}) - ([^{14}\text{C}]\text{-GTR})}{([^{14}\text{C}]\text{-GTR})} \times 100$$

Whole blood samples were deproteinized immediately with barium hydroxide and zinc sulfate and were centrifuged for 15 min at 12,000 X g. An aliquot of the supernatant was used for the determination of blood glucose concentration by the glucose oxidase method (11). A second aliquot of the supernate was passed through ion exchange columns (i.d. = 5 mm) containing 2.5 cm AG 1 - x 8 formate resin above 2.5 cm AG 50 w x 8 H⁺ form resins. The columns retained greater than 99% of the amino acids, lactate and pyruvate. A 1.5 ml water wash eluted greater than 99% of the glucose from the column. The eluate was evaporated to dryness and redissolved in 1 ml of water prior to the addition of the scintillation solution. All samples were counted in a liquid scintillation spectrometer (Nuclear Chicago, Mark I).

RESULTS

In preliminary experiments, a constant infusion of radio-labeled glucose was given into the maternal circulation to determine the time required for the establishment of a constant glucose specific activity in maternal plasma. The plateau was reached in approximately 1 h (Fig. 1). Thus, for the constant infusion technique, 1 h was taken as an appropriate time to begin sampling. In all experiments, the plateau specific activity was maintained between 60–105 min after the beginning of the labeled glucose infusion. Blood glucose concentration remained constant during each experiment, although the absolute value of the blood glucose between animals fell linearly with gestation ($r = 0.64$, $P < 0.01$), as previously reported (19). Blood glucose fell from 6.0 mM at 40 days gestation to 4.5 mM at term.

Using a constant infusion of [^3H]-6-glucose (Fig. 2A), nonpregnant animals demonstrated a glucose turnover rate of $6.95 \pm 1.03 \text{ mg} \cdot \text{min}^{-1}$. Over the limited range in weight of nonpregnant animals studied, there was no significant correlation between weight and GTR. In the pregnant animals, there was a progressive increase of glucose turnover during the period from midgestation (33 days) until term (63 days). A similar relationship between maternal GTR and gestational age was obtained with [^{14}C]-glucose although the absolute rates in individual animals were lower. At term, the GTR is approximately 2-fold higher than that in the nonpregnant female. If glucose turnover rate is expressed per unit body weight, the turnover rate did not change significantly ($12.1 \pm 0.4 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) over the gestation range studied (Fig. 2B), and was not significantly higher than the rate of nonpregnant animals ($10.1 \pm 1.3 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$).

The % of [^{14}C] recycling was estimated from the difference between the GTR determined with [^3H] and [^{14}C]-glucose (Equation 1). Carbon recycling appears to increase linearly ($r = 0.79$, $P < 0.001$) from approximately the 40th day of gestation until term (Fig. 3). As with GTR, the metabolic clearance of glucose expressed as $\text{ml} \cdot \text{min}^{-1}$ increased with gestational age ($r = 0.61$, $P < 0.05$) and exceeded the clearance in nonpregnant animals after 50 days of gestation (Fig. 4A). The clearance in nonpregnant animals was $7.5 \pm 0.9 \text{ ml} \cdot \text{min}^{-1}$ and in pregnant animals >50 days gestation was $11.2 \pm 1.2 \text{ ml} \cdot \text{min}^{-1}$ ($P < 0.025$). If the clearance is expressed per kg of body weight (Fig. 4B), there was no difference in nonpregnant and pregnant animals, i.e., 11.3 ± 1.6 and $11.3 \pm 0.5 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ respectively. However, in the pregnant animal it increases slightly from the 40th day of gestation until term, reflecting the decreasing glucose concentration with advancing gestation.

Fig. 1. Glucose specific activities in arterial plasma in a typical pregnant guinea pig during a constant infusion of U-[^{14}C]-glucose (—) and 6-[^3H] (----).

Fig. 2. Turnover rate of 6-[^3H]-glucose in pregnant guinea pigs at different stages of gestation (●) and in non-pregnant animals (○). The value in non-pregnant guinea pigs is a mean $\pm$ S.E. of seven animals.

Fig. 3. Recycling of radioactive glucose in pregnant guinea pigs at different stages of gestation. Percent of recycling was calculated as follows: $100 \times (\text{turnover rates of 6-[}^3\text{H}\text{]-glucose minus U-[}^{14}\text{C}\text{]-glucose} \div (\text{turnover rate of 6-[}^3\text{H}\text{]-glucose}))$.

Fig. 4. Metabolic clearance of glucose in pregnant guinea pigs at different stages of gestation (●) and in non-pregnant animals (○). The value in non-pregnant guinea pigs is a mean $\pm$ S.E. of seven animals.

DISCUSSION

In the fed pregnant guinea pig, blood glucose concentration falls during late gestation to levels significantly lower than in fed nonpregnant animals (19). Bergman and Sellers (3) have shown that the guinea pig develops very low glucose concentrations after brief periods of starvation. A similar phenomenon has been described in the rat (6). This "accelerated starvation" has been ascribed to the presence of the fetal tissue mass which has been regarded as the site of a relatively high rate of glucose utilization. To our knowledge there are no previous data concerning glucose turnover during pregnancy in unanesthetized, chronically catheterized small mammals. Atkins (1) reported studies of glucose turnover in nonpregnant rabbits free of anesthetic and surgical stress; however, the animals were not chronically catheterized. In the guinea pig, Wolfe *et al.* (20–22) have reported a glucose turnover rate of $5\text{--}6\text{ mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ in conscious, unrestrained male guinea pigs fasted for 24 h. In that study the animals were studied within 24 h of surgery with measured glucose concentrations much higher than in the current study, perhaps reflecting operative stress (19). In anesthetized, ventilated male guinea pigs, Freminet and LeClerc (7) found no difference in glucose turnover rate in the fed state ($9\text{ mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) or after a 48 h fast ($7.5\text{ mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$). The differences in experimental procedure may account for the discrepancies between the two studies. On the one hand, Wolfe *et al.* allowed a relatively brief time (1 day) for recovery from the stress of surgery. On the other hand Freminet and LeClerc studied anesthetized, artificially ventilated animals. Previous work from our laboratories has shown that a minimum of 3 days is required after surgery for recovery of normal food intake and normal arterial substrate concentrations (19).

The guinea pig represents a unique biologic system for mammalian reproduction. First of all, the species has a long gestational length (67 days), given the size of the adult animal. In addition,

the total fetal mass produced in the guinea pig is unequalled among mammals with the exception of the smaller bats (15). The total fetal mass at term may equal 30–60% of the prepregnant weight. This depends, in large part, upon the litter size (12), which averages three to four fetuses per litter and varies between one to seven fetuses. Furthermore, an average pregnant animal gains $\sim 13.3\text{ g}\cdot\text{d}^{-1}$ in total weight (19). At an average fetal weight gain of $\sim 3\text{ g}\cdot\text{d}^{-1}$ (12), growth of four fetuses represents $12\text{ g}\cdot\text{d}^{-1}$, or 80–90% of the total weight increase (12). Given the enormous fetal mass produced per unit weight of mother, the guinea pig provides an excellent species for studies of the metabolic demand placed upon the mother by pregnancy.

Even in the large mammals, where chronic catheterizations have been successfully carried out for some years, there are comparatively few data on glucose turnover rate in pregnancy. As in the present study, Kalhan *et al.* (14) found no difference in the GTR per kg body weight of pregnant and nonpregnant women. In man, the fetal mass represents $\sim 6\%$ of maternal prepregnancy weight. Several reports have described an increase in GTR per kg body weight for both fed and fasted pregnant rats studied under anesthesia (8, 16, 18). The present study demonstrates that the glucose turnover rate (mg/min) increases fairly linearly during gestation from the 32nd day until term. At first glance this may not appear surprising because we have previously shown a highly significant linear relationship between gestational age and mean maternal weight with an average daily weight gain of 13.3 g per day (19). However, the total fetal mass is growing rapidly, increasing from approximately 40 g for a litter size of four at 40 days gestation, to a total fetal mass of approximately 260 g for the same litter size at 60 days. Thus, most of the weight gain of the mother is not represented by maternal tissues, but by fetal tissues. Despite this large increase in fetal mass, Figure 2B demonstrates that the weight specific glucose turnover rate remained fairly constant during pregnancy. A 4-fold higher fetal GTR· kg^{-1} compared to the maternal GTR has been reported for other mammalian species (9). If this were true for the 300–400 g of fetal guinea pig tissues at term, the maternal weight specific GTR would have increased to $18\text{--}22\text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, assuming the GTR of maternal tissues remained constant. Since the weight specific GTR did not increase (Fig. 2B), it is unlikely that the GTR in the guinea pig fetus is 4-fold higher than the maternal GTR.

We have shown that, compared to the nonpregnant females, there is an increased glucose clearance (ml/min) in the late pregnant guinea pig and that this occurs in the presence of a lower glucose concentration. A similar conclusion can be deduced from the data published by Bergman (4) in the sheep and by Kalhan (14) in man. However, the effects of pregnancy and advancing gestation upon both glucose turnover and clearance should be further investigated by serial measurements in the same animal where interanimal differences in litter size and maternal metabolism are avoided.

Previously it has been pointed out that O_2 consumption of mammalian fetuses does not follow the $3/4$ power of body weight (2). Moll, *et al.* (17) have demonstrated this for the guinea pig by indirect estimates of fetal O_2 consumption. The present study on glucose turnover and Moll's study on O_2 consumption suggest that the fetal consumption rates for both oxygen and glucose may be lower than expected per kilogram body weight by comparison to the weight specific rates in postnatal life. This hypothesis needs careful investigation.

Finally, the observations that glucose recycling increases with advancing gestation could reflect a change in maternal metabolism that would have a glucose sparing effect as the requirements of the conceptus increase. Alternately, the increased recycling may reflect a characteristic of fetal metabolism and become evident in the total glucose turnover in the mother only as the total fetal mass increases. This latter hypothesis is consistent with data obtained on carbon recycling in the fetal lamb where measurements of glucose turnover rates with $[^3\text{H}]$ -glucose and $[^{14}\text{C}]$ -glucose have been made directly in both the fetuses (10) and the mother (10, 13). These studies have demonstrated that the turnover rates

in the adult sheep determined with either [^{14}C]- or tritiated glucose are identical, whereas, in the fetal lamb, the tritiated glucose turnover rate is significantly higher than that estimated with [^{14}C]-glucose (10).

REFERENCES AND NOTES

- Atkins, G. L.: A new technique for maintaining and monitoring conscious, stress-free rabbits in a steady state: its use in the determination of glucose kinetics. *Q. J. Exp. Physiol.*, **65**: 63 (1980).
- Battaglia, F. C. and Meschia, G.: Principal substrates of fetal metabolism. *Physiol. Rev.*, **58**: 499 (1978).
- Bergman, E. N. and Sellers, A. F.: Comparison of fasting ketosis in pregnant and non-pregnant guinea-pig. *Am. J. Physiol.*, **198**: 1083 (1960).
- Bergman, E. N.: Quantitative aspects of glucose metabolism in pregnant and non-pregnant sheep. *Am. J. Physiol.*, **204**: 147 (1963).
- Cowett, R. M., Susa, J. B., Sommer, M., Oh, W., Giletti, B., and Schwartz, R.: Kinetic studies with [^{13}C]-U-Glucose in pregnant women and their offspring. *Pediatr. Res. (Abstract)*, **13**: 357 (1979).
- Freinkel, N.: Effects of the conceptus on maternal metabolism during pregnancy. In: Leibel, B. S., Wrenshall, G. S. Eds.: *On the Nature and Treatment of Diabetes*. p. 679, Excerpta Medica, (Amsterdam, 1965).
- Freminet, A. and LeClerc, L.: Effect of fasting on glucose, lactate and alanine turnover in rats and guinea-pigs. *Comp. Biochem. Physiol.*, **65**: 363 (1980).
- Gilbert, M. and Leturque, A.: Renouvellement du glucose plasmatique chez la ratte vierge et la ratte gestante. *J. Physiol. (Paris)*, **75**: 81a (1979).
- Hay, W. W.: Fetal glucose metabolism. *Semin. Perinatol.*, **3**: 157 (1979).
- Hay, W. W., Sparks, J. W., Quissell, B. J., Battaglia, F. C., and Meschia, G.: Simultaneous measurements of umbilical uptake, fetal utilization rate and fetal turnover rate of glucose. *Am. J. Physiol.*, **40**: E662-668 (1981).
- Huggett, A. S. G. and Nixon, D. A.: Use of glucose oxidase, peroxidase and O-dianisidine in determination of blood and urinary glucose. *Lancet*, **2**: 368 (1957).
- Ibsen, H. L.: Prenatal growth in guinea-pigs with special reference to environmental factors affecting weight at birth. *J. Exp. Zool.*, **51**: 51 (1928).
- Judson, G. J. and Leng, R. A.: Estimation of the total entry rate and resynthesis of glucose in sheep using glucoses uniformly labelled with [^{14}C] and variously labelled with [^3H]. *Aust. J. Biol. Sci.*, **25**: 1313 (1972).
- Kalhan, S. C., D'Angelo, L. J., Savin, S. M., and Adam, P. A. J.: Glucose production in pregnant women at term gestation. *J. Clin. Invest.*, **63**: 388 (1979).
- Leitch, I., Hytten, F. E., and Billewicz, W. Z.: The maternal and neonatal weights of some mammalia. *Proc. Zoo. Soc. (London)*, **133**: 11 (1959).
- Metzger, B. E., Hare, J. W., Hinsdale, E., and Freinkel, N.: Glucose kinetics and the role of insulin during the hypoglycemia of fasting in late pregnancy. *Diabetes (Abstract)*, **23**: 342 (1974).
- Moll, W., Künzel, W., and Ross, H. G.: Gas exchange of the pregnant uterus of anaesthetized and unanaesthetized guinea-pigs. *Respir. Physiol.*, **8**: 303 (1970).
- Ogata, E. S., Sanders, L., Metzger, B., and Freinkel, N.: Maternal metabolic protection by fasting hypoglycemia of pregnancy. *Pediatr. Res. (Abstract)*, **13**: 480 (1979).
- Sparks, J. W., Pegorier, J. P., Girard, J., and Battaglia, F. C.: Substrate concentration changes during pregnancy in the guinea-pig studied under unstressed steady-state conditions. *Pediatr. Res.*, **15**: 1340 (1980).
- Wolfe, R. R., Spitzer, J. J., Miller, H. I., and Elahi, D.: Effects of insulin infusion on glucose kinetics in normal and burned guinea-pigs. *Life Sci.*, **19**: 147 (1976).
- Wolfe, R. R., Miller, H. I., Elahi, D., and Spitzer, J. J.: Effect of burn injury on glucose turnover in guinea-pigs. *Surg. Gynecol. Obstet.*, **144**: 359 (1977).
- Wolfe, R. R., Miller, H. I., and Spitzer, J. J.: Glucose and lactate kinetics in burn shock. *Am. J. Physiol.*, **232**: E415 (1977).
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