Glucose metabolism in the female rat adipocyte: lipid synthesis from glucose during pregnancy and progesterone treatment

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(Received 28 July 1982)

SUMMARY

Pregnancy and progesterone treatment of ovariectomized rats decrease glucose metabolism through the pentose-phosphate pathway in isolated female rat adipocytes. As demonstrated in previous studies, progesterone directly decreases \([1-^{14}C]\)glucose oxidation through the pentose-phosphate pathway and lipogenesis from \([6-^{14}C]\)glucose; the present study therefore compared glucose-induced lipid synthesis during pregnancy (10, 16 and 20 days) with the effect of progesterone treatment (5 mg/rat per day for 14 days) to shed more light on the role of this steroid in glucose metabolism during pregnancy. The inhibition of \([6-^{14}C]\)glucose incorporation into triacylglycerols in the progesterone-treated rats was comparable to that which occurs during late (20 days) and mid-pregnancy (16 days) but not during early pregnancy (10 days). The inhibition of fatty acid synthesis was more important as pregnancy advanced and was different from the decrease in fatty acid synthesis induced by progesterone treatment. The sensitivity to insulin was comparable in virgin, ovariectomized and progesterone-treated ovariectomized rats but not in pregnant rats. This implies that progesterone and insulin affect glucose-induced lipid synthesis by distinct processes and that the impaired glucose metabolism is characterized by a reduction in basal glucose utilization rather than by an impaired insulin response.

INTRODUCTION

Many data, in several animal species, have shown that glucose metabolism is decreased during pregnancy and resistance to insulin often appears (Burt, 1956; Sutter, Félix, Sutter-Dub, Jacquot & Leclercq, 1970; Rushakoff & Kalkhoff, 1979; Sutter-Dub, Dazey, Vergnaud & Madec, 1981). Progesterone seems to be one of the factors implied in this resistance (Beck, 1969; Kalkhoff, Jacobson & Lemper, 1970; Sutter-Dub, Leclercq, Félix, Jacquot & Sutter, 1972; Sutter-Dub, Leclercq, Félix, Jacquot & Sutter, 1973; Sutter-Dub, Aerts, Van Assche, Faure & Sutter, 1977; Kalkhoff, Kissebah & Kim, 1978; Sutter-Dub, 1979; Shirling, Ashby & Baird, 1981; Sutter-Dub, Dazey, Vergnaud & Madec, 1981). The following steps have been shown to be inhibited during pregnancy and in progesterone-treated rats: glucose uptake and oxidation to \(\text{CO}_2\) in fat cells and in muscle and glucose incorporation into glycogen of the muscle (Sutter et al. 1970; Salans, 1971; Sutter-Dub & Dazey, 1977; Rushakoff & Kalkhoff, 1979; Sutter-Dub, Dazey, Madec, Vergnaud & Sutter, 1980; Sutter-Dub & Dazey, 1981). However, some of the observations on adipose tissue metabolism during pregnancy and progesterone treatment, obtained from separate studies conducted under different experimental conditions, were contradictory (Knopp, Herrera & Freinkel, 1970; Salans, 1971; Smith, 1973; Shirling et al. 1981; Sutter-Dub, Dazey, Vergnaud & Madec, 1981). The purpose of the present investigation was therefore to compare, in the same experimental conditions, the changes in glucose incorporation into
lipids during pregnancy and after progesterone treatment and to shed more light on the possible participation of progesterone in the insulin resistance of pregnancy.

MATERIALS AND METHODS

Parametrial fat pads were obtained from female Wistar rats (CF strain from the CNRS, Paris) weighing 130–140 g at the beginning of the experimental period and housed under conditions described earlier (Sutter-Dub et al. 1973).

Experimental animals

The glucose-induced lipogenesis was determined by studying rats in the following endocrine conditions: (1) virgin females, considered in each experimental series as basic control animals, belonging in each case to the same litter as the pregnant or operated rats, (2) pregnant rats (pregnancy was determined as described previously; Sutter-Dub et al. 1973) at 10, 16 and 20 days of gestation, (3) ovariectomized rats operated on the same day as group 4, (4) ovariectomized rats treated with 0.9% (w/v) NaCl solution, oil or progesterone. Treatment with progesterone (Codex BP 68 NF JP; Roussel-Uclaf, Romainville, France; 5 mg/rat per day) was performed as described previously (Sutter-Dub, Faure, Aerts & Van Assche, 1978). Treatment began 1 day after ovariectomy and continued for 14 days.

Incorporation of glucose into triacylglycerols and fatty acids

Fat cells were isolated by the method of Rodbell (1964) with slight modifications (Sutter-Dub, Dazey, Hamdan & Vergnaud, 1981). No differences in the fragility of fat cells were observed during pregnancy or after the different treatments as assessed with our controls (Trypan blue exclusion test, lipid film on the surface of the cell pellet and yield of cells isolated per gram of adipose tissue). The cell number and size were determined by counting an aliquot of the samples as described by Gliemann (1967). The cells were incubated with 0.25 µCi [6-14C]glucose (CEA, Gif-sur-Yvette, France; sp. act. 51.2 Ci/mol) in a Krebs-Ringer bicarbonate buffer containing 2% dialysed bovine serum albumin (Sigma, St Louis, Missouri, U.S.A.) and 0.55 mm-unlabelled D-glucose (KRBGA buffer). Samples (6 x 10^5 to 7 x 10^5) of fat cells of the concentrated cell solution were transferred into the incubation flasks in a final volume of 1 ml KRBGA buffer at pH 7.4. The flasks were gassed with 95% O_2 : 5% CO_2 and incubated for 2 h, with gentle shaking (60 cycles/min). The labelled lipids were extracted according to Dole & Meinertz (1960) and the radioactivity of these extracts was considered to represent the triacylglycerols. Labelled fatty acids were extracted and counted for radioactivity as described earlier (Sutter-Dub, Dazey, Hamdan & Vergnaud, 1981).

Insulin effect

In all experiments the insulin effect was assessed by adding 4 ng porcine insulin/ml (Novo Industries, Copenhagen) to the incubation medium.

Statistical analysis

Results are expressed as means ± s.e.m. Student’s paired t-test was used to assess the insulin effect; other data were compared using Student’s t-test for non-paired values.

RESULTS

Incorporation of [6-14C]glucose into triacylglycerols and fatty acids

After a 2-h incubation a relative decrease in basal [6-14C]glucose incorporation into triacylglycerols and fatty acids could be demonstrated in the adipose cells of pregnant and progesterone-treated ovariectomized rats (Figs 1 and 2).
**Pregnancy, progesterone and lipogenesis**

![Graph](image_url)

**Fig. 1.** Effect of pregnancy on the conversion of [6-14C]glucose to (a) fatty acids and (b) triacylglycerols by isolated adipocytes in the absence (open bars) or presence (hatched bars) of insulin (4 ng/ml). Lipids were extracted after incubation (2 h at 37 °C) of adipocytes from rats at the indicated stages of pregnancy and compared with control virgin (V) rats. Values are expressed in nmol glucose converted to product/10^6 cells per 2 h. Data are the means ± s.E.M. of three to five separate experiments with a pool of cells from two to three animals. *P<0.05 compared with fat cells in the absence of insulin (Student’s paired t-test). †P<0.05 compared with the equivalent group at the other stages of pregnancy (Student’s t-test).

![Graph](image_url)

**Fig. 2.** [6-14C]Glucose incorporation into (a) fatty acids and (b) triacylglycerols of the fat cells of ovariectomized (OVX), progesterone-treated and control rats in the absence (open bars) and presence (hatched bars) of insulin (4 ng/ml). OVX rats were treated with NaCl, olive oil or progesterone (P) for 14 days starting 1 day after ovariectomy. Lipids were extracted after a 2-h incubation (37 °C). Values are expressed in nmol glucose converted to product/10^6 cells per 2 h. Data are the means ± s.E.M. of three to four separate experiments with a pool of cells from nine to ten animals. *P<0.05, **P<0.02, ***P<0.01, ****P<0.001 compared with fat cells in the absence of insulin (Student’s paired t-test). †P<0.05 compared with oil-treated group in the absence and presence of insulin (Student’s t-test).

**Pregnant rats**

Figure 1 shows that the rate of inhibition of [6-14C]glucose incorporation into fatty acids increased from days 10 to 20 of pregnancy whereas the incorporation into triacylglycerols reached a plateau after day 16 of pregnancy. The response to insulin was not significantly different from that of the control rats on day 10 of pregnancy but fatty acid synthesis in...
insulin-treated cells was decreased on day 16 and synthesis of fatty acids and triacylglycerols was decreased on day 20 of pregnancy.

**Ovariectomized rats**

The effects of ovariectomy and treatment with NaCl, olive oil and progesterone are illustrated in Fig. 2. In the absence or presence of insulin, ovariectomy alone decreased slightly but not significantly the glucose-induced synthesis of triacylglycerols and fatty acids. Treatment with olive oil had no effect on this inhibition of [14C]triacylglycerol and [14C]fatty acid synthesis. Progesterone treatment decreased fatty acid and triacylglycerol synthesis. Insulin increased glucose-induced fatty acid synthesis but not triacylglycerol synthesis in the progesterone-treated group.

**DISCUSSION**

Studies of the metabolism of differentially labelled glucose in adipose tissue have indicated that the oxidative pathway plays an important part in glucose metabolism (Sutter-Dub & Vergnaud, 1981). It appears that the rate of fatty acid synthesis is related to the amount of glucose oxidized by means of the pentose-phosphate shunt (Winegrad & Renold, 1958). In previous experiments we demonstrated that the oxidation of [1-14C]glucose was decreased in adipocytes of pregnant or ovariectomized progesterone-treated rats (Sutter-Dub, Dazey, Vergnaud & Madec, 1981).

[1-14C]Glucose oxidation and [6-14C]glucose incorporation into triacylglycerols and fatty acids were similarly decreased by the direct action of progesterone added in the medium during a 2-h incubation (Sutter-Dub, Dazey, Hamdan & Vergnaud, 1981). Since there is clear evidence of a relationship between lipogenesis and the pentose-phosphate pathway, the results gained here were in good agreement with the inhibitions we observed previously. The present study (results not given) also confirmed the results of others concerning the hypertrophy of parametrial adipocytes during mid-pregnancy and the decrease in their size during late pregnancy (Flint, Sinnett-Smith, Clegg & Vernon, 1979). The decreased glucose-induced lipogenesis at the stages of pregnancy studied here were also in general agreement with results of Flint et al. (1979) except for our results obtained on day 10 of gestation. Smith (1973) observed a decrease only at the end of pregnancy, but in his work there was a large difference between rats which had not been mated and rats at 28 days post partum and no difference in the incorporation of [1-14C]glucose and [6-14C]glucose into fatty acids. Our results also differed from those of Knopp et al. (1970) who were the only authors to notice a significant increase in the rate of fatty acid synthesis in perirenal adipocytes by mid-pregnancy. When glucose-induced lipogenesis is studied in progesterone-treated rats, results vary between different laboratories, probably because of the different experimental conditions.

Incorporation of [6-14C]glucose into triacylglycerols and fatty acids was decreased by progesterone treatment. Shirling et al. (1981) showed an increase in glucose oxidation and lipogenesis but they used gonadally intact female rats with progesterone implants. In gonadally intact rats (or in ovariectomized rats given oestradiol) treatment with progesterone increases body weight and food intake; in contrast, the treatment has no effect on food intake or adiposity in ovariectomized rats (Wade & Gray, 1979). The increase in fatty acid synthesis observed by Shirling et al. (1981) may therefore be attributable to the presence of the intact ovaries and related to the weight gain of the rats. It is difficult to compare our results with those obtained after a single injection of progesterone (1 mg/rat) 3 h before the experiment (Hansen, Fahmy & Nielsen, 1980). Nevertheless, results obtained with other steroids (cortisol, corticosterone, cortisone, desoxycorticosterone) which also decrease glucose uptake and oxidation (Leboeuf, Renold & Cahill, 1962) were comparable.
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to our results. Treatment with these steroids decreases the stimulation of lipid synthesis from glucose in rat adipose tissue (see references in Jeanrenaud & Renold, 1964).

Notwithstanding the variations observed between different studies on the effects of progesterone treatment, which are probably related to differences in experimental conditions, the aim of the present study was to compare glucose-induced lipid synthesis during pregnancy with the effect of progesterone alone, to shed more light on the role of this steroid in the insulin resistance of pregnancy. It is evident from our results that the inhibition of \([6-^{14}C]\)glucose incorporation into triacylglycerols in the progesterone-treated rats was comparable to that which occurs during late and mid-pregnancy but not during early pregnancy. Moreover, the inhibition of fatty acid synthesis was more important as pregnancy advanced and was different from the progesterone-induced decrease in fatty acid synthesis. Furthermore, insulin stimulation of fatty acid synthesis was depressed on days 16 and 20 of pregnancy whereas this effect was not affected by progesterone treatment.

In contrast, the decreased insulin action on triacylglycerol synthesis was comparable in progesterone-treated and 20-day pregnant rats. We cannot therefore exclude the possibility that during the latter half of pregnancy adipose tissue anabolism is challenged by the emergence of high plasma titres of contra-insulin hormones such as corticosterone and placental lactogen or by changes in insulin kinetics. Insulin sensitivity is reduced in fat cells of gonadally intact progesterone-treated rats (Shirling et al. 1981) as well as in those of ovariectomized progesterone-treated rats (our results) suggesting that direct insulin inhibition was not the explanation. This would imply that progesterone and insulin affect glucose metabolism through different mechanisms.

Furthermore, from these and our previous results showing that progesterone decreases glucose phosphorylation by reducing the hexokinase isoenzyme II activity (Sutter-Dub & Vergnaud, 1982) and glucose oxidation through the pentose-phosphate pathway (Sutter-Dub & Vergnaud, 1981), it cannot be concluded whether progesterone affects directly both lipogenesis and pentose-phosphate shunt activity, or whether the decreased rate of the pentose-phosphate pathway is a consequence of the decreased fatty acid synthesis, a point which we have to clarify in further studies.

The authors are indebted to Novo Industries (Copenhagen) for the generous gift of crystalline porcine insulin and to the Roussel-Uclaf Laboratoires for the generous gift of progesterone. This work was supported by grants from CNRS—Aide Individuelle no. 278, from the Fondation pour la Recherche Médicale Française and from the Institut National de la Santé et de la Recherche Médicale C.R.L. no. 824017.

REFERENCES


