Effect of insulin on glucose metabolism in adipocytes from virgin and late-pregnant rats

Armelle LETURQUE,* Michèle GUERRE-MILLO,† Marcelle LAVAU† and Jean GIRARD*

*Centre de Recherches sur la Nutrition du CNRS, 9 rue Jules Hetzel, 92190 Meudon-Bellevue, France, and

†Unité de Recherche sur la Physiopathologie de la Nutrition, INSERM U.177,

Institut Biomédical des Cordeliers, 15–21 rue de l'Ecole de Médecine, 75270 Paris Cedex 06, France

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Under basal conditions (zero insulin), paraovarian adipocytes from 19-day-pregnant rats exhibited the same rates of $[U-^{14}C]$ glucose conversion into CO_2 and total lipids as did those from age-matched virgin rats. The dose–response curves for insulin stimulation of glucose metabolism were similar in both groups: maximal response (+100% over basal values) and high sensitivity (half-maximal effect at 0.05 nM-insulin). The present results suggest that the insulin resistance *in vivo* that occurs during late pregnancy may involve circulating factors lost *in vitro*.

Overall insulin resistance has been demonstrated *in vivo* during late pregnancy in both human and rat (Burt, 1956; Kalkhoff *et al.*, 1964; Knopp *et al.*, 1970a; Costrini & Kalkhoff, 1971; Leturque *et al.*, 1980). In addition, by using the euglycaemic–hyperinsulinaemic-clamp technique, the insulin resistance of pregnancy has been characterized by a decreased sensitivity to insulin of tissues both producing and utilizing glucose (Leturque *et al.*, 1984). However, the effects of pregnancy on insulin action *in vitro* remain controversial. Rushakoff & Kalkhoff (1981) have reported that the stimulation of glucose uptake by insulin in perfused hindlimb was lower in starved pregnant rats than in starved virgin rats, whereas Leturque *et al.* (1981) have shown that insulin stimulates glucose transport and metabolism to the same extent in incubated soleus muscle of fed virgin and pregnant rats. Further studies have shown that maximal insulin concentrations stimulated glucose uptake and metabolism to the same extent in pieces of adipose tissue from pregnant and virgin rats (Leake & Burt, 1969; Knopp *et al.*, 1970b), thus suggesting that insulin responsiveness is not altered by pregnancy. However, insulin dose–response curves were not examined in those studies. More recent work using isolated adipocytes was not conclusive, since maximal insulin concentrations increased glucose metabolism by less than 40% over basal values in pregnant as well as in virgin rats (Flint *et al.*, 1979; Sutter-Dub *et al.*, 1981, 1983), conditions under which reliable dose–response curves to insulin cannot be con-

structed. More recently, we have shown that gonadal adipocytes from mature virgin female rats are exquisitely sensitive and responsive to insulin in terms of glucose metabolism, with insulin concentrations eliciting half-maximal stimulation as low as 0.05 nM, and maximal insulin effects greater than 100% over the basal rates (Guerre-Millo *et al.*, 1985). This prompted us to investigate the effects of insulin on glucose metabolism in isolated adipocytes from pregnant rats in order to clarify whether pregnancy induced a change in the insulin sensitivity and/or responsiveness of these cells.

Experimental

Animals

Female Wistar rats obtained from IFFA-CREDO (Fresnes, France) at 70 ± 2 days were fed *ad libitum* on laboratory chow (carbohydrate 65%, protein 24% and fat 11% of total energy). They were housed at 24°C with light from 07:00 to 19:00 h. Half the females were housed with males overnight, and the stage of pregnancy was determined as described previously (Leturque *et al.*, 1980). Pregnant rats with less than eight foetuses were not included in this study. Rats were studied on day 19 of gestation, since previous studies have shown that insulin resistance *in vivo* was maximal at this stage of pregnancy (Leturque *et al.*, 1980, 1984). In each experiment, age-matched virgin rats were used as controls. All these studies were performed on rats between 91 and 113 days of age. The mean body weight was 226 ± 11 g ($n = 14$) for

virgin rats and 295 ± 14 g ($n = 7$) for pregnant rats. Each experiment was done on the paraovarian adipocytes from two virgin rats and one pregnant rat.

Preparation of isolated adipocytes

All studies were begun between 09:00 and 10:00 h. Animals were killed by a blow to the head, and paraovarian adipose tissue was removed. Isolated fat-cells were prepared by the method of Rodbell (1964) with the modifications described previously (Lavau *et al.*, 1979). Fat-cell sizes were determined by a photomicrographic method (Lavau *et al.*, 1977).

Glucose metabolism

The major pathways of glucose metabolism in adipocytes (i.e. conversion into CO_2 and into total lipids) were studied as previously described (Lavau *et al.*, 1979). Triplicate samples of adipocyte suspensions were incubated with 5 mM-[U- ^{14}C]-glucose (sp. radioactivity 300 Ci/mol; CEA, Saclay, France) in Krebs-Ringer bicarbonate buffer (Krebs & Henseleit, 1932), pH 7.4, containing bovine serum albumin (30 mg/ml) without or with insulin at concentrations in the range 0.06–2.5 nM. After 2 h, the incubation was terminated by addition of 3 M- H_2SO_4 into the flasks. The generated $^{14}\text{CO}_2$ was collected in Hyamine contained in a centre well. Total lipids then were extracted with a heptane/propan-2-ol mixture (Dole & Meinertz, 1960) and used for the measurement of ^{14}C incorporation.

Fatty acid synthetase

Fat-cells suspended in ice-cold 0.25 M-sucrose buffer containing 3 mM-dithiothreitol and 1 mM-EDTA, pH 6.8, were sonicated for 15 s (M.S.E.

instrument: medium power, amplitude 4) and then centrifuged at 100000 g at 0°C for 60 min. Fatty acid synthetase activity was measured spectrophotometrically in the supernatants by monitoring the oxidation of NADPH in the presence of acetyl-CoA and malonyl-CoA by the method of Martin *et al.* (1961). The assays were run at 37°C in duplicate, a Perkin-Elmer model 555 spectrophotometer being used. Conditions were selected to ensure linear rates with respect to time (up to 8–10 min) and sample concentration.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. *P* values were obtained by the non-paired Student's *t* test.

Results and discussion

The size of paraovarian adipocytes was significantly greater in 19-days-pregnant than in virgin rats (300 ± 18 versus 230 ± 16 ng of lipids/cell; $P < 0.01$), in agreement with previous studies (Bershtein & Aleksandrov, 1977; Flint *et al.*, 1979; Steingrimsdottir *et al.*, 1980).

The rates of CO_2 production and of lipid synthesis in the absence or in the presence of insulin were substantially higher in adipocytes from pregnant than from virgin rats (Table 1). However, the differences between the two groups of rats did not reach the level of statistical significance, probably because of the large variability between the pregnant rats. These observations are in agreement with the data reported previously by Leake & Burt (1969) and Knopp *et al.* (1970b), using pieces of adipose tissue. In contrast, Sutter-Dub *et al.* (1981, 1983, 1984) reported lower rates of glucose metabolism in adipocytes from pregnant rats than in adipocytes from virgin rats in the absence or in the

Table 1. *Insulin effect on glucose metabolism in paraovarian adipocytes from age-matched virgin and 19-day-pregnant rats*
Results are the means $\pm$ S.E.M. for seven experiments performed in triplicate, on different days. In each experiment, the paraovarian adipose tissue from two virgin rats and one pregnant rat was used. Fat-cells were incubated in 2.5 ml of Krebs-Ringer bicarbonate buffer containing 30 mg of albumin/ml, pH 7.4, at 37°C for 2 h. D-[U- ^{14}C]Glucose was present at a final concentration of 5 mM.

Animals	Insulin (nM)	[U- ^{14}C]Glucose conversion (nmol/2 h per 10^6 cells) into		
		CO_2	Total lipids	CO_2 + lipids
	0	133 ± 11 (7)	214 ± 16 (7)	350 ± 25 (7)
	0.06	257 ± 36 (7)	351 ± 38 (7)	600 ± 70 (7)
	0.18	308 ± 43 (7)	402 ± 48 (7)	710 ± 90 (7)
	0.36	327 ± 50 (5)	430 ± 62 (5)	760 ± 100 (5)
	2.50	333 ± 50 (7)	423 ± 56 (7)	760 ± 100 (7)
19 Days pregnant	0	201 ± 46 (7)	281 ± 35 (7)	480 ± 80 (7)
	0.06	393 ± 78 (7)	460 ± 82 (7)	850 ± 150 (7)
	0.18	512 ± 100 (7)	614 ± 125 (7)	1130 ± 260 (7)
	0.36	634 ± 131 (5)	720 ± 142 (5)	1350 ± 260 (5)
	2.50	562 ± 117 (7)	652 ± 122 (7)	1200 ± 230 (7)

presence of insulin, but under their experimental conditions (0.5 mM-glucose) glucose metabolism might have been limited by the rates of glucose transport (Foley *et al.*, 1980). Our data, obtained under conditions where glucose transport is not rate-limiting for glucose utilization (5 mM-glucose), do not rule out the possibility that defects in glucose transport could be present in adipocytes from pregnant rats. Nevertheless, as shown in the present study, these defects, if any, are not associated with defects in glucose metabolism at physiological glucose concentration.

When expressed as percentage increase over basal rates, the maximal insulin effects on glucose oxidation and incorporation into lipids was similar in the two groups: $184 \pm 18\%$ in pregnant and $144 \pm 24\%$ in virgin rats for glucose oxidation, and $123 \pm 15\%$ in pregnant and $97 \pm 23\%$ in virgin rats for lipid synthesis. The large effect of insulin observed in the present studies confirms our observations that gonadal adipocytes from female rats are highly responsive to the hormone (Guerre-Millo *et al.*, 1985). When the data were expressed as a percentage of the maximal insulin effect (Fig. 1), the insulin-dose-response curves of glucose metabolism (labelled CO_2 plus labelled lipids) of the two groups were quite comparable. The mean ED_{50} of insulin (concentration eliciting half-maximal stimulation) calculated from the individual dose-response curves was similar in pregnant and in

virgin rats (0.06 ± 0.006 and 0.05 ± 0.006 nM respectively). This demonstrates clearly that pregnancy does not impair the insulin sensitivity of glucose metabolism in adipocytes.

In keeping with the above glucose-metabolism data, the activity of fatty acid synthetase, measured in the same cell suspensions as those used for glucose-metabolism experiments, was substantially higher in adipocytes from pregnant rats (89 ± 15 nmol of NADPH/min per 10^6 cells) than in virgin rats (54 ± 17 nmol of NADPH/min per 10^6 cells). However, this difference was not statistically significant.

This work provides evidence that glucose metabolism in adipocytes from pregnant rats remains as sensitive and as responsive to insulin as that in adipocytes from virgin rats under physiological concentrations of glucose and insulin. Therefore, there is no intrinsic alteration in adipocytes from pregnant rats in their capacity to metabolize glucose that could account for the insulin resistance *in vivo* exhibited by late-pregnant rats. However, this conclusion, drawn from observations made for only one adipose-tissue site, must be extended to the whole-body fat with caution, as large differences in insulin responsiveness and sensitivity between adipocytes from various fat depots have been demonstrated in female (M. Guerre-Millo, A. Leturque, J. Girard & M. Lavau, unpublished work) as well as in male rats (Fried *et al.*, 1982).

The present work on adipocytes, coupled with previous studies on isolated soleus muscle (Leturque *et al.*, 1981) and on isolated hepatocytes (Davidson, 1984) showing that pregnancy has no effect on insulin action, strongly suggests that the insulin resistance *in vivo* observed in late-pregnant rats could result from factors lost *in vitro*, i.e. circulating factors. Increased plasma concentrations of hormones such as progesterone (Sutter-Dub *et al.*, 1973) and placental lactogen (Shiu *et al.*, 1973), and/or of non-esterified fatty acid and triacylglycerol (Scow *et al.*, 1964; Knopp *et al.*, 1973) that counteract insulin effects (see Kalkhoff *et al.*, 1978) are likely candidates.

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Fig. 1. [$U\text{-}^{14}\text{C}$]Glucose metabolism: percentage of the maximal insulin effect

This was calculated from the ratio: $100 \times (\text{insulin-stimulated minus basal}) / \text{maximum increment at } 2.5 \text{ nM-insulin}$. Glucose metabolism values were obtained from the sum of glucose oxidation and glucose incorporation into total lipids. Data represent the means $\pm$ S.E.M. for the same experiments as in Table 1. Rats: $\circ$, virgin; $\bullet$, pregnant.

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