Evidence that stimulation of glucose metabolism by insulin is not altered in isolated soleus muscle of pregnant rats

Armelle LETURQUE, Pascale SATABIN, Pascal FERRÉ and Jean R. GIRARD*

Laboratoire de Physiologie du Développement, Collège de France, Place Marcelin Berthelot, 75231 Paris
Cedex 05, France

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Various concentrations of insulin stimulated to the same extent glucose transport and metabolism in incubated soleus muscle of virgin and 19-day-pregnant rats. This suggests that the resistance to insulin that occurs during pregnancy *in vivo* does not result from an intrinsic defect in skeletal muscles.

Studies *in vivo* have shown that pregnant rats became progressively resistant to insulin after 16 days of gestation (Knopp *et al.*, 1970; Costrini & Kalkhoff, 1971; Leturque *et al.*, 1980). Insulin resistance is characterized by a decreased sensitivity to insulin of tissues producing and utilizing glucose (Leturque *et al.*, 1980). As there is actually no preparation of isolated hepatocytes sufficiently sensitive to insulin *in vitro* to enable one to perform a dose–response curve, we have chosen to study a tissue in which glucose utilization is controlled directly by changes in plasma insulin concentrations *in vivo*. The major insulin-dependent peripheral tissues are adipose tissue and skeletal muscles, which represent respectively 15% and 45% of body weight (Addis *et al.*, 1936; Peckman *et al.*, 1962). Previous studies have shown that *in vitro* insulin stimulates to the same extent glucose uptake and metabolism in adipose tissue of virgin or pregnant rats (Leake & Burt, 1966; Knopp *et al.*, 1970). As skeletal muscles *in vivo* are responsible for 35% of glucose uptake in response to an intravenous glucose injection (Curtis-Prior *et al.*, 1969), they are likely candidates to be involved in the overall insulin resistance during late pregnancy in the rat. The aim of the present study was to investigate the sensitivity to insulin of incubated soleus muscles of virgin and pregnant rats to find whether pregnancy induced an intrinsic defect in skeletal muscle with regard to insulin-stimulated glucose metabolism.

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% (% of total calories)]. They were housed at 24°C with light from 07:00 to 19:00 h. The stage of pregnancy was determined as described previously (Leturque *et al.*, 1980). The average number of foetuses in each pregnant rat was ten, and pregnant rats with less than eight foetuses were not included in this study. Pregnant 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). Age-matched virgin rats were used as non-pregnant controls.

Incubation of strips of soleus muscles

Rats were killed by cervical dislocation, and hindlimbs were fixed and dissected out to expose the soleus muscles. As intact soleus muscle of adult rats presents critical problems of substrate diffusion (Chaudry & Gould, 1969; Maizels *et al.*, 1977), we have used a soleus-muscle preparation developed more recently which overcomes this difficulty and permits comparison between muscles of different sizes (Crettaz *et al.*, 1980). Strips of soleus muscle weighing 25–35 mg were isolated and separated from the overall muscle mass as described by Crettaz *et al.* (1980). Strips of soleus muscles were stretched on a stainless-steel holder as described by Cuendet *et al.* (1976). Incubations were carried out in a shaking incubator at 37°C, in 25 ml Erlenmeyer flasks containing 4 ml of Krebs–Ringer (Krebs & Henseleit, 1932) bicarbonate buffer, pH 7.4, with 1.5% fatty acid-free bovine serum albumin (Sigma, St. Louis, MO, U.S.A.). Each flask was gassed with O₂/CO₂ (19:1) for 5 min, and then sealed with a rubber stopper. Actual incubations were preceded by a 20 min preincubation, at the end of which flasks and medium were changed and the preparation was gassed again as described above. When the effects of

* To whom reprint requests should be addressed.

insulin were tested, the hormone was added during both preincubation and incubation periods.

Biochemical measurements

The uptake of 2-deoxyglucose by muscles was used as an index of the rate of glucose transport and phosphorylation (Kipnis & Cori, 1960). This glucose analogue is known to be transported by the same glucose carrier system as glucose, phosphorylated to 2-deoxyglucose 6-phosphate, but not metabolized further. Muscles were incubated for 30 min in the presence of 2-deoxy-D-[1-³H]glucose (1 mM, 4 μ Ci/flask) and of 1 mM-pyruvate as energy source. In these conditions the uptake of 2-deoxyglucose was linear with time for 60 min, and therefore initial rate of uptake was measured (Crettaz *et al.*, 1980). The uptake of 2-deoxy-[³H]glucose in muscle was measured as described by Cuendet *et al.* (1976).

[5-³H]Glucose (5 mM, 2 μ Ci/flask) was used to assess the flux of glucose taken up from the incubation medium through glycolysis and glycogen synthesis. [5-³H]Glucose was dried to remove any contaminating ³H₂O before addition to the incubation medium. The rate of glycogen synthesis was measured by the incorporation of [³H]glucose into glycogen, and the rate of glycolysis was determined by the production of ³H₂O from [³H]glucose. Since the metabolism of glucose via the hexose monophosphate pathway is negligible in muscle, and since errors owing to glucose recycling are avoided with [5-³H]glucose (Hue & Hers, 1974), the sum of the glucose metabolized via glycolysis and that incorporated into glycogen equals the net uptake of glucose. Muscle were incubated for 60 min, and ³H₂O in the medium and labelled glycogen were measured as described by Cuendet *et al.* (1976).

Expression of the results

As protein concentration in soleus muscles was similar in pregnant (21.3 ± 0.6 mg/100 mg wet wt. of soleus, $n = 18$) and in virgin rats (21.5 ± 0.6 mg/100 mg wet wt., $n = 18$), the results were expressed per mg wet wt. of soleus muscle.

Chemicals

Pig monocomponent insulin was from Novo Research Institute (Copenhagen, Denmark). Radioactive products were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). Dowex 1 (X2; 200–400 mesh) resin was purchased from Bio-Rad (Richmond, CA, U.S.A.).

Statistics

Results are expressed as means $\pm$ S.E.M. Statistical analysis of the dose–response curves to insulin in pregnant and virgin rats were made by using a two-way analysis of variance.

Results and discussion

Insulin effect on 2-deoxyglucose uptake in muscles of virgin and pregnant rats

The basal value of 2-deoxyglucose uptake (in the absence of insulin) as well as the values of 2-deoxyglucose uptake measured in the presence of various concentrations of insulin in muscles of pregnant or virgin rats are shown in Table 1. At maximal insulin concentration, the increase in 2-deoxyglucose uptake, expressed as percentage of basal value, was similar in pregnant and virgin rats (respectively 143 ± 17 and $150 \pm 29\%$). Therefore the maximal responsiveness to insulin was not altered by pregnancy. When the results were expressed as percentages of maximal insulin effect,

Table 1. *Insulin effect on 2-deoxyglucose uptake, glucose incorporation into glycogen, glucose metabolism via glycolysis and total glucose metabolism in strips of soleus muscles from virgin and 19-day-pregnant rats.*

Details of the procedures are given in the Experimental section. Results are means $\pm$ S.E.M. for the numbers of experiments indicated in parentheses.

Animals	Insulin added (munits/ml)	2-Deoxyglucose uptake (nmol/30 min per mg)	Glycogen synthesis (nmol/h per mg)	Glycolysis (nmol/h per mg)	Total glucose metabolism (nmol/h per mg)
Virgin	0	0.8 ± 0.1 (8)	1.1 ± 0.1 (11)	4.9 ± 0.4 (11)	6.0 ± 0.5 (11)
	0.05	—	1.3 ± 0.3 (6)	5.9 ± 0.4 (6)	7.2 ± 0.6 (6)
	0.1	1.3 ± 0.1 (8)	1.5 ± 0.4 (5)	5.9 ± 0.4 (5)	7.4 ± 0.7 (5)
	0.25	1.5 ± 0.1 (8)	3.0 ± 0.5 (6)	8.6 ± 0.7 (6)	11.6 ± 1.0 (6)
	0.5	—	4.3 ± 0.5 (8)	9.3 ± 0.7 (8)	13.6 ± 1.0 (8)
	1	2.0 ± 0.2 (8)	4.4 ± 0.5 (7)	9.7 ± 0.6 (7)	14.1 ± 1.0 (7)
19 days pregnant	0	0.7 ± 0.1 (8)	0.9 ± 0.1 (11)	4.1 ± 0.4 (11)	5.0 ± 0.5 (11)
	0.05	—	1.0 ± 0.1 (6)	5.4 ± 0.5 (6)	6.4 ± 0.6 (6)
	0.1	1.1 ± 0.1 (8)	1.3 ± 0.5 (5)	5.7 ± 0.5 (5)	7.0 ± 0.6 (5)
	0.25	1.4 ± 0.1 (8)	2.6 ± 0.3 (6)	7.9 ± 0.3 (6)	10.5 ± 0.3 (6)
	0.5	—	3.4 ± 0.3 (8)	8.1 ± 0.8 (8)	11.5 ± 0.9 (8)
	1	1.7 ± 0.1 (8)	3.8 ± 0.3 (7)	8.8 ± 0.7 (7)	12.6 ± 0.8 (7)

the dose-response curves were similar in pregnant and virgin rats (results not shown). These data suggested that *in vitro* the sensitivity to insulin of glucose transport and phosphorylation in muscle was not altered during pregnancy. It has been reported previously in abstract form that 2-deoxyglucose uptake by whole soleus muscle was decreased by 50% in 18–19-day-pregnant rats compared with virgin rats (Soman, 1979). The difference between our experiments and those reported by Soman (1979) might be due to the fact that he used the whole soleus muscle. Indeed it has been shown that glucose metabolism was inversely proportional to soleus-muscle weight when soleus muscles used have a wet weight higher than 25–35 mg (Chaudry & Gould, 1969; Maizels *et al.*, 1977; Crettaz *et al.*, 1980). The weights of soleus muscles of 19-day-pregnant rats are 20% higher than those of virgin rats: 101 ± 2 mg ($n = 18$) versus 80 ± 2 mg ($n = 18$) ($P < 0.01$). So the 50% decrease in 2-deoxyglucose uptake observed by Soman (1979) in muscles of pregnant rats might be due to the greater weight of muscles rather than to a specific effect of pregnancy.

The overall comparison of the values of 2-deoxyglucose uptake by muscles from pregnant and from virgin rats, by using a two-way analysis of variance, showed a significant shift downwards ($P < 0.025$) in muscle of pregnant rats. This shift indicated a defect in glucose transport and phosphorylation in soleus muscle of pregnant rats which was independent of the effect of insulin on soleus muscle.

A similar defect in glucose transport and phosphorylation has been reported previously in soleus muscle of genetically obese mice (Cuendet *et al.*, 1976). The reasons for this defect are at present unknown.

Insulin effect on glucose metabolism in muscles of virgin and pregnant rats

The basal rates of glycolysis, glycogen synthesis and total glucose metabolism (in the absence of insulin) as well as the values measured in the presence of various concentrations of insulin in soleus muscle of pregnant and virgin rats are shown in Table 1. At maximal insulin concentration, the increase in glycolysis, glycogen synthesis and total glucose metabolism, expressed as percentages of the basal value, were similar in pregnant and virgin rats (respectively 98 ± 12 , 296 ± 43 and $146 \pm 19\%$ in muscle of virgin rats and 112 ± 17 , 327 ± 30 and $162 \pm 29\%$ in muscles of pregnant rats). When the results were expressed as percentages of maximal insulin effect, the dose-response curves were similar in pregnant and virgin rats (see Fig. 1 for total glucose metabolism).

This suggested that *in vitro* the sensitivity of glucose metabolism in muscle was not altered by

Fig. 1. Total glucose-metabolism data plotted as a percentage of the maximal insulin effect

This is calculated by dividing the increment in total glucose metabolism (absolute minus basal) at the indicated insulin concentration by the maximal increment in total glucose metabolism at 1 munit/ml for pregnant (○) and virgin (●) rats.

pregnancy. This conclusion is not in agreement with other data published in abstract form (Rushakoff & Kalkhoff, 1979). These authors reported that at comparable insulin concentration (50–500 μ units/ml) glucose uptake in perfused hindlimbs of 12-h-starved pregnant rats was 40–50% lower than in non-pregnant controls. However, as those authors did not indicate how they expressed muscle glucose uptake (μ mol/min or μ mol/min per mg muscle wt.), the discrepancy between their data and our results remains unexplained. The overall comparison of the values of the rate of glycolysis, glycogen synthesis and total glucose metabolism by muscles from pregnant and from virgin rats, by using a two-way analysis of variance, showed a shift downwards (respectively $P < 0.025$, $P < 0.001$, $P < 0.010$) in pregnant rats. The overall decrease in intracellular glucose metabolism in soleus muscle from pregnant rats could be secondary to the defect in glucose transport and phosphorylation, which is not related to a defect in insulin action. This does not preclude any further defect in intracellular glucose metabolism.

Several points may explain the apparent discrepancy between the present results with soleus muscle *in vitro* and overall insulin resistance *in vivo* in late pregnancy. It is possible that some other skeletal muscles behave differently from soleus muscle. Indeed, whole skeletal muscles are com-

posed of 50% white fibres and 50% red fibres, whereas soleus muscle contains 90% red fibres (Ariano *et al.*, 1973). Insulin resistance *in vivo* during late pregnancy is attended by increased contents of plasma non-esterified fatty acids and triacylglycerols in the rat (Scow *et al.*, 1964; Otway & Robinson, 1968; Knopp *et al.*, 1973). A decrease in glucose metabolism has been reported in the rat soleus muscle when incubated with fatty acids (Cuendet *et al.*, 1975). Furthermore, the increments in plasma glucose concentration after an oral glucose load in pregnant women are correlated with the basal concentrations of plasma non-esterified fatty acids and triacylglycerol at the time of glucose ingestion (Freinkel *et al.*, 1974, 1979). Finally, increases in circulating concentrations of hormones such as progesterone and placental lactogen, which counteract insulin effects (see Kalkhoff *et al.*, 1978), may also contribute to overall insulin resistance *in vivo*.

Our results strongly suggest that the state of insulin resistance observed in late pregnancy *in vivo* does not result from an intrinsic defect of skeletal muscle with regard to insulin-stimulated glucose utilization.

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