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Effect of lactation on insulin sensitivity of glucose metabolism in rat adipocytes

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During lactation glucose metabolism in paraovarian adipocytes is characterized by a 40 and 80% decrease of glucose incorporation into CO_2 and fatty acids in the presence of insulin. In contrast with the stimulation by insulin of glucose incorporation into lactate, glycerol remains unchanged. As a result, insulin sensitivity of total glucose metabolism (oxidation and lipid synthesis) is not altered in adipocytes from lactating rats.

Lactation (Rat adipocyte) Glucose metabolism Insulin sensitivity

1. INTRODUCTION

During lactation a number of metabolic adaptations occur to provide a continuous supply of substrates to the mammary gland. Lipogenesis in mammary gland is highly responsive [1] and very sensitive [2] to insulin, the latter being of particular importance in the face of the low plasma insulin concentration that prevails in the lactating rat [1,3,4]. Several studies have shown that fatty acid synthesis was depressed in adipose tissue of lactating rats under both basal conditions and after maximal stimulation by insulin [1,3–8]. This suggests that a reciprocal decrease in the effect of insulin on adipose tissue could contribute to the preferential orientation of glucose towards the mammary gland. However, it was not known whether lactation affected the sensitivity of lipogenesis to insulin in adipose tissue. Moreover, a reduction in overall glucose metabolism in adipose tissue during lactation has not been conclusively demonstrated. The stimulation of glucose incorporation into glyceride-glycerol by maximal insulin concentration was shown to be either sharply decreased [7] or unchanged [4] in adipose tissue of lactating rats when compared with adipose tissue of virgin rats. Conflicting observations were also reported for the effects of lactation on glucose oxidation [5,7]. Moreover, the production of lactate, a significant product of glucose metabolism in adipocytes [9,10], has never been investigated during lactation. The aim of this study was to assess the impact of lactation on insulin sensitivity of glucose metabolism in adipose tissue by using isolated paraovarian adipocytes which were recently shown to be a well-suited system for studies of changes in insulin action [11]. To delineate the effects of lactation per se on adipocyte metabolism, care was taken to compare lactating rats to age-matched rats whose pups were removed immediately after delivery.

2. MATERIALS AND METHODS

2.1. *Animals*

Female Wistar rats were obtained from Iffa-Credo (Fresnes, France) at 60 days of age. They were housed at 24°C with light from 07.00 to 19.00 h, and had free access to water and chow pellets (caloric percentage: carbohydrate 65%, fat 11%, protein 24%). They were mated about 10 days later. At delivery, the number of pups in each

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litter was adjusted to 10 for lactating rats. In the non-lactating control group pups were removed immediately after delivery. All rats were studied 12 days post-partum. At this time the mean body weight was 300 ± 9 g (n = 14) for lactating rats and 274 ± 7 g (n = 13) for non-lactating rats.

2.2. *Preparation of isolated adipocytes*

All studies were begun between 09.00 and 10.00 h. Animals were killed by cervical dislocation, and paraovarian adipose tissue was removed. Each experiment was performed on paraovarian adipose tissue from one lactating and one non-lactating rat. Isolated adipocytes were prepared by the method of Rodbell [12] with the modifications described in [11,13]. Fat-cell sizes were determined by a photomicrographic method [14].

2.3. *Glucose metabolism*

[U-^{14}C]Glucose conversion into CO_2, glyceride-fatty acids and glyceride-glycerol were studied as in [13]. In brief, triplicate aliquots (0.5 ml) of adipocytes (between 2 and 7 × 10^5 cells) suspended in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 3% bovine serum albumin (fraction V, Sigma) and 5 mM glucose were incubated at 37°C in plastic vials containing 2 ml of the same buffer, [U-^{14}C]glucose (300 mCi/mmol, CEA Saclay, France) and insulin concentrations in the range 0–2.5 nM. After 2 h, incubation was terminated by adding 0.5 ml of 3 M H_2SO_4 to the flasks and $^{14}CO_2$ was collected in 0.3 ml hyamine added to a center well. The contents of the incubation flasks were then extracted with a heptane/propan-2-ol mixture [15] and an aliquot used for the measurement of ^{14}C incorporation into total lipids. The remainder of the lipids was saponified and used for measurement of ^{14}C incorporation into fatty acids (organic phase) and glycerol (aqueous phase). Glucose incorporation into lactate was studied in different experiments. Triplicate aliquots of adipocytes were incubated for 2 h, as described for metabolism studies, without or with insulin (2.5 nM). Lactate concentration was determined on the neutralized incubation medium by the lactate dehydrogenase method [16]. Lactate production was calculated as the difference between the concentration of lactate at the end and the beginning of the incubation.

2.4. *Fatty acid synthetase*

The activity of fatty acid synthetase (FAS) was determined in the cell suspensions used for the metabolic study as in [17].

2.5. *Statistical analysis*

Results are expressed as the means ± SE. All p values were obtained by the unpaired Student's t-test.

3. RESULTS

12 days after parturition, the size of paraovarian fat cells was similar in lactating and non-lactating rats, i.e. 248 ± 28 ng (n = 14) vs 257 ± 17 ng (n = 13) lipids per cell. The insulin dose-response curves for [U-^{14}C]glucose oxidation and incorporation into total lipids, fatty acids and glyceride-glycerol in paraovarian adipocytes from lactating and non-lactating rats are shown in fig.1. In the absence of insulin, the pattern of glucose utilization differed between the 2 groups only with regard to the rate of glucose incorporation into fatty acids which was 5-fold lower in lactating than in non-lactating rats (2.6 vs 12 nmol/10^6 cells per 2 h). However, this difference did not result in a detectable change at the level of radioactivity incorporated into total lipids because radioactive fatty acids represent a small part of the total radioactive lipids. There was no significant difference in the rates of glucose oxidation and lactate production (table 1) between the 2 groups. In the presence of maximal insulin concentrations glucose oxidation increased by 140 nmol/10^6 cells per 2 h in non-lactating rats and only by 70 nmol/10^6 cells per 2 h in lactating rats. Moreover, the responsiveness of fatty acid synthesis to insulin was nearly totally abolished in adipocytes from lactating rats with an increment of 10 nmol/10^6 cells per 2 h vs 75 nmol/10^6 cells per 2 h in non-lactating rats. In keeping with this, the activity of a key lipogenic enzyme, the fatty acid synthetase, was decreased in adipocytes from lactating rats as compared with non-lactating rats (11.3 ± 3.4 vs 23.5 ± 3.4 nmol NADPH/min per 10^6 cells, $p < 0.05$). In contrast, with the resistance to insulin of glucose oxidation and fatty acid synthesis, the incorporation of glucose into the glycerol moiety of triglycerides tended to be slightly more responsive to insulin in lactating than in non-lactating rats. This explained why there was

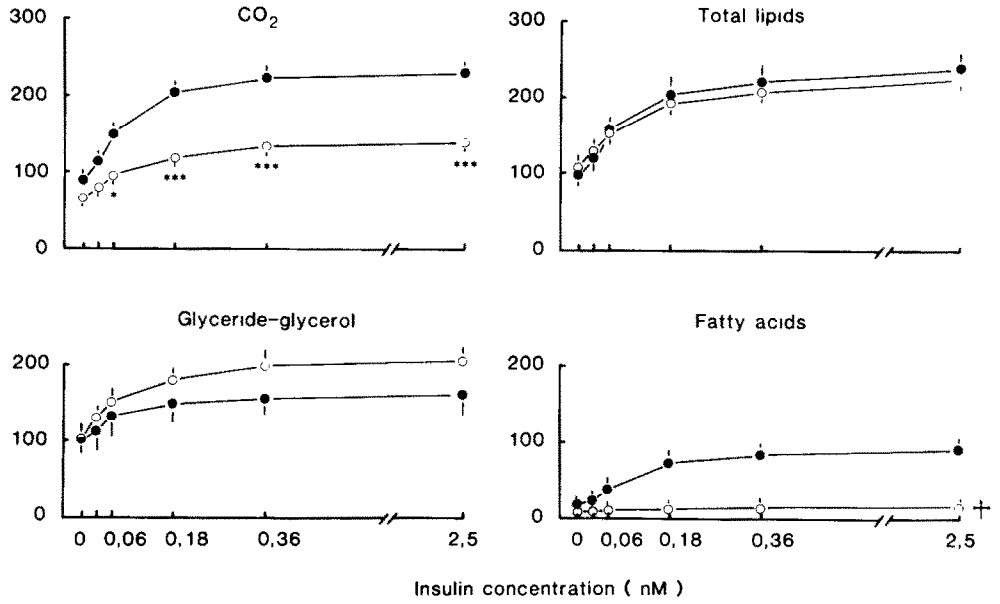

Fig.1. Insulin dose-response curves of glucose metabolism in paraovarian adipocytes from lactating (○—○) and non-lactating (●—●) rats. Each point is the mean ± SE for 4 experiments for glyceride-glycerol and fatty acids and 7 experiments for CO_2 and total lipids. * $p < 0.05$; *** $p < 0.001$ for lactating compared to non-lactating rats. $p < 0.001$ for the entire dose-response curve compared to the control one.

no difference in radioactivity incorporated into total lipids between the 2 groups. Similarly, the insulin responsiveness of lactate production was unaltered by lactation. It is noteworthy that adipocytes from mature female rats, regardless of the physiological state of the animals, produced substantial amounts of lactate (table 1) which accounted for about 40% of total glucose metabo-

Table 1

Lactate production in paraovarian adipocytes from non-lactating and lactating rats

Insulin concentration (nM)	Lactate (nmol/2 h per 10^6 cells)		
	Lactating	Non-lactating	
0	378 ± 135	433 ± 84	NS
2.5	862 ± 138	980 ± 78	NS

In the same experiments [U-^{14}C]glucose incorporation into CO_2 in the presence of 0 and 2.5 mM insulin was 112 ± 31 and 236 ± 52 nmol/2 h per 10^6 cells in adipocytes from lactating rats, and 151 ± 26 and 365 ± 52 nmol/2 h per 10^6 cells in adipocytes from non-lactating rats. Results are the means from 6 experiments. NS, differences between lactating and non-lactating rats not statistically significant

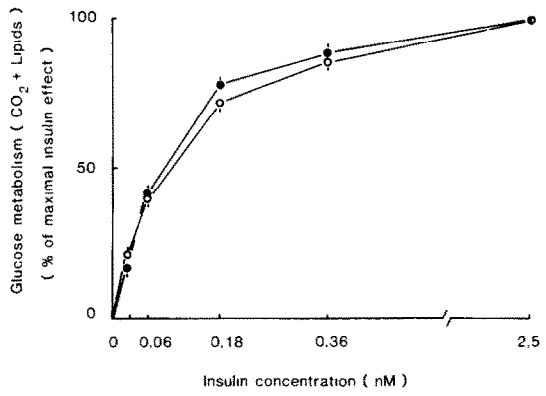

Fig.2. [U-^{14}C]Glucose metabolism: percentage of the maximal insulin effect. (●) Non-lactating, (○) lactating rats. Data are from the experiments presented in fig.1.

lised under basal as well as insulin-stimulated conditions.

When the data presented in fig.1, i.e. the conversion of glucose into CO_2 + lipids, were expressed as percentages of maximal insulin effect, the 2 curves were strictly superimposed (fig.2), demonstrating that the sensitivity of glucose metabolism to insulin was unchanged during lactation.

4. DISCUSSION

The present data, based on studies of 12-day post-partum lactating or non-lactating rats, provide clear evidence that lactation affects selectively the rates of glucose metabolism in various pathways and their response to insulin in isolated paraovarian adipocytes. However, total glucose metabolism, calculated as the sum of glucose incorporated into CO_2, lipids and lactate is only slightly decreased in adipocytes of lactating rats compared to non-lactating rats, in the absence of insulin (−20%) or presence of 2.5 mM insulin (−24%). Fatty acid synthesis is greatly reduced in adipocytes from lactating rats in both the absence or presence of insulin. This is in agreement with previous data obtained in vivo [1,8] and in vitro [5,7]. According to the drop in lipogenesis, a decreased activity of several lipogenic enzymes such as fatty acid synthetase and acetyl-CoA carboxylase has been reported in adipose tissue of lactating rats (this study; [7,18]). Insulin responsiveness of glucose oxidation is also reduced during lactation. However [U-^{14}C]glucose does not allow one to distinguish between CO_2 produced in the pentose phosphate pathway and the citric acid cycle. Thus, differences between adipocytes from lactating and non-lactating rats in the relative importance of these 2 metabolic pathways in the production of CO_2 cannot be excluded from these results. In contrast, no change in insulin responsiveness is observed for glyceride-glycerol and lactate production. In adipocytes from lactating rats the high rate of glyceride-glycerol synthesis compared to fatty acid synthesis (this paper; [4,19]) could be explained by a high rate of re-esterification. Indeed, this observation is in agreement with the study of May [20] reporting a high rate of re-esterification in insulin-resistant adipose cells from obese rats exhibiting a very low rate of fatty acid synthesis. The results expressed in percentage of the maximal insulin effect clearly show that insulin sensitivity of glucose metabolism is unaltered during lactation. This is in agreement with the data of Flint et al. [4] who reported that lactation does not alter insulin binding to adipocytes of lactating rats compared to virgin rats.

In adipose tissue of lactating rats, 2 adaptative patterns, observed over the whole range of insulin concentrations, could be delineated: (i) a decrease in the capacity of the metabolic pathways leading to an irreversible disposal of glucose, namely CO_2 and fatty acid synthesis (decreased by 40 and 80%, respectively); (ii) an unaltered capacity of the pathways leading to the synthesis of C3 precursors, lactate and glycerol. This suggests that glucose metabolism could be regulated at the level of pyruvate dehydrogenase. As the activity of this enzyme is not decreased in adipose tissue of lactating rats [18], a possible defect in the activation of pyruvate dehydrogenase under the influence of insulin during lactation cannot be excluded.

The adaptations of glucose metabolic pathways in adipose tissue of lactating rats permit the sparing of a part of the glucose carbons that could therefore be secondarily used by the mammary gland. Indeed, lactate produced by adipose tissue can be used as lipogenic precursor in liver and mammary gland and then incorporated into milk lipids. Furthermore, it has recently been shown that lipogenesis in the mammary gland is dependent on active hepatic neoglucogenesis in the refeeding state [21]. Then, lactate and glycerol can be incorporated into glycogen by the liver via neogluconeogenesis [22,23], and glucose released can be removed by the mammary gland. These changes in adipose tissue metabolism are probably controlled by the high plasma prolactin-to-insulin ratio that prevails in lactating rats. Indeed, when lactating rats are separated from their pups for 24 h, the plasma prolactin-to-insulin ratio is greatly decreased [8] and glucose metabolism in adipose tissue is then comparable to that observed in non-lactating rats (not shown).

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