Insulin resistance of glucose metabolism in isolated brown adipocytes of lactating rats

Evidence for a post-receptor defect in insulin action

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The mechanism responsible for the insulin resistance described *in vivo* in brown adipose tissue (BAT) of lactating rats was investigated. The effect of insulin on glucose metabolism was studied on isolated brown adipocytes of non-lactating and lactating rats. Insulin stimulation of total glucose metabolism is 50 % less in brown adipocytes from lactating than from non-lactating rats. This reflects a decreased effect of insulin on glucose oxidation and lipogenesis. However, the effect of noradrenaline (8 μ M) on glucose metabolism was preserved in brown adipocytes from lactating rats as compared with non-lactating rats. The number of insulin receptors is similar in BAT of lactating and non-lactating rats. The insulin-receptor tyrosine kinase activity is not altered during lactation, for receptor autophosphorylation as well as tyrosine kinase activity towards the synthetic peptide poly(Glu₄-Tyr₁). The defect in the action of insulin is thus localized at a post-receptor level. The insulin stimulation of pyruvate dehydrogenase activity during euglycaemic/hyperinsulinaemic clamps is 2-fold lower in BAT from lactating than from non-lactating rats. However, the percentage of active form of pyruvate dehydrogenase is similar in non-lactating and lactating rats (8.6 % versus 8.9 % in the basal state, and 37.0 % versus 32.3 % during the clamp). A decrease in the amount of pyruvate dehydrogenase is likely to be involved in the insulin resistance described in BAT during lactation.

INTRODUCTION

Brown adipose tissue (BAT) is known to be the main site of cold-induced non-shivering thermogenesis in rodents (Himms-Hagen, 1976). Actually, it is also regarded as an important component in the regulation of energy balance in laboratory rodents by means of diet-induced thermogenesis. Indeed, it has been shown that thermogenesis in BAT is decreased in obese rodents (Himms-Hagen, 1985), and increased by overfeeding (Rothwell & Stock, 1979). Lactation is characterized by a marked hyperphagia (Fell *et al.*, 1963), occurring simultaneously with modifications of BAT: a decrease in BAT weight in rats (Agius & Williamson, 1980), but an increase in BAT weight in mice (Trayhurn *et al.*, 1982). However, in both rats and mice, lactation is accompanied by a decrease in BAT activity: a decrease in GDP binding (Trayhurn *et al.*, 1982; Villarroja *et al.*, 1987), a decrease in the concentration of mitochondrial uncoupling protein, and a decrease in the proton-conductance pathway activity (Trayhurn & Jennings, 1987; Villarroja *et al.*, 1986). Moreover, BAT lipogenesis and glucose utilization, measured in the basal state as well as under glucose or insulin stimulation, are decreased in lactating rats as compared with non-lactating rats (Agius & Williamson, 1980; Burnol *et al.*, 1983; Ferré *et al.*, 1986). The aim of this work was then to characterize further the mechanisms responsible for the insulin-resistant state observed in BAT of lactating rats. In that attempt we have studied the effect of insulin on the various glucose metabolic pathways in isolated brown

adipocytes. As insulin-resistant states have often been associated with an alteration in the insulin-receptor tyrosine kinase activity, to localize the site of defect in insulin action the number of insulin receptors and tyrosine kinase activity of the insulin receptors have also been studied in BAT of lactating and non-lactating rats.

MATERIALS AND METHODS

Chemicals

Collagenase was purchased from Boehringer (Meylan, France). Pig monocomponent insulin was obtained from Novo (Copenhagen, Denmark). Fatty-acid-free albumin, noradrenaline, Triton X-100, *N*-acetylglucosamine, poly(Glu₄-Tyr₁), bacitracin, aprotinin and phenylmethanesulphonyl fluoride were purchased from Sigma (St. Louis, MO, U.S.A.). [6-¹⁴C]Glucose was obtained from CEA (Saclay, France), [γ -³²P]ATP (3000 Ci/mmol) from Amersham International (Amersham, Bucks, U.K.), and ¹²⁵I-insulin from CEA (Saclay, France). The wheat-germ-agglutinin-agarose was purchased from ICN (Lisle, IL, U.S.A.). All reagents for SDS/polyacrylamide-gel electrophoresis were from Bio-Rad (Richmond, CA, U.S.A.).

Animals

Female Wistar rats were obtained from Iffa-Credo (l'Arbresle, France) at 8 weeks of age. They were housed at 23 °C with light from 07:00 to 19:00 h and had free access to water and chow pellets (percentage composition

Abbreviation used: BAT, brown adipose tissue.

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in terms of energy: carbohydrate 65 %, fat 11 %, protein 24 %). The number of pups was adjusted to 10 in each litter on the day of delivery. In the non-lactating group, pups were removed at delivery. Studies were performed at 11–13 days *post partum* (i.e. at about 16 weeks of age). At this time the mean body weight was 277 ± 4 g ($n = 33$) for lactating rats and 262 ± 49 g ($n = 31$) for non-lactating rats.

Glucose metabolism in isolated brown adipocytes

Isolated brown adipocytes were prepared as described previously (Ebner *et al.*, 1987). The yield of the cell preparations was similar in the two groups of rats (Ebner *et al.*, 1987). In addition, the mean size of the cells was comparable in lactating and non-lactating rats (result not shown). The cell incubations were performed in Krebs–Ringer bicarbonate with 5.5 mM-glucose, in the presence of [6-¹⁴C]glucose (1 μ Ci/vial), and insulin concn. in the range 0–1000 μ units/ml. Glucose incorporation into CO₂, lactate, acylglycerol fatty acids and acylglycerol glycerol was determined as previously described (Ebner *et al.*, 1987). Total glucose metabolism was considered as the incorporation of glucose carbon into CO₂, lactate, acylglycerol glycerol and acylglycerol fatty acids.

Insulin-receptor kinase activity

The preparation of BAT insulin receptors and the measurement of their tyrosine kinase activity was performed as described by Tanti *et al.* (1986) and is briefly summarized below.

Preparation of partially purified insulin receptors. Three to four rats were used for each preparation. They were killed by cervical dislocation, and interscapular BAT was removed. The same weight of BAT for lactating and non-lactating rats (about 1 g) was homogenized in 9 vol. of 50 mM-Hepes (pH 7.6)/150 mM-NaCl/1 % Triton X-100, containing 1 mM-bacitracin, 1000 units of aprotinin/ml and 1 mM-phenylmethanesulphonyl fluoride. The homogenates were solubilized for 90 min at 4 °C, and then centrifuged at 100000 *g* for 90 min at 4 °C. The supernatants were applied to 1 ml of wheat-germ-agglutinin-agarose. Glycoproteins were eluted in 500 μ l fractions with 0.3 M-*N*-acetylglucosamine in a buffer containing 30 mM-Hepes (pH 7.6), 30 mM-NaCl and 0.1 % Triton X-100. Usually the first four fractions were pooled, representing 85–90 % of the total insulin-binding activity recovered. The protein concn. of the eluate determined by the Bradford dye method by using Bio-Rad reagent was 0.46 ± 0.10 mg/ml in non-lactating rats and 0.48 ± 0.11 mg/ml in lactating rats.

Insulin binding to solubilized receptors. Partially purified insulin receptors (10 μ l) were incubated for 16 h at 4 °C in 50 mM-Hepes (pH 7.6)/150 mM-NaCl buffer containing ¹²⁵I-insulin and increasing concentrations of unlabelled insulin. Receptor-bound insulin was precipitated with bovine γ -globulin (0.25 mg/ml final concn.) and poly(ethylene glycol) (12.5 % final concn.) in Hepes/NaCl buffer. The binding was corrected for non-specific binding, determined in presence of 1 μ M unlabelled insulin. The number of insulin receptors of high affinity in each preparation was determined by estimating the abscissa intercept of the Scatchard plot of the insulin-binding data.

Insulin-receptor autophosphorylation. Equivalent amounts of insulin-binding activity were preincubated in Hepes/NaCl buffer, with 0.2 % bovine serum albumin for 90 min, at 20 °C. The phosphorylation reaction was initiated by adding 4 mM-MnCl₂, 8 mM-MgCl₂ and 15 μ M-[γ -³²P]ATP (final concns.). After various periods of time at 20 °C the reaction was stopped by adding the Laemmli (1970) reagent and heating the samples for 2 min at 100 °C. Samples were analysed by SDS/polyacrylamide-gel electrophoresis in a 7.5 %-acrylamide resolving gel in accordance with the method of Laemmli (1970).

Tyrosine kinase activity of insulin receptors. Equivalent amounts of insulin-binding activity were preincubated for 1 h at 20 °C, in Hepes/NaCl buffer containing 0.2 % bovine serum albumin. The exogenous substrate poly(Glu₄-Tyr₁) was added for an additional 30 min (final concn. 0.25 mg/ml). The phosphorylation reaction was initiated by adding 4 mM-MnCl₂, 8 mM-MgCl₂ and 20 μ M-[γ -³²P]ATP (final concn.). After 30 min at 20 °C the reaction was stopped by applying samples on Whatman ET31 paper squares and soaking the papers in 10 % (v/v) trichloroacetic acid containing 10 mM-Na₄P₂O₇ (Braun *et al.*, 1984). The papers were then extensively washed in 5 % trichloroacetic acid/10 mM-Na₄P₂O₇. The radioactivity of the trichloroacetic acid-precipitable material was measured by Čerenkov counting. In preliminary experiments it was checked that phosphorylation of poly(Glu₄-Tyr₁) was linear for 1 h, when measured in the presence or in the absence of insulin.

Phosphatase activity. To assess phosphatase activity, receptors were preincubated with insulin and allowed to self-phosphorylate for 15 min at 20 °C. At the end of this reaction an excess of unlabelled ATP (50 mM) was added. After various periods of time (0–90 min) the reaction was stopped, and the samples were analysed by SDS/polyacrylamide-gel electrophoresis as described above. Any loss of ³²P from the insulin receptor then reflects dephosphorylation by phosphatases.

Euglycaemic-clamp studies

Euglycaemic/hyperinsulinaemic-clamp studies were performed as described previously (Burnol *et al.*, 1986a). The rate of insulin infusion was 1.5 munits/min per kg, allowing a plasma insulin concentration over 1000 μ units/ml to be reached, giving the maximal effect of the hormone in BAT of non-lactating and of lactating rats (Ferré *et al.*, 1986). Rats studied in the basal state were infused with 0.9 % NaCl for 1 h. At the end of the clamp experiment, or after the saline infusion, BAT was quickly removed, frozen in liquid N₂, and stored at –70 °C until the enzyme assays were performed.

Enzyme assay

For measurement of pyruvate dehydrogenase activity, BAT samples were homogenized in 100 mM-potassium phosphate buffer, pH 7.0, containing 5 mM-EDTA, 5 mM-dithiothreitol and 10 mM-pyruvate. Extracts were centrifuged twice for 1 min in an Eppendorf 5414 centrifuge. Pyruvate dehydrogenase was assayed spectrophotometrically by coupling with arylamine acetyltransferase as described by Coore *et al.* (1971). Total activity of pyruvate dehydrogenase was measured after preincu-

bation of the extracts with pig heart pyruvate dehydrogenase phosphatase in the presence of 25 mM-MgCl₂ and 1 mM-CaCl₂ (Stansbie *et al.*, 1976).

RESULTS

Glucose metabolism in isolated brown adipocytes of lactating and non-lactating rats

The effects of various insulin concentrations on [6-¹⁴C]glucose conversion into CO₂, acylglycerol fatty acids, acylglycerol glycerol and lactate are presented in Fig. 1. For any of the metabolic pathways studied, basal glucose utilization is not significantly altered in brown adipocytes of lactating rats as compared with non-lactating rats. In non-lactating rats, glucose metabolism is increased by insulin, reaching a maximum value at insulin concentrations of 100–1000 μ units/ml; glucose oxidation, fatty acid, acylglycerol glycerol and lactate synthesis are increased 2–5-fold. In lactating rats, glucose oxidation is only slightly increased by maximal insulin concentrations, and acylglycerol fatty acid synthesis is not significantly enhanced compared with basal conditions. In contrast, glucose incorporation into lactate and acylglycerol is stimulated 2–3-fold by insulin in brown adipocytes of lactating rats. In both groups of rats, lactate synthesis represents 50–70% and acylglycerol glycerol 6–8% of the total amount of glucose metabolized in the absence or in the presence of insulin. The effect of insulin on total glucose metabolism is presented in Fig. 2. In the absence of insulin, total

glucose metabolism is not significantly altered during lactation. However, at every insulin concentration tested, the effect of insulin on total glucose metabolism is decreased by 40–50% (Fig. 2, Table 1). The results expressed as a percentage of the maximal insulin effect underlined that this represents a decrease in maximal insulin effect without alteration in insulin sensitivity (the insulin concn. giving the half-maximal effect is 17 and 15 μ units/ml for non-lactating and lactating rats respectively; results not shown).

The maximal effect of noradrenaline (8 μ M) on glucose metabolism (Ebner *et al.*, 1987) was also tested in brown adipocytes from non-lactating and lactating rats (Table 1). In the presence of noradrenaline, glucose oxidation is increased 7-fold in brown adipocytes from both non-lactating and lactating rats, and lipogenesis is decreased by respectively 72% and 62% in non-lactating and lactating rats (results not shown). Total glucose metabolism is increased similarly by noradrenaline in the two groups of rats [total glucose metabolism was 1.1 ± 0.2 versus 0.7 ± 0.1 ($n = 5$) in the basal state, and 2.6 ± 0.3 versus 2.0 ± 0.3 ($n = 4$) in presence of 8 μ M-noradrenaline, for non-lactating and lactating rats respectively].

Insulin receptors in BAT of lactating and non-lactating rats

Insulin binding to its receptor, and subsequent autophosphorylation of the β -subunit of the receptor, are the first step of insulin action. A defect in insulin-receptor number or activity could explain the altered

Fig. 1. Insulin dose/response curves of [6-¹⁴C]glucose metabolism in isolated brown adipocytes from non-lactating (◆) and lactating (□) rats

Data represent means $\pm$ S.E.M. of five experiments. In each experiment, for each insulin concentration tested glucose metabolism was determined in triplicate on the same preparation of brown adipocytes, for non-lactating and lactating rats simultaneously. * $P < 0.05$, ** $P < 0.01$ for significance of differences compared with the corresponding non-lactating value, as determined by Student's paired t test.

Fig. 2. Insulin effect on total glucose metabolism in isolated brown adipocytes from non-lactating (◆) and lactating (□) rats

For details, see Fig. 1.

ability of insulin to stimulate glucose metabolism in brown adipocytes during lactation.

Insulin binding. The displacement curve of ^{125}I -insulin bound by increasing amounts of unlabelled insulin is illustrated in Fig. 3. The curve is similar for wheat-germ-agglutinin eluates of BAT from non-lactating and

lactating rats. In addition, the Scatchard analysis of the insulin-binding activity of partially purified insulin receptors is similar in lactating and non-lactating rats (Fig. 3). This implies that the receptor affinity and receptor number are unchanged during lactation. The mean number of insulin receptors of high affinity is respectively 0.83 and 0.79 fmol of insulin-binding activity/ μg of protein for non-lactating and lactating rats.

Insulin-receptor autophosphorylation. The wheat-germ-agglutinin eluates preincubated with various insulin concentrations and exposed to $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ show a dose-dependent increase in ^{32}P incorporation into a 93 000- M_r protein (Fig. 4), which can then be identified as the β -subunit of the insulin receptor. It has been checked that phosphatase activity was negligible in the wheat-germ-agglutinin eluate of BAT from lactating and non-lactating rats (results not shown). The insulin dose-response curve of ^{32}P incorporation into the β -subunit is similar in insulin receptors obtained from BAT of lactating or non-lactating rats (Fig. 4). The bands corresponding to the β -subunit were excised and counted for radioactivity. The ^{32}P incorporated into the β -subunit of the insulin receptor in the presence of maximal insulin concentration was similar in the two groups of rats (520 ± 151 d.p.m. for non-lactating and 535 ± 139 d.p.m. for lactating rats; both $n = 3$) and was subtracted from the insulin-stimulated values. The curve describing the sensitivity to insulin of the autophosphorylation of the insulin receptor is shown in Fig. 4. There is no difference in the stimulation by insulin of the β -subunit autophosphorylation in BAT of lactating and non-lactating rats.

Fig. 3. Equilibrium binding of ^{125}I -insulin to wheat-germ-agglutinin eluates of BAT from non-lactating (◆) and lactating (□) rats

The main Figure shows the displacement curve of ^{125}I -insulin specific binding in the presence of increasing concentrations of unlabelled insulin. The insert shows the same results expressed by the Scatchard plot. Data are the means $\pm$ S.E.M. of eight determinations.

Fig. 4. Autophosphorylation of insulin receptors of BAT from non-lactating and lactating rats

(a) A representative autoradiograph. (b) The β -subunit of the insulin receptor (phosphorylated protein of $M_r \sim 93000$) was excised and counted by Čerenkov counting. The results are expressed as percentage of the maximal effect. The points represent means $\pm$ S.E.M. of three separate experiments: $\square$, lactating; $\blacklozenge$, non-lactating.

Table 1. Pyruvate dehydrogenase activity in BAT of lactating and non-lactating rats in the basal state and during the euglycaemic/hyperinsulinaemic clamp

Values are means $\pm$ S.E.M. of six experiments. Abbreviations: PDH_a , active form of pyruvate dehydrogenase; PDH_t , total pyruvate dehydrogenase, measured in the presence of pig heart pyruvate dehydrogenase phosphatase (see the Materials and methods section). * $P < 0.05$, ** $P < 0.01$ for significance of differences compared with corresponding non-lactating value; † $P < 0.02$ for significant effect of insulin.

	Pyruvate dehydrogenase (units/g wet wt.)			
	Non-lactating		Lactating	
	Basal	Clamp	Basal	Clamp
PDH_a	0.6 $\pm$ 0.1	3.2 $\pm$ 0.3†	0.4 $\pm$ 0.1	1.7 $\pm$ 0.3**†
PDH_t	6.8 $\pm$ 0.1	8.8 $\pm$ 0.5	4.3 $\pm$ 0.4*	5.2 $\pm$ 0.6**
PDH_a/PDH_t (%)	8.6 $\pm$ 1.0	37.0 $\pm$ 2.8	8.9 $\pm$ 2.1	32.3 $\pm$ 2.5

Fig. 5. Tyrosine kinase activity of insulin receptors of BAT from non-lactating ($\blacklozenge$) and lactating ($\square$) rats towards poly(Glu₄-Tyr₁)

Results are expressed as pmol of phosphate incorporated/30 min per pmol of insulin-binding activity of the wheat-germ-agglutinin eluates. The points represent means $\pm$ S.E.M. of three separate experiments.

Tyrosine kinase activity of insulin receptors. Basal phosphorylation of poly(Glu₄-Tyr₁) measured in the absence of insulin is similar in BAT of lactating and non-lactating rats (respectively 233 ± 24 and 286 ± 42 pmol of ^{32}P incorporated in 30 min per pmol of insulin-binding activity). The basal value was then subtracted from the insulin-stimulated values in each experiment, and the data shown in Fig. 5 represent the ability of insulin to stimulate poly(Glu₄-Tyr₁) phosphorylation over basal. The effect of insulin on poly(Glu₄-Tyr₁) phosphorylation is similar in BAT of lactating and non-lactating rats. Maximal insulin concentrations induce a 2.5-fold increase in poly(Glu₄-Tyr₁) phosphorylation in both groups.

Effect of insulin on pyruvate dehydrogenase activity

To test the action of insulin distal to receptor binding and activation, the capacity of insulin to stimulate pyruvate dehydrogenase activity was measured (Table 1). For technical reasons, linked to the low number of isolated brown adipocytes that can be obtained, it was not possible to measure the effect of insulin *in vitro*. The experiments were then performed *in vivo*, by the euglycaemic/hyperinsulinaemic-clamp technique.

In the basal state, pyruvate dehydrogenase activity is similar in non-lactating and lactating rats, but the total amount of pyruvate dehydrogenase is 37% lower in BAT of lactating than of non-lactating rats. During the euglycaemic/hyperinsulinaemic clamp, insulin stimulates pyruvate dehydrogenase activity, but the initial activity of the enzyme reached at the end of the clamp is 2-fold lower in lactating than in non-lactating rats (Table 1). However, in the basal state as well as during the euglycaemic/hyperinsulinaemic clamp, the percentage of active form of pyruvate dehydrogenase was similar in lactating and non-lactating rats (Table 1).

DISCUSSION

Several previous papers have provided evidence that isolated brown adipocytes are a suitable preparation for

studying glucose metabolism and the effect of insulin on BAT metabolism (Isler *et al.*, 1987; Ebner *et al.*, 1987; Saggerson *et al.*, 1988). The present study shows that in isolated brown adipocytes the effect of insulin on total glucose metabolism is decreased by 50% during lactation. This alteration in the effect of insulin reflects a specific lack of insulin effect on glucose oxidation and lipogenesis, whereas acylglycerol glycerol and lactate synthesis are unaffected. Similar results have been obtained on BAT pieces from lactating and virgin rats (Villarroya & Mampel, 1987). These authors have also established that glucose incorporation into BAT glycogen is unaltered during lactation. These adaptations of the various glucose metabolic pathways are similar to the adaptations previously described for glucose metabolism in isolated white adipocytes of lactating rats (Burnol *et al.*, 1986b). The suppression of the action of insulin on glucose oxidation and lipogenesis, the two metabolic pathways leading to irreversible disposal of glucose, may be important for preserving substrates for the synthesis of milk components in the mammary gland. In addition, lactate and glycerol synthesized in BAT can be released into the bloodstream and used as lipogenic precursors in liver. Indeed, it has been shown that an active hepatic gluconeogenesis is necessary for maintaining lipogenesis in the mammary gland during the starved-refed transition in the rat (Williamson *et al.*, 1985).

Under noradrenaline stimulation, glucose metabolism is similarly modified in brown adipocytes from non-lactating and lactating rats. This suggests that the defects observed in glucose metabolism in brown adipocytes from lactating rats are relatively specific to the action of insulin.

The first step of insulin action is its binding to specific cell-surface receptors. In a number of studies, insulin-resistant states have been shown to be associated with an alteration of the insulin-receptor kinase activity: in muscles of obese mice (Le Marchand-Brustel *et al.*, 1985) or humans (Caro *et al.*, 1987; Arner *et al.*, 1987), in liver, adipocytes and muscles of non-insulin-dependent diabetic humans (Caro *et al.*, 1986; Freidenberg *et al.*, 1987; Arner *et al.*, 1987), or rats (Kadowaki *et al.*, 1984; Okamoto *et al.*, 1986; Burant *et al.*, 1986), in liver of fasting rats or chickens (Freidenberg *et al.*, 1985; Simon *et al.*, 1986), and also BAT of obese mice (Tanti *et al.*, 1986).

The present work shows that the number of insulin receptors is not altered in BAT of lactating rats. This is in agreement with studies reporting that insulin stimulation of glucose metabolism is decreased in white adipocytes from lactating rats (Burnol *et al.*, 1986b), despite similar insulin-binding capacities (Flint *et al.*, 1979). It should be noted that the number of insulin receptors is unchanged in adipose tissues of lactating rats, despite decreased plasma insulin concentration (Agius *et al.*, 1979; Burnol *et al.*, 1983).

Autophosphorylation of the β -subunit of the insulin receptor is the first measurable post-binding event of insulin action. The kinase activity of the β -subunit of the insulin receptor is similar in BAT of lactating and non-lactating rats. There are already in the literature some reports of insulin-resistant states in the presence of an unchanged insulin-receptor kinase activity: in primary culture of hepatocytes from streptozotocin-diabetic rats (Amatruda & Roncone, 1985), and in liver and muscles of uraemic rats (Cecchin *et al.*, 1988). The insulin

resistance of glucose utilization in BAT of lactating rats described both *in vivo* and *in vitro* must then be attributable to more distal post-receptor defects. A possible site for such a defect is at the level of the generation of intracellular mediators of insulin action (for review, see Gottschalk & Jarett, 1985).

The present study shows that total pyruvate dehydrogenase activity is 37% lower in BAT of lactating than of non-lactating rats. During the euglycaemic/hyperinsulinaemic clamp, insulin stimulates pyruvate dehydrogenase activity in lactating and non-lactating rats. This suggests that the insulin signalling mechanism is unaltered during lactation. It has been shown that insulin is unable to stimulate pyruvate dehydrogenase activity in white adipose tissue of lactating rats (Kilgour & Vernon, 1987). Further studies, performed on plasma membranes and permeabilized mitochondria from virgin and lactating rats, have given evidence that there is a defect in the signal-transduction system in the plasma membrane of lactating rats at steps subsequent to the binding of insulin to its receptor (Kilgour & Vernon, 1988). The insulin-resistance observed in lactating rat white and brown adipocytes seems thus to be linked to different mechanisms: a decreased ability of plasma membranes to produce or release an intracellular mediator of the action of insulin in white adipocytes, and a decreased amount of pyruvate dehydrogenase in brown adipocytes, without alteration in the insulin activation of pyruvate dehydrogenase.

During lactation, the increased food intake is due to an increase in the meal size and also in the meal frequency (Strubbe & Gorissen, 1980). It has also been shown that after a meal there is an increase in BAT lipogenesis and glycogen content (Glick *et al.*, 1983), and thus of glucose utilization. BAT is the tissue exhibiting the highest sensitivity to insulin for glucose utilization (a 60-fold effect; Ferré *et al.*, 1986). Under insulin stimulation glucose utilization by BAT can then represent up to 8–12% of total glucose turnover rate (Ferré *et al.*, 1986). The insulin resistance which appears in BAT during lactation, together with its decreased thermogenic capacity, may thus allow sparing of a substantial amount of energy, which can be preferentially used for milk synthesis. This hypothesis is supported by a study showing an inverse linear correlation between the size of the litter, and thus the energy requirement linked to milk production, and the thermogenic capacity of BAT (Isler *et al.*, 1984).

In conclusion, this study has shown that the insulin resistance described in BAT of lactating rats is localized at a post-receptor level. It can be suggested that the defect is, at least partly, linked to a decreased amount of one of the effectors of insulin action, i.e. the pyruvate dehydrogenase complex.

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