Development of insulin sensitivity in white adipose tissue during the suckling–weaning transition in the rat

Involvement of glucose transport and lipogenesis

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The changes of insulin responsiveness of white adipose tissue during the suckling–weaning transition in the rat were investigated *in vitro* on isolated adipocytes. Insulin binding, glucose transport and glucose metabolism in adipocytes from suckling rats and from rats weaned on to a high-carbohydrate (HC) or a high-fat (HF) diet were compared. Despite similar insulin binding, insulin-stimulated glucose transport rate is lower in adipocytes from suckling rats and HF-weaned rats than in adipocytes from HC-weaned rats. Moreover, whereas insulin markedly stimulates glucose metabolism in adipocytes from HC-weaned rats, glucose metabolism is totally unresponsive to insulin in adipocytes from suckling and HF-weaned rats. This insulin resistance is associated with a very low rate of lipogenesis and low activities of acetyl-CoA carboxylase, fatty acid synthase and pyruvate dehydrogenase.

INTRODUCTION

In the rat, the suckling–weaning transition is accompanied by large nutritional, hormonal and metabolic modifications (Henning, 1981; Ferré *et al.*, 1986). During suckling, the rats are fed on a high-fat (HF) low-carbohydrate diet, the milk (Chalk & Bailey, 1979). At weaning, the intake of fat decreases, whereas that of carbohydrate increases. This, in turn, induces hormonal changes: plasma insulin concentrations, which are low during the suckling period, increase at weaning. In contrast, plasma glucagon concentrations, which are elevated during suckling, decrease at weaning (Girard *et al.*, 1977). Large changes in energy metabolism occur at this critical phase of the development (Ferré *et al.*, 1986). During the suckling period, an insulin resistance of glucose metabolism has been demonstrated *in vivo*, at both hepatic and peripheral levels (Issad *et al.*, 1987). Euglycaemic/hyperinsulinaemic-clamp experiments coupled with the 2-deoxyglucose technique have shown that the white adipose tissue of suckling rats is totally unresponsive to insulin *in vivo* (Issad *et al.*, 1988). Insulin responsiveness appears in white adipose tissue after weaning on to a high-carbohydrate (HC) diet, but not after weaning on to a HF diet, indicating an important role of the nutritional conditions (Issad *et al.*, 1988).

The aim of the present work was to determine the causes of the insulin resistance observed in white adipose tissue during suckling. For this purpose, we have studied the effect of insulin on glucose transport and metabolism in isolated adipocytes from suckling rats and from rats weaned on to a HC or a HF diet. The insulin-binding capacity of the isolated adipocytes was also determined in each group of rats.

MATERIALS AND METHODS

Animals

Wistar rats bred in our laboratory were used. They were housed in plastic cages in a room in which the temperature was maintained at 24 °C, with light on from 07:00 to 19:00 h. The studies were performed on 13–15-day-old suckling rats and on 28–30-day-old weaned rats. The litters were standardized to ten animals at birth. The rats were weaned at 19 days on to a semi-synthetic HC diet [25% protein, 5% fat, 60% carbohydrate, 5% cellulose, 5% minerals (w/w)] or a HF diet [37% protein, 43% fat, 15% cellulose, 5% minerals (w/w)]. The mothers of the rats weaned on to the HC diet had free access to water and chow pellets [24% protein, 4% fat, 57% carbohydrate, 6% cellulose, 9% minerals (w/w)]. Since the suckling rats begin to nibble the food of the mother at 15 days of age (Redman & Sweney, 1976), and to avoid carbohydrate intake from chow pellets between 15 and 19 days, the mothers of rats weaned on to the HF diet were fed with the same HF diet from day 14 of lactation until weaning. At the time of the experiments, mean body weight was 25.6 ± 1.3 g in suckling rats ($n = 12$), 63.5 ± 2.2 g in HC-weaned rats ($n = 12$) and 61.5 ± 2.2 g in HF-weaned rats ($n = 12$).

Preparation of isolated adipocytes

Isolated adipocytes were prepared by the method of Rodbell (1964) with the modifications described by Guerre-Millo *et al.* (1985). All studies began between 10:00 and 11:00 h. Animals were killed by cervical dislocation. The subcutaneous white adipose tissue of the inguinal region was removed, cut into small pieces, and

Abbreviations used: HC diet, high-carbohydrate diet; HF diet, high-fat diet.

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digested with collagenase (0.44 unit/mg; Boehringer, Meylan, France). A Krebs–Ringer phosphate buffer (pH 7.4) (Dawson *et al.*, 1969) containing 1 mM- Ca^{2+} was used for the digestion of adipose tissue. This was done in a shaking water-bath (37 °C), for 1 h and under oxygenation (O_2/CO_2 , 19:1). The digested tissue was then filtered through a 200 μm nylon mesh, washed three times with the incubation buffer containing 1 mM- Ca^{2+} and resuspended in the same buffer containing 2 mM- Ca^{2+} .

A sample of the initial cell suspension was used for the determination of cell concentration with a Neubauer haemocytometer. Mean fat-cell diameter was determined by a photomicrographic method (Lavau *et al.*, 1977).

Insulin binding

The adipocytes [(1–2) $\times 10^5$ cells] were incubated in 0.35 ml of Krebs–Ringer phosphate Hepes buffer (pH 7.4) (Dawson *et al.*, 1969) for 1 h at 30 °C with [mono- ^{125}I]iodotyrosine-A14]insulin (sp. radioactivity 2280 Ci/mmol), kindly provided by Dr B. Desbuquois (Hôpital Necker, Paris). Preliminary experiments have shown that insulin binding reached a plateau after 60 min of incubation. The incubations were terminated by the oil-centrifugation procedure (Gammeltoft & Gliemann, 1973). Data were reported as specific binding, which was obtained by subtracting the amount of radioactivity that remained bound to the cells in the presence of a large excess of unlabelled insulin (10 μM) from total binding. Non-specific binding averaged 5–12 % of total binding. Insulin degradation during the experiment was determined by measuring the trichloroacetic acid-precipitable radioactivity at the end of the experiment (Corbin & Reimann, 1974). It never exceeded 4 % of total radioactivity. The radioactivity was measured in a γ -radiation spectrometer (MR252; Kontron, Montigny, France).

Glucose transport

Glucose transport was assessed by measuring the cell-associated radioactivity after rapid incubation of adipocytes in the presence of 50 μM -[U- ^{14}C]glucose (Kashiwagi *et al.*, 1983; Gliemann *et al.*, 1984; Ebner *et al.*, 1987). At this low glucose concentration and for a short-time incubation (20 s), adipocyte glucose uptake is a reflection of glucose transport (Gliemann *et al.*, 1984). The adipocytes [(0.5–1) $\times 10^6$ cells/ml] were preincubated at 37 °C in a Krebs–Ringer phosphate buffer (Dawson *et al.*, 1969) containing pyruvate (2 mM) as an energy substrate and 3 % (w/v) bovine serum albumin (fraction V, Sigma; fatty acid-free). After 1 h of preincubation with various insulin concentrations, the incubation was started under magnetic stirring by addition of 50 μM -[U- ^{14}C]glucose (sp. radioactivity 295 mCi/mmol; C.E.A., Saclay, France) and [^3H]inulin as a marker for the extracellular water (radioactivity 0.4 mCi/mg; N.E.N./Du Pont de Nemours, Paris, France). Under these conditions, the uptake of the tracer was linear with time for at least 30 s in the three groups of adipocytes (results not shown), and was considered to be a measurement of glucose transport activity by the cells. The incubations were terminated at 20 s by centrifuging 200 μl samples of the incubation mixture through a dinonyl phthalate layer (Merck, Darmstadt, Germany) (Gammeltoft & Gliemann, 1973). The radioactivity in the cell layer was corrected for sugar trapped in the

extracellular water by using the [^3H]inulin marker. The radioactivity was counted in a spectrometer (Betamatic, Kontron) after addition of scintillation fluid.

Glucose metabolism

The cells were incubated for 2 h in a shaking water bath (37 °C) with different concentrations of insulin. [U- ^{14}C]Glucose conversion into CO_2 , acylglycerol fatty acids and acylglycerol glycerol were determined as described by Lavau *et al.* (1979). [U- ^{14}C]Glucose conversion into lactate was determined as described by Ferré *et al.* (1978) and Ebner *et al.* (1987). In brief, (1–2) $\times 10^5$ cells/ml were suspended in 2.5 ml of Krebs–Ringer bicarbonate buffer, pH 7.4 (Dawson *et al.*, 1969), containing 3 % bovine serum albumin (fraction V, Sigma; fatty acid-free), 5 mM-[U- ^{14}C]glucose (0.5 $\mu\text{Ci/ml}$) and insulin (0–800 $\mu\text{units/ml}$). Triplicates were performed for each insulin concentration. The flasks were capped with a rubber stopper equipped with a plastic centre well and they were gassed with O_2/CO_2 (19:1) for 5 min. After 2 h, incubations were terminated by adding 0.5 ml of HClO_4 (40 %, v/v) into the flasks. $^{14}\text{CO}_2$ was collected in 0.35 ml of Hyamine added to the centre well during an additional 45 min incubation; the radioactivity trapped into the Hyamine was then counted in a spectrometer (Betamatic, Kontron) after addition of scintillation fluid. A portion (0.5 ml) of the incubation medium was removed for determination of the [^{14}C]glucose incorporation into lactate. Separation of labelled lactate accumulated in the incubation medium from labelled glucose was performed on ion-exchange columns (Ferré *et al.*, 1978). Lipids were extracted from the remaining HClO_4 extracts with a heptane/propan-2-ol mixture (Dole & Meinertz, 1960). The lipids were saponified with ethanolic KOH, acidified with HClO_4 , and the fatty acids were extracted with light petroleum (b.p. 40–65 °C). The radioactivity was measured in both the organic (acylglycerol fatty acids) and aqueous (acylglycerol glycerol) phases.

Enzyme activities

For determination of acetyl-CoA carboxylase and fatty acid synthase activities, the subcutaneous white adipose tissue of the inguinal region was frozen in liquid N_2 immediately after the animal was killed.

The activity of fatty acid synthase was determined by the spectrophotometric assay of Linn (1981). The results were expressed as nmol of NADPH oxidized/min per g of tissue.

The maximal activity of acetyl-CoA carboxylase was determined as described by Maeda *et al.* (1975) after incubation in the presence of 10 mM-citrate. The results were expressed as nmol of malonyl-CoA formed/min per g of tissue.

Pyruvate dehydrogenase activity was determined in isolated adipocytes in the absence or presence of insulin (800 $\mu\text{units/ml}$). Adipocytes were incubated as described above for glucose metabolism, except that the labelled glucose was omitted. At the end of the incubation, adipocytes were frozen in liquid N_2 . Pyruvate dehydrogenase activity was determined as described by Stansbie *et al.* (1976). Total activity was taken to be the activity present in extracts after incubation with pig heart pyruvate dehydrogenase phosphate phosphatase in the presence of 25 mM- MgCl_2 and 1 mM- CaCl_2 (Stansbie *et al.*, 1976). The results are expressed as nmol/min per g of adipocytes at 30 °C.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. Statistical analysis was performed by Student's *t* test for unpaired data unless otherwise indicated.

RESULTS

Adipose-cell sizes

Mean cell diameter (determined by measuring 200 cells in 8–12 different cell preparations) was similar in suckling rats and HC-weaned rats [$50.2 \pm 0.9 \mu\text{m}$ ($n = 8$) and $50.4 \pm 0.9 \mu\text{m}$ ($n = 12$)], and it was significantly higher ($P < 0.001$) in HF-weaned rats ($62.0 \pm 1.0 \mu\text{m}$; $n = 8$).

Insulin binding

Mono[^{125}I]iodo-insulin binding on isolated adipocytes was similar in suckling, HC-weaned and HF-weaned rats [respectively $2.3 \pm 0.5 \text{ fmol}/10^6 \text{ cells}$ ($n = 9$), $1.9 \pm 0.4 \text{ fmol}/10^6 \text{ cells}$ ($n = 9$) and $2.0 \pm 0.3 \text{ fmol}/10^6 \text{ cells}$ ($n = 4$)].

Glucose transport

Basal glucose transport rates were similar in adipocytes from suckling, HF- and HC-weaned rats (Fig. 1). Insulin stimulated the transport of glucose in the three groups of adipocytes. Nevertheless, the maximal rate of glucose transport was 2–3-fold higher in adipocytes from HC-weaned rats than in the two other groups, whatever the basis (cell number or cell surface) chosen for expressing the results. Maximal glucose transport rate was significantly higher in adipocytes from HF-weaned rats than in adipocytes from suckling rats when expressed on a cell-number basis, but similar when expressed on a cell-surface basis (results not shown).

Glucose metabolism

Total glucose metabolism was considered to be the sum of glucose incorporation into CO_2 , lactate, acylglycerol fatty acids and acylglycerol glycerol.

Fig. 1. Insulin effect on glucose transport in adipocytes from suckling (□), HF- (○) and HC- (●) weaned rats

Results are means $\pm$ S.E.M. for eight determinations: ***, difference statistically significant at $P < 0.001$ when adipocytes from HC-weaned rats are compared with adipocytes from suckling or HF-weaned rats.

Fig. 2. Insulin effect on total glucose metabolism in adipocytes from suckling (□), HF- (○) and HC- (●) weaned rats

Total glucose metabolism is the sum of [^{14}C]glucose conversion into CO_2 , lactate, acylglycerol glycerol and acylglycerol fatty acids. Results are means $\pm$ S.E.M. for four to six determinations: *, **, ***, difference statistically significant at respectively $P < 0.05$, $P < 0.01$ and $P < 0.001$ when adipocytes from HC-weaned rats are compared with adipocytes from suckling or HF-weaned rats.

Total glucose metabolism (Fig. 2) was unresponsive to insulin in adipocytes from suckling and HF-weaned rats. By contrast, a considerable insulin stimulation of total glucose metabolism was observed in adipocytes from HC-weaned rats.

The partition of glucose into the major pathways of glucose metabolism in adipocytes from suckling, HC- and HF-weaned rats, in the basal state and under maximal insulin stimulation, is indicated in Table 1. Although basal glucose utilization was similar in adipocytes from the three groups of rats, the partition of glucose in the metabolic pathways was different: in suckling rats, lactate was the major end-product of glucose metabolism; in HF-weaned rats, acylglycerol glycerol and lactate were the two major end-products. In these two groups, incorporation of glucose into fatty acids was barely detectable. In HC-weaned rats, CO_2 and fatty acids represented the major fate for glucose carbon.

In adipocytes from suckling and HF-weaned rats, insulin had no stimulatory effect, whatever the pathway considered. By contrast, in adipocytes from HC-weaned rats, insulin had an important stimulatory effect on glucose incorporation into acylglycerol fatty acids, CO_2 and to a minor extent into lactate.

Thus, whereas the different pathways of glucose metabolism are totally unresponsive to insulin in adipocytes from suckling and HF-weaned rats, a large stimulation of lactate production, glucose oxidation and lipogenesis is observed in adipocytes from HC-weaned rats.

Lipogenic enzyme activities

In an attempt to determine whether the unresponsiveness of glucose metabolism to insulin in adipocytes

Table 1. Partition of [U-¹⁴C]glucose in the different pathways of glucose metabolism in the absence (basal value) or in the presence of insulin (800 μ units/ml)

[U-¹⁴C]Glucose incorporation into CO₂, acylglycerol fatty acids, acylglycerol glycerol and lactate was determined in adipocytes from suckling, HF-weaned and HC-weaned rats. Results are means for four to six determinations. ** Difference statistically significant at $P < 0.01$ (paired t test) when insulin-stimulated values are compared with the basal value in the same experimental group.

Metabolite	Insulin	Glucose metabolized (nmol/2 h per 10 ⁶ cells)		
		Suckling	HF-weaned	HC-weaned
CO ₂	—	42 $\pm$ 12	122 $\pm$ 17	177 $\pm$ 23
	+	38 $\pm$ 12	109 $\pm$ 16	490 $\pm$ 97**
Fatty acids	—	12 $\pm$ 3	9 $\pm$ 5	130 $\pm$ 20
	+	8 $\pm$ 2	19 $\pm$ 9	423 $\pm$ 55**
Glycerol	—	62 $\pm$ 25	295 $\pm$ 62	138 $\pm$ 28
	+	73 $\pm$ 24	224 $\pm$ 54	186 $\pm$ 20
Lactate	—	258 $\pm$ 60	158 $\pm$ 17	90 $\pm$ 12
	+	320 $\pm$ 80	146 $\pm$ 28	260 $\pm$ 43**

Table 2. Lipogenic enzyme activities in suckling and weaned rats

Fatty acid synthase (FAS) activity (nmol of NADPH/min per g wet wt. of tissue) and citrate-stimulated acetyl-CoA carboxylase (ACC) activity (nmol of malonyl-CoA/min per g wet wt. of tissue) were determined on fat-pads. Pyruvate dehydrogenase (PDH) activity (nmol of substrate/min per 10⁶ adipocytes) was determined on isolated adipocytes in the absence or presence of 800 μ units of insulin/ml. The percentage of the total form is given in parentheses. Results are means $\pm$ S.E.M. of 3–4 determinations in duplicate. * Difference significant at $P < 0.05$ when compared with the initial activity in the absence of insulin (paired t test). †, ††, Difference significant respectively at $P < 0.05$ and $P < 0.01$ when compared with suckling rats. ‡, ‡‡, Difference significant respectively at $P < 0.05$ and $P < 0.01$ when compared with HC-weaned rats.

Animals	FAS	ACC	PDH		Total activity
			Initial activity		
			No insulin	+ Insulin	
Suckling	22 ± 9	29 ± 10	0.19 ± 0.04 (6 %)	0.50 ± 0.15* (15 %)	3.3 ± 1.1
HC-weaned	878 ± 120††	451 ± 102††	1.7 ± 0.5†† (18 %)	3.0 ± 0.3*,†† (32 %)	9.3 ± 2.8†
HF-weaned	42 ± 12‡‡	16 ± 6‡‡	0.39 ± 0.15‡ (6 %)	0.80 ± 0.30‡‡ (13 %)	6.3 ± 2.9

from suckling and HF-weaned rats was linked to a defect in the transduction of the hormonal signal or to post-receptor defects, the activities of lipogenic enzymes were determined. Both fatty acid synthase and citrate-stimulated acetyl-CoA carboxylase activities were barely detectable in adipose tissue of suckling and HF-weaned rats (Table 2), precluding a reliable study of the effect of insulin on the activity of acetyl-CoA carboxylase. In contrast, the activities of these two enzymes were elevated in the adipose tissue of HC-weaned rats.

In isolated adipocytes of suckling and HF-weaned rats, initial activity of pyruvate dehydrogenase was respectively 9- and 4-fold lower than in adipocytes of HC-weaned rats. This difference persisted in the presence of 800 μ units of insulin/ml (Table 2). In contrast, total pyruvate dehydrogenase activity was only 2.8- and 1.5-fold lower in adipocytes of suckling and HF-weaned rats than in adipocytes of HC-weaned rats. This implied that the percentage of active pyruvate dehydrogenase was

always lower in adipocytes of suckling and HF-weaned rats than in adipocytes of HC-weaned rats (Table 2).

DISCUSSION

The insulin resistance of glucose utilization observed *in vivo* in white adipose tissue of suckling and HF-weaned rats (Issad *et al.*, 1988) is also observed *in vitro* in isolated adipocytes, indicating that it is due to an intrinsic defect of white adipose tissue and not solely to circulating insulin antagonists.

The insulin resistance observed in suckling and HF-weaned rats is not due to an insulin-binding defect, since insulin binding is similar in adipocytes from suckling, HC-weaned rats and HF-weaned rats. Moreover, the similar fold stimulation of glucose transport (Fig. 1) and pyruvate dehydrogenase activity (Table 2) over basal by insulin in the adipocytes of suckling and HC-weaned rats

suggests that the insulin signal transduction is not severely affected.

Table 1 shows that the increase of glucose utilization in adipocytes from HC-weaned rats under maximal insulin stimulation is essentially due to a large stimulation of glucose oxidation and of glucose incorporation into acylglycerol fatty acids (lipogenesis). Both basal and insulin-stimulated rates of lipogenesis are extremely low in adipocytes from suckling and HF-weaned rats. Clearly, the extremely low activities of ATP citrate lyase, acetyl-CoA carboxylase and fatty acid synthase in white adipose tissue of suckling and HF-weaned rats (Gandemer *et al.*, 1982, 1985; Tsujikawa & Kimura, 1980; and Table 2) represent a post-receptor defect precluding an efficient stimulation by insulin of the lipogenic pathway. The low activities of acetyl-CoA carboxylase and fatty acid synthase in adipose tissue of suckling and HF-weaned rats could result from a low rate of transcription of the gene coding for these enzymes, as suggested by the low amount of their specific mRNA (Ferré *et al.*, 1988). For pyruvate dehydrogenase, the decrease in its total activity is much less marked than for acetyl-CoA carboxylase or fatty acid synthase (Table 2). However, both the initial and insulin-stimulated activities are markedly decreased. This situation is reminiscent of that observed in heart after induction of diabetes or in the liver after starvation, in which re-activation of pyruvate dehydrogenase by insulin and carbohydrate is delayed (Randle *et al.*, 1988; Holness & Sugden, 1989), leading to a temporary post-receptor insulin resistance. Whether this is due to multi-site phosphorylation of pyruvate dehydrogenase (Kerbey *et al.*, 1979) or to a long-term activation of pyruvate dehydrogenase kinase (Denyer *et al.*, 1986) remains to be determined.

Glucose oxidation represents a considerable part of glucose utilization in adipocytes from HC-weaned rats under insulin stimulation. It has been shown in white adipocytes from young rats of the same age that the decarboxylation of glucose through the pentose phosphate pathway represents a major part of insulin-stimulated CO₂ production and that this CO₂ production is tightly linked to the stimulation of the lipogenic activity (Richardson & Czech, 1978). Thus decreased lipogenic enzyme activities, by limiting glucose utilization in oxidative and synthetic pathways, could be a major determinant of the insulin resistance observed in adipocytes from suckling and HF-weaned rats. At weaning on to a HC diet, the lipogenic enzyme activities develop markedly in white adipose tissue, and insulin responsiveness increases considerably.

Insulin-stimulated glucose transport rate is lower in adipocytes from suckling and HF-weaned rats than in adipocytes from HC-weaned rats. The suckling period corresponds to the intake of a HF diet. Several authors have observed decreased insulin-stimulated glucose transport rates in adipocytes from adult rats fed on HF diets (Ip *et al.*, 1977; Olefsky & Saekow, 1978; Salans *et al.*, 1981; Maegawa *et al.*, 1986). On the other hand, the suckling period is attended by low plasma insulin concentration (Girard *et al.*, 1977), whereas at weaning on to a HC diet plasma insulin concentration increases markedly. In experimental models of relative insulin deficiency, such as fasted rats and streptozotocin-diabetic rats, insulin-stimulated glucose transport rate is decreased. This is secondary to a decreased glucose transporter number in the intracellular pool, which could in turn

decrease the number of transporters translocated at the plasma membrane through insulin action (Suzuki & Kono, 1980; Cushman & Wardzala, 1980; Berger *et al.*, 1989). Whether the same kind of mechanism operates in suckling rats remains to be determined. Glucose transport can be stimulated in adipocytes from suckling and HF-weaned rats, whereas glucose metabolism is totally unresponsive to insulin in these cells (Fig. 2). This indicates that the defect of glucose transport is not solely responsible for the insulin resistance observed.

The fact that the insulin resistance of white adipose tissue observed during suckling is maintained by weaning the rats on to a HF diet supports the idea that the development of insulin sensitivity that normally occurs at weaning is due to the switch from the HF diet (milk) to a HC diet. The consumption of a HC diet at weaning probably increases post-prandial insulin secretion, and it seems possible that one of the long-term insulin effects could be to induce the appearance of intracellular proteins involved in short-term insulin action, such as glucose transporters (Kobayashi & Olefsky, 1979; Wardzala *et al.*, 1985; Kahn & Cushman, 1985; Sivitz *et al.*, 1989) and lipogenic enzymes (Trimble *et al.*, 1984; Ferré *et al.*, 1988). This, coupled with an increased responsiveness of lipogenic enzymes to short-term insulin action through dephosphorylation mechanisms, could ultimately lead to a higher capacity for insulin-stimulated glucose metabolism.

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