REGULATION OF ADIPOSE TISSUE METABOLISM DURING PREGNANCY AND LACTATION IN THE EWE: THE ROLE OF INSULIN

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ABSTRACT

This study was conducted to examine the effect of insulin on lipid metabolism of adipocytes during pregnancy and lactation in ewes. During the first 3 mo of pregnancy, metabolism of adipocytes from omental adipose tissue was characterized by a high rate of de novo lipogenesis (90 to 125 nmol of acetate incorporated into lipids·2 h·10⁶ cells⁻¹) and a 38% reduction in response to β-lipolytic stimulus (isoproterenol 10⁻⁶ M). Simultaneously, there was a rise in the number of high-affinity insulin receptors (Kₐ = .2 nM), and insulin binding characteristics showed a decrease in the negative cooperativity phenomenon. Moreover, lipogenesis stimulated by insulin (1 mU/ml) increased in comparison with observations in nonpregnant ewes. The last third of pregnancy and early lactation were characterized by a marked fall in lipogenesis and a simultaneous increase in isoproterenol-stimulated lipolysis. During lactation, the number of total insulin receptors was decreased by 62% and insulin stimulation of lipogenesis became inefficient. Results suggest that insulin plays a direct role in adipose tissue metabolism during pregnancy. 

Key Words: Ewes, Pregnancy, Lactation, Adipose Tissue, Insulin


Introduction

Accumulation of lipids in adipose tissue during the first two-thirds of pregnancy and the subsequent increased mobilization of fat during the last third are observed in a large number of mammalian species (Vernon, 1980; Chilliard, 1987; Guesnet and Demarne, 1987).

In ruminants, particularly in the ewe, earlier results (Vernon et al., 1981, 1985) did not indicate that insulin plays a direct role during the phase of accumulation, as it does in rats (Vernon et al., 1979). Recent studies have shown that adipose tissue becomes refractory to insulin (acetate and glucose utilization, fatty acid synthesis) during lactation (Vernon and Finley, 1988; Vernon and Taylor, 1988).

To determine more accurately the specific function of insulin during pregnancy and lactation, this study was designed to estimate the temporal association between number of insulin receptors and rates of de novo lipogenesis and lipolysis. We examined these variables on isolated adipocytes, which are more sensitive to hormones than pieces of adipose tissue. An additional objective was to ascertain whether these metabolic adaptations occurred in omental tissue as in subcutaneous adipose tissue. Omental fat can contribute as much as 40% of total lipid mobilized during late pregnancy (Robinson et al., 1978).

Materials and Methods

Animals and Experimental Procedure. Twenty Préalpes du Sud ewes of about 4 yr of age were mated in early October and November. They were given ad libitum access to dehydrated alfalfa and corn. For the last 2 mo of pregnancy, the dietary GE/d was increased to 3.6 Mcal supplying 80 g of CP (300 g of
filtration, the suspension of fat cells was containing 3% (mol) defatted fraction V of BSA and 10 mg of crude collagenase. After (about 100 mg) that were incubated at 39°C in of pregnancy, and at 7 (n = 1). Ewes were first anesthetized by an i.v. injection of pentobarbitone (.15 g), pentothal (.5 g), and atropine sulfate (.04 g). They were placed under general anesthesia with halothane (Fluothane). Samples of omental adipose tissue (50 g) were taken; no more than two surgical biopsies were carried out on the same ewe, and the interval between two operations was never less than 6 wk. Adipocytes were isolated from fat pads by the collagenase method described by Rodbell (1964) and modified in our laboratory (Guesnet et al., 1987). Omental tissue was cut into pieces (about 100 mg) that were incubated at 39°C in Krebs-Ringer bicarbonate buffer at pH 7.4 containing 3% (wt/vol) defatted fraction V of BSA and 10 mg of crude collagenase. After filtration, the suspension of fat cells was rinsed and the number determined with a Coulter Counter.

Incubation Procedure. Rate of lipogenesis was estimated by the incorporation of [1-14C]acetate in lipids. In brief, isolated fat cells (1.5 to 2.5 x 10^5 cells) were incubated in quadruplicate in plastic vials. The incubation medium contained .6 μCi of sodium [1-14C]acetate, 5.55 mM glucose and 1.5 mM sodium acetate in 2 ml of Krebs Ringer bicarbonate buffer at pH 7.4 containing 3% of defatted fraction V of BSA. After 2 h at 39°C with gentle agitation, the incubations were stopped by placing tubes in an ice bath; lipids were extracted by two successive methods according to Folch et al. (1957) and to Dole and Meinetz (1960). To measure the lipogenic response to insulin, additional vials containing different concentrations of insulin (0 to 10 mU/ml) were incubated and extracted as described. The maximal rate of lipolysis was measured as glycerol release after stimulation with isoproterenol at 10^-6 M (Guesnet et al., 1987).

Purification of Plasma Membranes. The plasma membrane suspension was fractionated by linear gradient centrifugation (5 to 25% Ficoll, .25 M sucrose) according to the method of McKeel and Jarrett (1970). The membrane protein was assayed using the technique of Lowry et al. (1951). Enrichment of the membrane preparation was assessed by measuring the specific activities of 5'-nucleotidase (EC 3.1.3.5) (Avruch and Wallach, 1971) and alkaline phosphatase (EC 3.1.3.1.) (Lansing et al., 1967). No mitochondria were found in any of the preparations as assessed by transmission electron microscopy.

Binding Assays. The iodinated bovine insulin kindly supplied by Dr. Laffite (INSERM, U55, Paris) had a specific activity of 140 μCi/μg. Insulin binding was investigated by the method of Freychet (1976) in a final volume of .25 ml of Krebs Ringer phosphate buffer, pH 7.5, containing [125I]insulin (10^5 cpm), unlabeled insulin at various concentrations (1 ng to 1 μg), and 100 μg of membrane protein. Data from insulin binding were calculated according to the methods of Scatchard (1949) by a least squares regression analysis. Average affinity profiles were calculated using the data from the Scatchard plots (De Meyts and Roth, 1975). The average affinity profile (K) was calculated as K = (B/F)/R0 - B, where B/F = bound/free and Ro = binding capacity, and was plotted as a function of log Y with Y = B/R0.

Statistical Analysis. All the results are expressed as means ± SD. Data were analyzed by analysis of variance (ANOVA) using the Genstat V program computer. Preplanned comparisons of period means were obtained by Scheffe's test using the residual variance of ANOVA.
Results

Variations in de novo Lipogenesis and Lipolysis. Measures of de novo lipogenesis by isolated adipocytes showed two distinct phases during pregnancy (Figure 1): the first 60 d were characterized by a high activity (125 to 90 nmol·2 h\(^{-1}\)·10\(^6\) cells\(^{-1}\)), but after d 90 of pregnancy lipogenesis fell (\(P < .05\)) and by d 120 reached a value 85% lower than that observed for pregnant females at 30 d of pregnancy (18 ± 8 vs 120 ± 19 nmol·2 h\(^{-1}\)·10\(^6\) cells\(^{-1}\)) (\(P < .01\)). This low rate of lipogenesis lasted through parturition and the first 6 wk of lactation. So, the rate of acetate incorporation remained between 25 and 45 nmol·2 h\(^{-1}\)·10\(^6\) cells\(^{-1}\).

For lipolysis, \(\beta\) -adrenergic stimulus (isoproterenol at 10\(^{-6}\) M) of adipocytes from omental adipose tissue revealed two phases of response during pregnancy. First, the maximal lipolytic response to isoproterenol decreased beyond d 75 in comparison to that at d 0 of pregnancy; on d 90 it had declined 38% below control levels (.815 ± 151 vs 1.304 ± 82 \(\mu\)mol of released glycerol·2 h\(^{-1}\)·10\(^6\) cells\(^{-1}\)) but was not statistically significant. Second, between d 90 and 120 of pregnancy, the lipolytic rate increased progressively and attained 1.741 ± 191 \(\mu\)mol·2 h\(^{-1}\)·10\(^6\) cells\(^{-1}\) on d 140.

A peak of maximal \(\beta\) -adrenergic stimulus of lipolysis was reached at d 14 of lactation (2.364 ± .341 \(\mu\)mol·2 h\(^{-1}\)·10\(^6\) cells\(^{-1}\)) (\(P < .01\)). After 2 mo of lactation, the lipolysis rate returned to values similar to that observed at d 0 of pregnancy.

Variations in the Number of Insulin Receptors. A Scatchard plot of insulin binding by plasma membranes purified from omental adipose tissue is shown in Figure 2. Curvilinear plots were characteristic of the presence of two kinds of insulin receptors but also of negative cooperativity of insulin binding. In the nonpregnant ewe, we observed two kinds of receptors, one with a high affinity (\(K_d = .16\) to .2 nM) and one with a low affinity (\(K_d = 10\) nM) for insulin. During pregnancy and lactation, the number of insulin receptors and the dissociation constant varied. We therefore report the variation in the number of high-affinity receptors and total receptors during pregnancy and lactation (Table 1). In comparison with nonpregnant females, the number of high-affinity receptors were greater between d 60 and 90 pregnancy (23 ± 6 vs 38 ± 9 fmol/mg of protein) (\(P < .05\)), whereas the total number of receptors was reduced by 27% (511 ± 220 vs 375 ± 75 fmol/mg of protein); differences were not significant. In lactating ewes, the total number of insulin receptors fell, but not below values for nonpregnant ewes (511 ± 220 vs 195 ± 50 fmol/mg of protein).

To obtain a better understanding of this variation in insulin binding characteristics, we studied the average affinity profile according to the method of De Meyts and Roth (1975) (Figure 3), who found that the average insulin affinity for its receptors (\(K_e\)) decreases when the degree of receptor occupation increases. Thus, in the case of nonpregnant females, the affinity constant of empty receptors (\(K_e\)) was 5 \(×\) 10\(^7\) M\(^{-1}\). At the time of their saturation (100% occupation) the affinity constant of filled receptors (\(K_f\)) reached 2 \(×\) 10\(^7\) M\(^{-1}\). In this case, the degree of receptor occupation required for half the decrease in \(K_e\) was reached at 2%. During pregnancy and lactation, our results clearly showed that the average affinity profile of insulin binding was modified. In fact, the two constant values \(K_e\) and \(K_f\) were increased 2.5-fold (\(K_e = 12.5 \times 10^7\) M\(^{-1}\), \(K_f = 5 \times 10^7\) M\(^{-1}\)). In parallel, the negative cooperativity was changed because the 50% fall in the constant \(K_e\) reached 5 and 6% of receptor occupation. Moreover, similarity in the average affinity profile between pregnant and lactating ewes pointed out that only the number of insulin receptors was modified during this physiological period.

Insulin Stimulation of de novo Lipogenesis. Insulin (25 to 250 \(\mu\)U/ml of incubation medium) stimulated [1-\(^{14}\)C]acetate incorporation into adipocyte triglycerides by 10 to 12% in tissue from nonpregnant ewes and ewes between d 30 and 90 of pregnancy (Figure 4). Higher insulin concentrations (1 to 10 \(\mu\)U/ml), estimated at 25-fold the physiological concentration, stimulated (70%) lipogenesis in tissue taken from ewes between d 30 and 90 of pregnancy (\(P < .01\)) but not in tissue from nonpregnant ewes or from ewes in late pregnancy or lactation. The stimulation curve was biphasic because beyond 10 \(\mu\)U/ml the insulin stimulation decreased and [1-\(^{14}\)C]acetate incorporation rate was 30% greater in tissue from d 30 and 90 of pregnancy than in tissue taken from nonpregnant ewes. The de novo lipogenesis of adipocytes isolated from d 120 of pregnancy or d 14 of lactation was unresponsive to insulin stimulation regardless of the hormonal concentration used.
Discussion

Early Pregnancy. The results of our study clearly point out that fatty acid metabolism in omental adipose tissue favors lipogenesis during the first 2 mo of pregnancy in ewes. These data showed patterns similar to those of Vernon et al. (1981) even though the rate of \([1^{-14}C]\)acetate incorporation into omental fat cells was low compared with that of subcutaneous adipose tissue. The maximum response of adipocyte to \(\beta\)-adrenergic-stimulated lipolysis was reduced from d 30 through 90 of pregnancy. This lower rate of lipid mobilization would promote more efficiently the in situ esterification of fatty acids into adipose tissue triglycerides. So, the present findings indicate that metabolic adaptations of omental adipose tissue in early pregnancy were similar to those obtained from subcutaneous adipose tissue (Vernon et al., 1981).

In rats, this phase of fat accumulation is assumed to be under the control of insulin. Several authors have observed an increase in the lipogenic effect of insulin in vitro, in the number of insulin receptors, and in the plasma insulin concentration (Flint et al., 1979; Vernon et al., 1979; Lennox et al., 1985). In ewes, simultaneous increase in insulinemia and in number of insulin receptors did not fully explain accumulation of lipids in adipose tissue during the first two-thirds of pregnancy (Blom et al., 1976; Vernon et al., 1981). In part, this was because many reports showed no or little effect of insulin on incorporation of acetate into lipid during short-term incubation of adipose tissue from ewes (Vernon, 1980). In

![Graph](image-url)

**Figure 1.** Effect of pregnancy and lactation on the rate of lipogenesis and maximal rate of isoproterenol-stimulated glycerol release from ewe epiploic adipocytes. The incubation included \(1.5 \times 10^5\) to \(2.5 \times 10^5\) adipocytes for lipogenesis and \(2.5 \times 10^5\) to \(3.5 \times 10^5\) adipocytes for lipolysis. Results are means ± SD where \(n\) is the number of observations. *\(P < .05\) and **\(P < .01\): significantly different from 30 d pregnant ewe for lipogenesis and from 0 d pregnant ewe for lipolysis.
ADIPOSE TISSUE METABOLISM IN THE EWE

TABLE 1. PLASMA MEMBRANE-BINDING CHARACTERISTICS FROM OMENTAL ADIPOSE TISSUE IN THE EWE

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>No. of high-affinity insulin receptors, fmol/(mg-protein)</th>
<th>Total insulin receptors, fmol/(mg-protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>3</td>
<td>23b ± 6</td>
<td>511b ± 220</td>
</tr>
<tr>
<td>Pregnant (60 to 90 d)</td>
<td>4</td>
<td>38c ± 9</td>
<td>375b ± 75</td>
</tr>
<tr>
<td>Lactating (7 to 30 d)</td>
<td>3</td>
<td>17b ± 1</td>
<td>195b ± 50</td>
</tr>
</tbody>
</table>

aData are presented as the means ± SD.
b,cMeans without a common superscript were different (P < .05).

contrast, our results clearly showed that variation in lipogenic activity of omental adipose tissue directly depends on the hormonal control by insulin during the first 3 mo of pregnancy. In vitro stimulation of fatty acid synthesis was clearly demonstrated at high insulin concentrations (10 mU/ml) with our isolated adipocyte system. At the same time, the number of high-affinity insulin receptors increased, and analysis of the average affinity profile according to De Meyts and Roth (1975) clearly showed that insulin affinity constants for its empty (K_0) and filled (K_I) receptors were equally raised 2.5-fold; the negative cooperativity, specific to insulin binding, was also reduced, suggesting a fall in the receptor-receptor interaction. This better insulin-receptor interaction may explain the ability of insulin to stimulate (+70%) acetate incorporation into lipids in early pregnancy. Vernon et al. (1981) have suggested that progesterone might play a protective function against insulin-antagonistic hormones during this period.

Late Pregnancy. As in the study of Vernon et al. (1981) with subcutaneous adipose tissue, our study showed that around d 90 to 100 of pregnancy a switch in omental adipose tissue metabolism toward lipid mobilization occurred. In fact, rate of de novo lipogenesis fell

Figure 2. Scatchard plots of [125I]insulin binding (140 μCi/μg) to plasma membranes of omental adipose tissue from nonpregnant, pregnant and lactating ewes. B/F is the ratio of insulin specifically bound to plasma membranes to free, unbound insulin at equilibrium. Each point is the mean value obtained from three ewes, except there were four pregnant ewes.
and the lipolytic effect of isoproterenol increased and became significantly larger (+130%) around parturition. The number of insulin receptors and insulin serum concentration fell during this time (Blom et al., 1976; Vernon et al., 1981, 1985). In late pregnancy, even high doses of insulin were inefficient in stimulating fatty acid synthesis. In rats, despite serum insulin concentrations and numbers of insulin receptors similar to those of virgin rats, lipogenesis rate of adipose tissue fell and adipocytes improved resistance to the lipogenic effect of insulin, suggesting a peripheral resistance to insulin in vivo (Leturque et al., 1980; Lennox et al., 1985; Toyoda et al., 1985). Several hormones that increase during late pregnancy can be implicated in the induction of insulin resistance in the rat: 1) progesterone, which might antagonize insulin action in fat cells in vivo by decreasing phosphorylation of glucose into glucose-6-phosphate (Sutter-Dub et al., 1981); 2) growth hormone (GH), which is antagonistic toward insulin in vivo (Kahn et al., 1978; Ayling et al., 1981); and 3) lipolytic hormones, which through release of nonesterified fatty acids could inhibit acetyl-coenzyme A carboxylase response to insulin (De Fernandez-Feo and Saggerson, 1980). Adrenergic hormones and glucagon also could be potential candidates because their lipolytic effects increase during late pregnancy (Aitchison et al., 1982; Zammit, 1985). In ewes, there is evidence that no insulin resistance occurs during late pregnancy (Van der Walt et al., 1980; Basset, 1983). Nevertheless, we observed no effect of insulin on rate of lipogenesis in omental adipose tissue during this period. Elevated concentrations of GH could explain the absence of insulin response; this hormone inhibited the in vitro lipogenic effect of insulin in ewes (Vernon, 1982; Vernon and Finley, 1986). As in rats, adrenergic hormones could be implicated in view of higher stimulation of lipolysis by isoproterenol during late pregnancy (Iliou and Demame, 1987; Guesnet et al., 1987); no convincing evidence has shown that glucagon enhanced lipolysis in ewes in vivo as in vitro (Etherton et al., 1977; Vernon, 1980). Finally, the potential diabetogenic and lipolytic role of placental lactogen, pointed out by Thordarson et al. (1984, 1987), does not

![Figure 3. Average affinity profiles for plasma membranes of omental adipose tissue from ewes.](image-url)
seem to be valid because metabolic effects were not evident in their studies.

**Lactation.** Lipid mobilization continued during the first 2 wk of lactation. In spite of a low basal lipolysis rate (Guesnet et al., 1987), the β-adrenergic-stimulated lipolysis in our study was very high at the beginning of lactation and returned to values similar to those of the controls at the end of lactation. Similar variations were found by Vernon and Finley (1985), who showed an increase in norepinephrine-stimulated lipolysis in subcutaneous adipose tissue. At the same time, the antilipolytic effect of adenosine was elevated, most likely to prevent an overall excessive lipid mobilization (Vernon and Finley, 1985; Iliou and Demarne, 1987). During periods of intensive lipolysis, serum insulin concentrations remained low and the number of insulin receptors on isolated adipocytes (Vernon et al., 1981) and plasma membranes decreased without any modification in binding affinity. In parallel to this, de novo lipogenesis by omental adipose tissue did not respond to insulin stimulation, whatever the incubation dose. The factors responsible for these adaptations have not been fully defined. Prolactin does not seem to be involved, in contrast to the situation in nonruminant mammals (Vernon et al., 1986), but GH may be because its serum concentration is high (Vernon et al., 1981). Vernon and Finley (1988) showed that, in lactating ewes, GH antagonized the insulin induction of fatty acid synthesis in subcutaneous tissue maintained in tissue culture for 24 or 48 h.

In conclusion, the switch of adipose tissue metabolism from lipogenesis at the beginning of pregnancy (accumulation of maternal fat stores) to lipolysis in late pregnancy and lactation seems to be a general feature in various depots of adipose tissue. In ewes, as in rats, insulin plays a central role in metabolic substrate utilization and delivery and surely plays a direct role in adipose tissue metabolic

![Graph](image)

**Figure 4.** Effect of insulin on lipogenesis in omental adipose tissue from pregnant or lactating ewes. The response to insulin (0 to 5 × 10^{-2} units/ml) was estimated by [1-^{14}C]acetate incorporation into lipids, and was expressed as percentage of control or basal value. Each point is the mean value obtained from four determinations (nonpregnant ewes, □; pregnant ewes 30 to 90 d, ○; pregnant ewes 120 d and lactating 14 d, △).
control in early pregnancy. Further studies on antilipolytic effects of insulin would improve our understanding of the participation of this hormone in control of lipolysis in adipose tissue during pregnancy and lactation.

Implications

Increased lipogenic rates observed in omental adipose tissue from ewes during the first 3 mo of pregnancy are the result of higher sensitivity to insulin-stimulated lipogenesis. Increases in the number of high-affinity insulin receptors and(or) better insulin-receptor interaction may explain the effects of this hormone.

Literature Cited


