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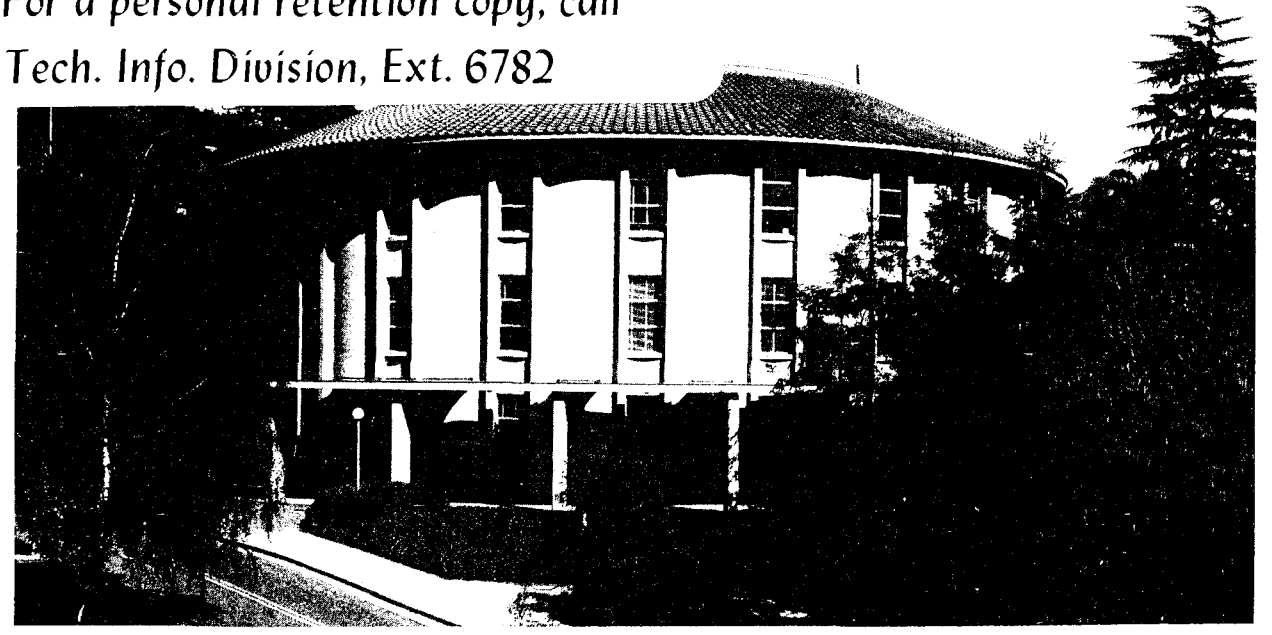

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METABOLIC COOPERATIVITY BETWEEEN MAMMARY EPITHELIAL CELLS AND ADIPOCYTES OF MICE

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ABSTRACT

Modulation by the lactational state of the metabolic activity of adipocytes was demonstrated by measuring glycogen and lipid synthesis from ^{14}C-glucose in mammary fat pads cleared of epithelium, in abdominal fat pads, and in adipocytes from both sources and from intact mammary gland. Mature virgin, pregnant, and lactating mice were used. Accumulation of glycogen, the activity of glycogen synthase, and the lipogenic rate in abdominal and mammary adipocytes remained high during pregnancy but decreased to insignificant levels by early lactation. The depressant effects of lactation were observed solely in those mammary adipocytes isolated from intact glands. The presence of mammary epithelial cells was also required to effect the stimulated lipogenesis in mammary adipocytes during pregnancy. We conclude that the metabolic activity of adipocytes is modulated both during pregnancy and lactation to channel nutrients to the mammary epithelial cell. The fact that the changes occur in mammary adipocytes only when epithelial cells are present indicates that local as well as systemic factors are operating in these modulations.

Index terms: Glycogen, lipogenesis, cleared mammary fat pads, pregnancy, lactation, isolated cells, mammary gland.

We have demonstrated previously that the transitions from the virgin state to pregnancy and to lactation elicit changes in the rate of glycogen synthesis in mammary epithelial cells of mice (2,10). This report provides evidence that glycogenesis in adipocytes, both mammary and abdominal is also influenced by lactational state. The alterations in the epithelial cells are likely due to changes in the concentration of hormones known to act on these cells during the lactation cycle (7,13) The changes in adipocytes could be an indirect response to alterations in epithelial cell activity as well as a direct response to hormonal influences. The systemic responses to meet the metabolic needs of the gland during lactation have been described. The activity of specific enzymes of liver and adipose tissue are altered by the onset of lactation (1,25) and lactation has also been shown to inhibit lipogenesis (27) and to enhance lipolysis (28) in rat adipose tissue. In addition, local, glandular factors appear to influence mammary adipocyte activity (9,18). The loss of fat in mouse mammary adipocytes during lactation, for example, has been shown to be dependent upon the presence of epithelial cells (9).

In an attempt to distinguish between local and systemic effects of changes in the lactational state on metabolic activity in adipocytes, we have measured glycogen synthesis and lipogenesis in fat pads, both in abdominal pads and in mammary pads previously freed of epithelial elements (8), and in mammary adipocytes from virgin, pregnant and lactating mice. The results of this study demonstrate that modulation of glycogen synthesis and lipogenesis in adipocytes are influenced by both local mammary and systemic factors.

MATERIALS AND METHODS

Mature virgin, mid-to-late pregnant (14-18d), and lactating (7-10d) Balb/c Crgl mice were obtained from Cancer Research Laboratory at the University of California at Berkeley. Mammary epithelial cells were isolated from the glands of these mice by the method of Lasfargues and Moore (16) as modified by Emerman and Pitelka (12). Mammary adipocytes were harvested from the top of the tube after the initial centrifugation and washed 2 times prior to incubation. For the purpose of studying mammary adipocytes free of influence from adjacent epithelial cells, the mammary epithelial anlage was removed from one pair of glands when the mice were three weeks old (8). The perirenal pad was used as the source of an abdominal fat pad.

The tissues (ca.5 mg protein) or $2x10^6$ cells were incubated in 0.5 ml Medium 199 containing 11mM [U-^{14}C] glucose (New England Nuclear, final specific activity, 30 Ci/mol). Insulin, cortisol, and prolactin were present in the medium at 5 µg/ml each. The media with the tissues or cells were equilibrated with 5% CO_2 in 95% air, put on a gyrator shaker (100 rpm) and incubated at 37°C in 95% air, 5% CO_2 for 1 and 2 h periods. The epithelial cell suspensions were centrifuged at 1000 rpm for 3 min to pellet the cells. The fat cells were concentrated by collecting the medium beneath the cells with a pipette. The cells and tissue were rapidly washed twice in Hanks' balanced salt solution (HBSS) containing unlabeled glucose, once with diluted HBSS (1:2 with H_2O), and killed with 3 ml of 80% methanol (v/v) in 0.01N NaOH containing 0.1% sodium dodecyl sulfate (SDS) as described previously (11). The tissues or cells were homogenized and the methanol evaporated under a stream of nitrogen prior to further analysis.

The metabolites in the cell and tissue suspensions were separated by 2-dimensional chromatography as described previously (3,5). By this procedure glycogen and other macromolecules remain at the origin in our solvent systems. To determine the glycogen content, the origins were hydrolyzed with trifluoroacetic acid (4) and the released glucose was isolated and quantitated by autoradiography. The ^{14}C-labeled areas on the chromatograms were assayed for radioactive content with an automated Geiger-Muller apparatus (19). The lipid was extracted from the SDS suspension by the method of Slayback et al. (26). The organic extract was removed for assay of ^{14}C content by liquid scintillation spectrometry. A portion of each sample was removed for protein determination by the method of Lowry et al. (17) using an Autoanalyzer II system (Technicon). The results are expressed as nmols ^{14}C per mg protein. In the case of glycogen, we recognize that the labeled material accumulating during the incubation period is the net of synthesis and degradation, but for the sake of simplicity will refer to this process as glycogen synthesis.

The cells and tissue for enzymatic assay were homogenized, and glycogen synthase was assayed by the method described by Golden et al. (14), except that glycogen was isolated by thin layer chromatography on cellulose using 66% ethanol as solvent. The protein concentration of the homogenate was determined (17) and the results expressed as nmols of substrate converted to glycogen per mg protein per min.

RESULTS

The rate of glycogen synthesis in abdominal fat pads was unchanged by the transition from the virgin to the pregnant state, but drastically curtailed by 40-fold in 7-10d lactating mice (Table 1A). A similar decline in glycogen synthesis with lactation was observed in pieces of whole gland. In contrast, glycogenesis in mammary fat pads cleared of epithelium (8) was unchanged by the tranisition from pregnancy to lactation (Table 1A). Lactation did effect a decline in the rate of glycogen synthesis in isolated mammary epithelial cells (Table 1B). Therefore, the fall in glycogen synthetic capacity of whole gland could involve solely the epithelial elements since the decreased proportion of adipocytes in the lactating gland (20,22) would decrease their contribution to the overall rate of glycogenesis. The fact that the glycogen synthesis in cleared mammary fat pads was unaffected by changes in the lactational state supports this contention. The other possibility, compatible with the results, is that mammary adipocytes respond to lactation just as their abdominal counterparts do but, in their case, the response requires the presence of adjacent epithelial cells.

To distinguish between the possibilities, adipocytes isolated from mammary glands were tested for their ability to convert glucose carbon to glycogen (Table 1B). Regardless of the physiological state of the animal mammary adipocytes exhibited very low glycogen synthetic activity. The loss of activity appeared to involve damage to adipocytes during the isolation procedure and not to some glandular effect because adipocytes isolated from cleared fat pads were similarly affected (Table 1B). Of the metabolic measurements made, the loss of activity involved only glycogen synthesis and only of isolated adipocytes. The activity of glycogen

synthase was not depressed in these cells, indicating the damage was not at the enzymatic level, and lipogenesis was not altered (see below). Furthermore, the rate of glycogen synthesis in mammary epithelial cells was over 60% of that of intact glands from pregnant and lactating mice, and, therefore, was likely depressed little, if at all, by the isolation procedure. If most of the glycogen synthetic activity remains intact in epithelial cells during isolation, then the rate of this synthesis in isolated adipocytes was only 5-6% of that expected. Clearly, glycogen accumulation in adipocytes was decreased due to tissue dissociation.

Because the rate of glycogen synthesis in pieces of gland and in isolated mammary epithelial cells was determined, the glycogen synthesis attributable to adipocytes in the gland can be calculated. The rate of glycogen synthesis from glucose carbon in the intact gland from pregnant mice was 200 nmol/mg tissue protein/hr and 130 nmol/mg/hr in epithelial cells isolated from these glands. If we assume (i) that the gland of mice in mid to late pregnancy is composed of approximately equal proportions of epithelial and fat cells (22), (ii) that almost all of the glycogenesis in the gland can be attributed to these two cell types; and accept that the rate of glycogen synthesis in the isolated epithelial cells is little affected by isolation, then we can calculate the contribution of adipocytes to the overall rate of glycogenesis on the basis of the following formula:

$$Wg_{syn}=(EC_{syn})(EC_f)+(AC_{syn})(AC_f)$$

where WG_{syn} designates the rate of synthesis in the whole gland; EC_{syn}, the rate in epithelial cells; and AC_{syn}, in adipocytes. The proportion of

the gland made up of epithelial cells is denoted by EC_f, and that by adipocytes as AC_f. From this it can be calculated that in pregnancy, the rate of glycogen synthesis in mammary adipocytes would have to be near 300 nmols of glucose carbon incorporated into glycogen/mg protein/hr. If we accept that the proportion of adipocytes in the lactacting mouse gland is about 10% (20,22), glycogenesis in mammary adipocytes in the intact gland must decrease during lactation to less than one-tenth the pregnant rate. Although this may not be a precise estimate, it is clear that the rate must decrease with lactation. During lactation, glycogenesis falls to below 5 nmol/mg/hr for whole gland and one-half this for epithelial cells (Table 1). For synthesis in the mammary adipocytes to remain at, or somewhat near, the calculated rate for pregnancy, the adipocytes would have to compromise less than 1% of the cells in the lactating gland, a highly unlikely low proportion based on tissue estimates (20,22).

Assay of the activity of glycogen synthase supports the conclusion that mammary adipocytes lose glycogen synthetic capacity at lactation only when in the presence of mammary epithelial cells. The activity of this enzyme in adipocytes isolated from intact mammary gland was reduced 30-fold by the onset of lactation while that in adipocytes from gland previously freed of its epithelial elements was unchanged (Table 2). The specific activity of glycogen synthase from homogenates of whole mammary pads cleared of epithelium was similar to that in homogenates of cells isolated from these pads (individual data not shown).

Changes associated with the lactational state have been observed before in lipid metabolism in adipocytes (1,15,25,27,28). Therefore, we measured lipogenesis from glucose in the same preparations used in the glycogen studies (Fig. 1). The rate of lipogenesis in the abdominal fat

pad was depressed by the onset of lactation, as reported by others (27), but the decrease was not to the same extent as that of glycogen synthesis (Table 1 and Fig. 1). Lipogenesis in the cleared mammary fat pad was depressed 3-fold by the transition from the virginal to the pregnant state and remained at the same rate thereafter. This pattern is quite different from that of glycogen synthesis in these same preparations (Table 1). Mammary adipocytes isolated from intact gland also showed a lipogenic response to changes in the physiological state that was distinct form the response in glycogen synthetic activity. Pregnancy induced a 3-fold increase followed by a dramatic decrease in lipogenic activity during lactation to a rate of 16% of that in the virginal state and 5% of that in pregnancy (Fig. 1).

DISCUSSION

We have added new evidence to the concept that lactation and the preparation for it during pregnancy involves more than just a response by epithelial cells of mammary gland. The activity of adipocytes is also modulated. This modulation varies with the antatomical site and, therefore, appears to be in part due to local factors within the mammary gland that affect specific metabolic pathways.

The direct way to search for a local glandular effect on glycogenesis by mammary adipocytes is to compare the synthetic activity of adipocytes isolated from intact gland to those from mammary pads cleared of epithelial elements. Such an approach was obviated by the fact that synthesis in isolated fat cells was markedly depressed by the isolation procedure. When the rate of glycogen synthesis by pieces of intact gland is compared to that in the two major cell types capable of glycogen

synthesis in the gland, fat and epithelial cells, the sum of the cellular synthesis could not possibly equal that in the gland, regardless of the relative proportions. It is unlikely that another cell type, comprising a minor proportion of the gland, was responsible for the remainder of glycogen synthesis because the rate would have to be astronomical to account for the synthesis observed in the intact gland. Because lipogenic capacity is maintained in these cells (Fig. 1), we conclude that glycogen accumulation in adipocytes is particularly vulnerable to tissue disruption. The fact that the specific activity of glycogen synthase remained high indicates that the depression might be reversible. Nevertheless, the fat cells failed to recover this activity in the relatively short times in culture in the current study.

Based on our calculations of the rate of glycogen synthesis in mammary adipocytes, we conclude that glycogen synthesis in these cells is depressed during lactation by local, glandular factors because cleared mammary pads are not affected. The basis for the local effect could be nutritional; the epithelial cells merely compete better for available nutrient than surrounding adipocytes. Lowering of the exogenous glucose concentration, due to increased uptake by mammary epithelial cells, could limit glycogen synthesis as has been shown in other cell types (5,10). On the other hand, an effector derived from mammary epithelial cells by action of the hormonal and nutritional changes accompanying lactation could be directly responsible for depressing glycogen synthesis in adipocytes in the intact gland. A hormonal mechanism for establishing this superiority on a glandular basis for lipid uptake has been worked out by Scow and co-workers (23,30,31). Only further work with isolated cells

of each type and/or hormonal manipulations in intact animals can sort out all the possibilities. Until the depression in glycogen accumulation in adipocytes resulting from the isolation procedures can be overcome, these studies are held in abeyance.

The lipogenic response of mammary adipocytes to changes in the physiological state is more complex than the response of glycogen synthesis. A stimulatory effect related to epithelial cells appears to operate during pregnancy as well as the depressant effect of lactation. One of us has previously reported that mammary epithelial cells from pregnant mice release an unknown factor in cell culture that stimulates lipogenesis in adipocytes (18). We may be observing the manifestation of the same effect on adipocytes dissociated from the intact gland of pregnant mice in the present case.

The severely depressed lipogenesis in mammary adipocytes during lactation must be also due in part to a local response to lactation because lipogenesis in adipocytes dissociated from intact gland was depressed to a greater extent than that in abdominal pads. This local effect could be regulated by metabolic effectors from the epithelial cells but the greater affinity of epithelial cells for glucose and triglyceride would contribute to this decrease. The increase in lipoprotein lipase activity in epithelial cells and its decrease in adipocytes (15,30,31) provides the basis for more efficient lipid uptake by mammary epithelial cells during lactation. The lack of lipid accumulation in mammary adipocytes observed by Elias et al. (9) can be accounted for by this locally induced decrease in lipogenesis as well as by the increase in lipolysis observed in all adipocytes during lactation (28).

The depressed rate of glycogen synthesis in abdominal fat pads during lactation is likely due to the systemic response to lactation that also affects lipid metabolism (1,9,15,25,27,28). The systemic response can be attributed to the requirement of the mammary epithelial cell for glucose, triglyceride, and amino acids for the synthesis of milk components limiting the availability of these substrates to other tissues. Of course, such a nutritional change would also evoke alterations in the circulating concentration of hormones modulating metabolic activity which could result in some of the observed metabolic alterations. This response likely accounts for the decrease in lipogenesis in these pads observed in the present study.

Glycogenesis and lipogenesis in mammary fat pads freed of their mammary elements were not altered by lactation. This difference in synthetic activity between anatomical sites could arise because abdominal fat pads are more responsive to nutritional stress than are subcutaneous ones (24). The increased blood flow to the gland during lactation (29), which would help maintain the nutrient supply to the mammary pad, is evidently not the basis for this difference because the increased vascularity does not occur in mammary pads freed of epithelium (29). Thus, the basis for the anatomical difference remains unknown.

Studies in cell culture provide the classic example of metabolic cooperativity. One cell type lacking a crucial enzyme system survives due to receipt of the enzymatic product from enzymatically competent cells present in the culture (6,21). In the present case, the metabolic activity of the adipocyte is modulated <u>in vivo</u> to support the mammary epithelial cell: to store lipid and glycogen during pregnancy in preparation for lactation and during lactation to give preference to the

mammary epithelial cell for the available nutrients for milk production.

ACKNOWLEDGEMENT

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TABLE 1

GLYCOGEN SYNTHESIS IN ABDOMINAL FAT PADS, MAMMMARY GLANDS AND CLEARED MAMMARY FAT PADS AND IN MAMMARY ADIPOCYTE AND EPITHELIAL CELLS OF VIRGIN, PREGNANT, AND LACTATING MICE

Tissue or Cell Preparation	Glycogen Synthesis from: Virgin	Pregnant	Lactating
	A. Fat pads and Tissue Pieces		
Abdominal fat pads	135±26(8)	163±63(4)	4±0.7(2)
Mammary gland	107±23(6)	202±4(6)	3±0.1(6)
Cld mammary fat pads*	261±83(6)	323±68(4)	257±54(8)
	B. Isolated Cells		
Mammary epithelial cells	26±8(6)	24±33(6)	2±0.2(6)
Mammary adipocytes	13±4(8)	15±1(6)	4±0.6(6)
Adipocytes from cld pads	14±2(2)	ND≠	ND

Fat pads (approximately 50 mg) and adipocytes approximately 10^6 cells) were incubated in Medium 199 containing 11 mM [U-^{14}]C) glucose (30 Ci/mol) previously equilibrated with 95% O_2/5% CO_2. The duration of incubation was 1 and 2 hrs at 37°C at which time the pads or cells were removed, washed, and glycogen isolated and assayed for radioactivity as described in the text. The results are the mean ± the standard error or range for 2 experiments and are expressed as nmol ^{14}C recovered in glycogen per mg protein per hr. The number of experiments is given in brackets.

* Mammary fat pads f of epithelium (8)

≠ Not determined

TABLE 2

SPECIFIC ACTIVITY OF GLYCOGEN SYNTHASE OF ADIPOCYTES FROM CLEARED MAMMARY FAT PADS AND FROM MAMMARY GLAND

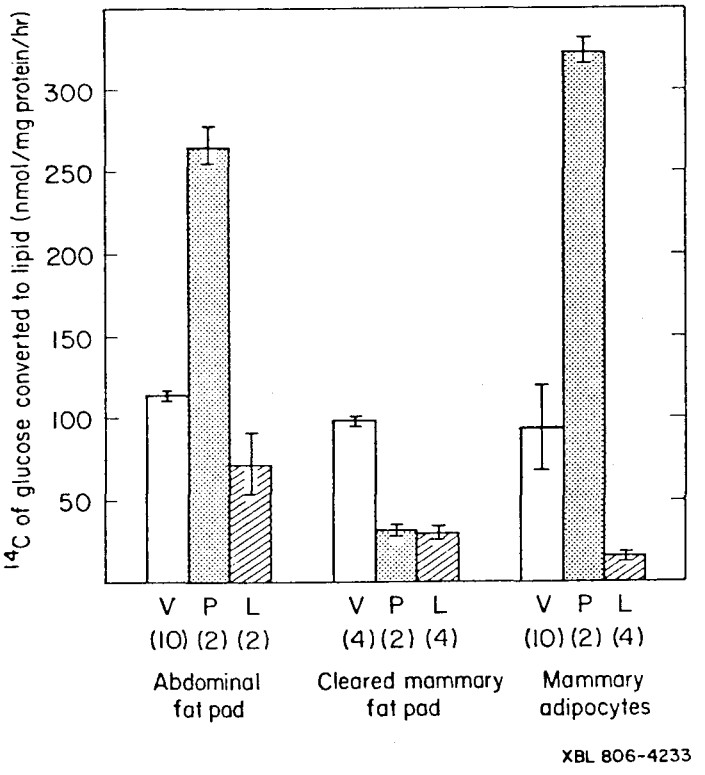
Source of Adipocytes	Specific Activity of Glycogen Synthase from: Pregnant	Lactating
Mammary gland	3.1±0.4(2)	0.11±0.05(3)
Cld mammary gland*	5.9±0.5(2)	6.8 ±0.07(4)

Isolated adipocytes or whole pads were homogenized as described in the text. Glycogen synthase activity was determined by the method of Golden et al. (13) modified as described in the text. Results are expressed as nmol per mg homogenate protein per min and are the mean ± the standard error or range of the number of experiments in brackets.

* Mammary fat pads freed of epithelium (8).

FIGURE LEGEND

Fig. 1. Lipogenesis in abdominal and cleared mammary fat pads and in mammary adipocytes from virgin, pregnant, and lactating mice. Fat pads and adipocytes were prepared from mature virgin (V), pregnant (P), and lactating (L) mice and incubated as described in Table 1 and the labeled lipid isolated as described in the text. Each bar is the mean and the vertical line, the standard error or range for 2 experiments of the number of nanomoles recovered in lipid per mg protein per hr. Number of experiments is given in brackets.


^{14}C of glucose converted to lipid (nmol/mg protein/hr)
300
250
200
150
100
50
V P L
(10) (2) (2)
Abdominal fat pad
V P L
(4) (2) (4)
Cleared mammary fat pad
V P L
(10) (2) (4)
Mammary adipocytes

XBL 806-4233