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Review

Role and regulation of metabolism in adipose tissue during lactation

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The metabolism of energy-yielding compounds in adipose tissues has a pivotal role in supplying the energy demands of lactation. In most mammals, the relatively high ratio of milk energy production to maintenance demands a cycle of energy storage during pregnancy, lipid mobilization and increased feed intake during lactation, and restoration of body fat in late lactation or after weaning. In well-fed women, the energy balance usually remains positive. However, in situations of low-energy supply, the stored lipid can be critical to establishment of lactation and maintenance of maternal health. There is a commonality among mammals in the adaptative responses during lactation and in their general regulation. However, in rodents and most domestic species the magnitude of metabolic response is greater than in women. The importance of adipose tissue to lactation is demonstrated by the number of highly coordinated and redundant control elements that regulate the adaptations of metabolism. This coordination is carried out by the central nervous system through the endocrine organs and the sympathetic nervous system. Recently, insights have been gained into the quantitation of the adaptations due to rate of milk production, stage of lactation, and intake of energy-yielding compounds and the physiological mechanisms of action of several regulatory factors. The intake of nutrients and demand for milk precursors have differential effects on the enzymes of lipid synthesis and release from adipose tissue, and the equations describing these chemical interconversions vary with stage of lactation, nutrient intake, and genetic propensity for milk production. Regulatory mechanisms that are now better understood include those of the interactions of growth hormone and insulin to control lipogenesis and the activity of the sympathetic nervous system to regulate lipolysis. Improvements in understanding and managing lactational energy metabolism have been limited by the complexity of the chemical interconversions of nutrients and their regulation. For women specifically, the severe lack of information on adaptations at the tissue level hinder further advancement. Improvement in this area will require a coordinated effort to study both physiological control mechanisms and quantitative parameters of lipid metabolism. (J. Nutr. Biochem. 6:120–129, 1995.)

Keywords: lactation; adipose; regulation; metabolic control; lipids; energy

Introduction

Successful initiation and maintenance of lactation is critical to the well-being of both infant and mother. Benefits to the infant include increased resistance to infection, improved weight management, and better social interactions with the mother.[1–6] Several benefits to the mother include the potential for post-partum contraception, better control of body composition, improved bonding with baby, and an economical means of feeding the infant.

In addition to the direct benefits of human lactation, we derive additional benefit from lactation in domestic animals. This practice, established before the existence of written records, provides humans with an enhanced stability and flexibility of food supply. In the case of ruminant animals, we further derive the ability to convert otherwise unavailable biomass [plant cell walls (hemicellulose and cellulose) and low-quality protein] into a high-quality human food. A benefit with great future potential is the utilization of lactating animals to produce quantities of specific non-nutritive

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compounds valuable for medicinal uses. In a narrower sense, lactation provides biological scientists with a complex model of biological principles and applications, which can provide for study in many areas of biological science.

One research goal is to improve our understanding of the mechanisms of metabolic regulation, including those involving preparation for and adaptation to the lactational state and maintenance of lactation. Regulatory mechanisms in lactation include such phenomena as changes in hormone secretion and binding to tissues, intracellular signaling and biological response, and nutrient regulation of gene expression. A second research goal aims at a higher level of biological organization: we endeavor to quantify in chemical terms the effects of the lactational state on the chemical interactions in mammary and other tissues. This approach determines the aggregated effects of nutritional, physiological, or genetic manipulation of lactation on nutrient requirements and expected outputs such as milk production and changes in body composition. A third goal seeks to discover the mechanisms by which short- and long-term nutritional status, reproductive status, and health of the mother are connected.

This review will address the role of adipose tissue in the establishment and maintenance of lactation in several species. Topics will be covered briefly, in the hope that those with an interest in lactational biology can ponder the broader questions, and the reader requiring more critical detail will be directed to several excellent sources. The first section deals with quantification of nutrient use in adipose tissue and it's role in overall body metabolism, this will be a kinetic and descriptive approach rather than an heroic attempt at mechanistic explanations. The second section will use two examples of regulatory processes which tax our ability to identify mechanisms of metabolic regulation. The third section will describe the relationship of metabolism in adipose tissue during lactation with reproductive processes, as an example of the need to join disciplines to provide more useful explanations of critical biological phenomena. Species comparisons will be used to stress those regulatory phenomena which have been conserved during evolution and thus allow commonality of concepts in regulatory biology of lactation and to point out those species differences which prevent broad extrapolations for management of lactation across species.

Quantitative estimation of chemical interactions important for a successful lactation

The patterns and rates of metabolic processes within adipose tissue are directly affected by the intake of nutrients, their metabolism in other nonmammary tissues, and the demand for milk precursors. The changes in nutrient intake and demand for milk precursors create secondary responses in the endocrine systems, which reinforce the substrate signals to alter adipose metabolism in the longer term. Adipose tissue primarily responds to signals originating externally from it (hormones, nutrient supply); however, it is not just a passive organ throughout lactation. The amount of adipose tissue at parturition influences voluntary feed intake and also affects rates of milk component synthesis.[5–9] Both the genotype of the animal and the nutritional environment during pregnancy can alter the amount of adipose tissue at parturition.

As lactation continues, the cumulative activity of various enzymes and the size of the adipose mass in turn have effects on the maintenance of the lactational state, the return to reproductive readiness of the dam, and the preparation for the next lactation. This is because whole-body nutrient flux and thus substrate supply to the mammary gland are affected by differences in the inputs and outputs of nutrients through the adipose tissue. We can view adipose tissue not as an organ of control, but as an organ that has a primary role in the establishment and maintenance of lactation and maternal reproduction and health. It becomes necessary to define this role in quantitative terms, under situations of varied amounts of adipose tissue and rates of metabolic reactions within it.

Accretions of maternal adipose tissue and muscle mass occur in mid pregnancy in most species.[5–9] In the peripartum period, adaptations take place in many endocrine organs and maternal tissues to supply milk precursors and energy-yielding compounds to the mammary gland. The timing of these adaptations are earlier and tend to be more pronounced in litter-bearing or genetically selected species, but they are present in all lactating species.[5–10] In some species, including humans and dairy cattle, lactation may extend for several months or years, and there are adaptations to these extended lactations which differ from the adaptations to early lactation.[2,3,10,11]

In adipose tissue, the rate of anabolic reactions slows during late pregnancy and is further reduced in lactation. Depending on the rate of milk production and the length of lactation, body fat is restored during late lactation or after weaning. Catabolic pathways are generally reciprocal to the decreases in anabolism. Adaptations to management interventions are carried out by changes in enzyme activity within tissues and aggregated to appear at the whole-body level as changes in milk production and body composition. Although lactation catabolism is mild in women of average milk production and adequate nutrient intake, a wide range of fat gain or loss during lactation has been documented, especially for at-risk groups such as teenagers and women with low nutrient intake or a high energy expenditure.[1,2,4,7,12–14] The increases in requirements for fat and carbohydrate average 23 and 26% above maintenance requirements in lactating women.[14] Such a change in demand, accompanied by little or no increase in nutrient intake, can have a impact on maternal body composition.

These adaptations to lactation can be attenuated or exaggerated further by variations in the rates of nutrient intake or milk production. In dairy cattle, increased energy intake or decreased milk production will decrease the severity and length of the catabolic period, whereas high production or nutrient deficiency will increase and extend it.[10–14] Rates of anabolic reactions are slower and those of catabolic reactions faster for a longer time under moderate to severe energy restriction or increased milk production. Changes in nutrient intakes or in milk component output may cause a similar change in net energy balance but have different effects on supply of substrates and thus rates of chemical interactions in adipose tissue. In lactating dairy cattle,

changing intake of starch (within a range which does not change production of milk) components has a large influence on rates of lipogenesis in adipose tissue but a much smaller effect on rates of lipolysis.[10–12,15] A change in output of milk fat due to genetic selection alters lipolysis to a greater extent than lipogenesis.[10–12,15]

Altering the fat content of the diet provides another example of the complex effects of changes in intake of dietary chemical constituents on adipose metabolism during lactation. Increases in dietary fat intake, or via infusion of fat directly into the abomasum or duodenum of dairy cattle, decrease adipose tissue lipogenesis and esterification, but have little or no effect on rates of lipolysis.[16–18] This is the case even though energy balance is increased. These responses vary with stage of lactation and original rate of milk production. Increased fat intake can also alter the composition of milk and the total milk component production in diary cattle, pigs, and humans.[2–4,14,19,20] However, the responses in milk composition and production are extremely variable and are affected by stage of lactation, original rate of milk production, and body energy balance. In addition, specific dietary fats may act as regulatory factors, directly or indirectly altering various aspects of cellular function and metabolism out of proportion to the energetic equivalent of the fat.[16,20,21] Thus the study of the effects of amount and type of dietary fats in rations during lactation exemplifies the need for critical chemical definition of the nutritional situation.

Moderate changes in intake of different nutrients apparently has a much smaller effect on milk composition in women than in domestic species, and only under severely reduced nutrient intakes are milk volumes reduced.[2,4] In women, neonatal demand seems to be the only practical means to increase milk production. Milk production is a function of genotype, but this is difficult to determine and not amenable to manipulation in human populations. Neonatal demand is most practically altered by a positive approach to breastfeeding which allows a good establishment of lactation and a delayed introduction to solid foods which maintains milk production at a high rate.

Recently we have been able to investigate adipose metabolism in lactating pigs as a model of human lactation.[a,b] Pigs have several nutritional and physiological similarities to humans which neither rodents nor ruminants share. The metabolic body size (of lactating subadults and adults and of infants) is more similar for humans and pigs, the digestive physiology has good similarity, and pigs can adapt to and utilize mixed-carbohydrate (starch and fibrous carbohydrates) diets in a similar fashion to humans or rodents.[19,22–24] As for most other animal models, however, pigs do not usually consume diets as high in fat and animal protein as humans. The milk production rate of pigs can be varied by altering litter size, so that one can study the effects of a range of milk production rates, including those similar to those in women.

We altered the rates of lipid metabolism and body fat content of first parity pigs by changing energy intake during pregnancy and lactation.[a,b] Rations during pregnancy provided either 100 or 70% of recommended energy intake. (Amino acid, vitamin, and mineral intakes were equal.) It should be noted that the 100% recommendation for gestating pigs is designed for a limited increase in total body weight (to allow for the products of conception but to limit maternal fat accretion).[19] In this study during lactation, rations supplied either 100, 67, or 34% of energy requirements. The rates of adipose lipogenesis and esterification in pregnant and lactating pigs followed a pattern that was more similar to humans than rats.[4,14,23–25] For example, in animals fed to requirements in pregnancy and lactation, rates of lipogenesis actually increased from 9 days prepartum to 7 and 23 days postpartum (*Figure 1*).[a,b] Thus, the lactational state in pigs does not necessarily dictate a reduction in lipid synthesis when the mother is supplied with adequate energy. Because the relative milk production and energy deficit in pigs is much less than in dairy cattle or rats, the magnitude and length of normal adipose tissue adaptations in pigs are more consistent with what we know of metabolism in lactating women.[1–4]

Imposing a mild or severe energy deficit during lactation at a similar rate of milk demand reduced the rate of anabolic and increased the rate of catabolic reactions. When pigs were fed 67% of the energy requirements, rates of lipogen-

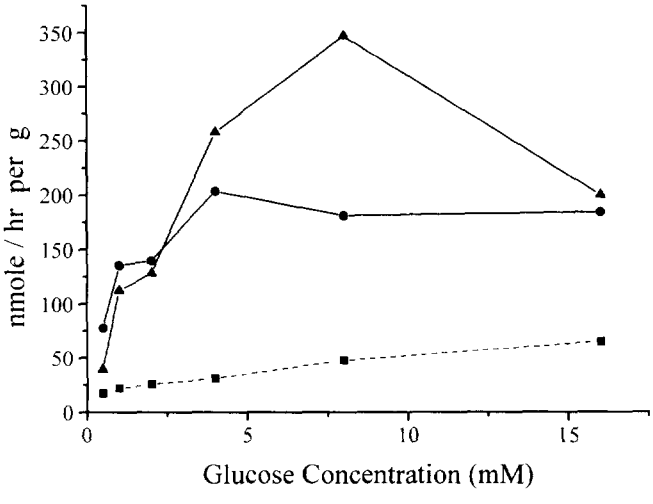

Figure 1 Lipogenesis from glucose in vitro in subcutaneous adipose tissue in pregnant and lactating pigs fed to requirements. Units are nanomoles of glucose converted to fatty acids per hour per gram of tissue. Pigs were fed to 100% of the National Research Council recommendations for energy, protein, vitamins, and minerals. Subcutaneous adipose tissue was biopsied on day 105 of pregnancy and days 7 and 23 of lactation. Rates of glucose conversion to fatty acids was determined by in vitro incubations.[10–12] Dashed lines and squares = day 105 pregnancy; triangles = day 7 of lactation; circles = day 23 of lactation. Rates at day 105 of pregnancy were less ($P < 0.05$) than on days 7 or 23 of lactation. Standard errors of the means were less than 15% of the means for each group.

[a]McNamara, J.P., Parmley, K.P., and Pettigrew, J.E. (1993). Adipose tissue metabolism adaptations to energy intake in pregnant and lactating swine (abstract). *Livestock Prod. Sci.* **35**, 202–203.

[b]Parmley, K.P., and McNamara, J.P. (1993). Metabolic adaptations in adipose tissue of swine as a model of lactation biology (abstract). *FASEB J.* **7**, A63.

esis from glucose were reduced to 20% of control (*Figure 2*). However, rates of esterification of fatty acids in storage triglycerides at day 7 were only reduced to about 70% of control.[a,b] Rates of lipolysis were increased almost 3-fold at days 7 and 23 of lactation on the 67% of energy requirements ration (*Figure 3*). A dietary regimen providing 34% of energy requirements further reduced lipogenesis only slightly compared with the moderate restriction (*Figure 2*), whereas esterification rates were further reduced to approximately 20 to 30% of normal.[a,b] Rates of lipolysis were not further increased by this additional energy deficit (*Figure 3*).

Thus, lactation itself does not necessarily cause changes in rates of lipogenesis or lipolysis in pigs fed according to normal management practices. This situation is clearly similar to that in women who follow normal recommendations for weight gain during pregnancy. Thus, the lactating pig may be a useful model for studying certain aspects of nutrition, metabolism, and metabolic regulation in women. Both pathways of lipid synthesis and the rate of lipolysis in the pig adapt sensitively to reductions in energy intake within the lactational state. In addition, the anabolic pathwasy adapt differentially: lipogenesis is most sensitive to changes in energy intake, and esterification less so. The maintenance of rates of esterification, coupled with the increase in lipolysis during lactation (adaptations which have been discovered in all species yet examined) ensure a rapid rate of fatty acid recycling through adipose tissue trigyceride. This adaptation provides a relatively cheap (in energy expenditure terms) source of flexibility and adaptability to supply the needs of both the mammary gland and the maternal system.

The level of detail in our knowledge of adipose tissue

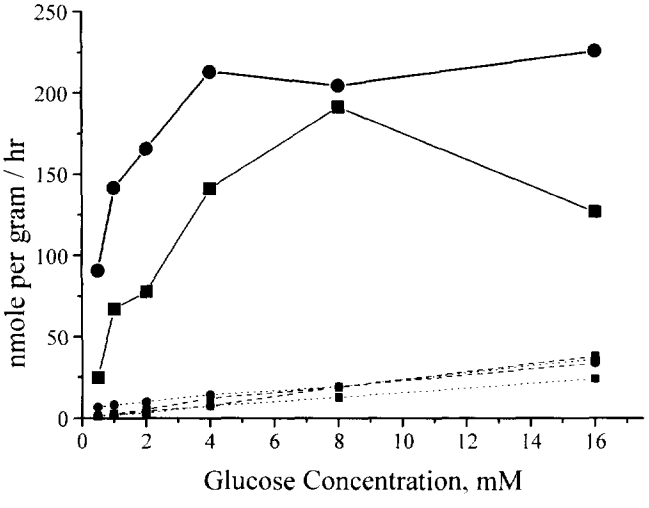

Figure 2 Lipogenesis from glucose in vitro in subcutaneous adipose tissue of lactating swine. Units are nanomoles of glucose converted to fatty acids per hour per gram of tissue. Pigs were fed either to 100, 67, or 34% of National Research Council recommendations for energy during lactation. All rations supplied 100% of required protein, vitamins, and minerals. Circles = day 7; squares = day 23; solid lines = high energy rations; dashed line = medium energy rations; dotted line = low energy rations. Days 7 and 23 were equal in rates, but high energy was higher ($P < 0.05$) than other rations. Standard errors were <15% of means for each group.

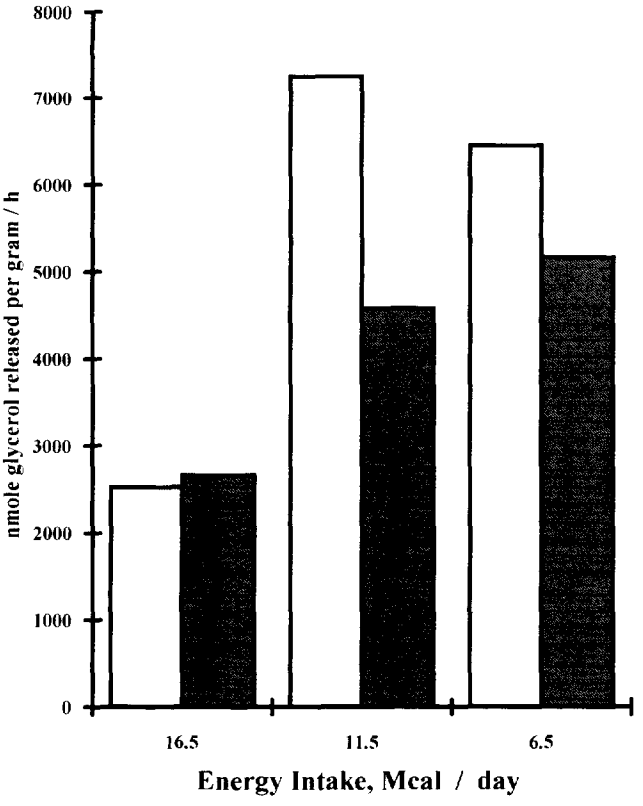

Figure 3 Lipolysis in vitro from subcutaneous adipose tissue of lactating swine. Units are nanomoles of glycerol released per hour per gram of tissue. Light bars are day 7, dark are day 23 of lactation. Animals were fed and tissue obtained as in legends for *Figures 1* and *2*. Lipolysis was higher on day 7 ($P < 0.05$) than day 23 for low and medium rations, and rates on low and medium energy rations were higher ($P < 0.05$) than high energy for both days. Standard errors were <15% of means for each group.

metabolism under different genetic and nutritional states continues to expand. Part of our duty as scientists includes improvement of ability to predict responses to various situations and manipulations. This increase in precision of prediction must be based on both an increase in detail and the ability to categorize and interpret it systematically and quantitatively. The effort to obtain quantitative descriptions of metabolism and integrate them into better explanatory or predictive descriptions has been referred to as "metabolic modeling," "mathematical modeling," "metabolic control theory," or "quantitative biology."[26–30] An in-depth discussion of this area is beyond the scope of this review; thus, the reader is directed to several excellent references on this topic.[26–30] This integrative approach has already advanced our conceptual and quantitative understanding of how the various metabolic pathways are integrated among tissues to support the specific needs of the whole organism. Our present level of knowledge and need to predict outcomes in response to interventions demands integration of data obtained from well-designed reductionistic studies. The studies must be specific for the situation of interest; and, in strictly chemical terms, extrapolation must be made with great caution.

Mechanisms of metabolic regulation in adipose tissue during lactation

In addition to acquiring quantitative information on rates of nutrient metabolism, we also must understand the regulatory mechanisms involved. It is important to obtain information on how these regulatory forces interact in vivo over the entire ranges of rates of milk production, stages of lactation, and rates of nutrient intake. Several good sources for more detailed descriptions of metabolic regulation of adipose and other tissues in lactation are available.[4–6,8–12] I would like to highlight just two of the mechanisms that regulate lipogenesis and lipolysis as examples of metabolic control in adipose tissue during lactation and how the complexity of this system challenges our ability to explain it.

Two examples of metabolic control critical to support of lactation by adipose tissue are the regulation of lipogenesis by somatotropin (ST) and the regulation of hormone-sensitive lipase by the sympathetic nervous system (SNS). Somatotropin and its actions have been studied for decades, but the onset of potential commercial use of ST in lactation has spurred intense analyses into the mode of ST action. The regulation of lipolysis has been investigated in great depth at the subcellular level and we have a good understanding of the molecular mechanisms involved.[31,32] However, strictly subcellular studies are inadequate to explain the wide range of estimates of rates of lipolysis during lactation. Thus, several driving forces such as the need to increase efficiency in agricultural production, the need for better support of human lactation, and the need for more basic information on support of lactation have converged on one area of study: the regulation of adipose tissue lipid metabolism during lactation.

Somatotropin effects on lipogenesis

In the transition to lactation, both insulin and somatotropin play pivotal roles. In late pregnancy and early lactation, adipose tissue becomes resistant to the normal actions of insulin. The reduction in lipogenesis is associated with a reduction in insulin receptors on adipocytes.[6,8,9,32] The enzyme that is primarily responsive to the action of insulin and ST is acetyl-CoA carboxylase, which is the primary rate-limiting enzyme in fatty acid synthesis from glucose or acetate.[32,33] Somatotropin inhibits insulin-stimulated lipogenesis in explant cultures of adipose tissue,[34–36] and tissue from lactating animals is more sensitive to this down-regulation.[32,33,35,36] Somatotropin does not alter insulin binding to its receptor. Rather by some event at the post-receptor level ST either inhibits the action of a second messenger, or prohibits its synthesis.[32] This second messenger may be some type of polyamine, or may be ornithine decarboxylase, which in turn affects polyamine concentration.

Insulin can be taken up and degraded in tissues. Insulin can be taken up by adipocytes and in some species a specific insulin protease has been identified.[37] This process has been suggested to be involved in the longer term responses to insulin (such as increased protein synthesis, including the amount of acetyl-CoA carboxylase). One mechanism by which ST may affect insulin action is through the alteration of insulin uptake and degradation. The inhibitory effects of ST on lipogenesis can be blocked by inhibiting protein synthesis in adipose tissue.[32] This mechanism is in fact consistent with the existence of a second messenger which arises from ST receptor binding to inhibit the biological response to insulin. That is, either ST, a portion of it, or some other second messenger, may enter the cell and inhibit the insulin protease necessary for normal action of insulin. All these possibilities remain conjecture this state, as it has not yet been demonstrated that insulin degradation is necessary for its action. However, this mechanism has not been disproven either.

We recently tested the hypotheses that adipose tissue from cows has an insulin protease and that ST and prolactin alter this activity. We found that adipose tissue from pregnant or lactating bovines possesses a specific insulin-protease which shares the same characteristics as insulin proteases from other sources.[35,37,38] Both ST and prolactin can directly inhibit the proteolytic activity of this enzyme on radiolabeled insulin in cell homogenates, and this action was not the effect of protein dilution, as several other proteins did not elicit the same effect.[35] Another protein which had a similar negative effect on insulin proteolysis was histone, a polyamine which has been demonstrated to be an inhibitor of other insulin proteases.[38,39] This finding may be of importance because prolactin and somatotropin have quite similar structures, and because the action of prolactin in the mammary gland may also be carried out by an inhibition of other serine proteases.[39]

The attenuation of insulin proteolysis by ST as one mechanism of action is not mutually exclusive to the "second messenger" theory proposed previously. In fact, the potential connection between the action of a histone-like peptide as an inhibitor of insulin protease and the suggestion that polyamines may function in the response to ST is intriguing. It has been proposed that the proline-induced loop at the N-terminal end of both prolactin and ST acts as an inhibitor of protease action because this same structure is also contained in other known protease inhibitors.[39] The actual mechanisms involved in the role of insulin uptake and processing in regulation of lipogenesis and the action of ST on this process are far from clear. We now have several intriguing possibilities, each with an equal (as yet) likelihood of being one mechanism of action of this process.

Regulation of lipolysis by the sympathetic nervous system

The regulation of lipolysis in lactation is no less complex than the regulation of lipogenesis. We do have a fairly clear picture of the intracellular regulation of lipolysis. The role of the β-adrenergic receptor and the resultant cascade of adenyl cyclase, cAMP, protein kinase, and hormone-sensitive lipase is quite well established.[31,32] The recent challenge has been to determine precisely how lipolysis is regulated in early lactation and how the continued rapid rates of lipolysis in late lactation of dairy cattle are maintained. Part of the increase in lipolysis may be accounted for by the insulin resistance of adipose tissue during early lactation.[32] However, insulin concentration or binding alone

cannot account for the wide variation in rates of lipolysis throughout lactation, especially as rates of lipolysis can vary even at the same rates of glucose and insulin flux.[8,9,11,12,32] Somatatropin, which is itself not acutely lipolytic, can increase the responsiveness of adipose tissue to β-adrenergic agents.[32,33] Somatotropin may act through altering the number or binding of β-receptors or the response to binding, or by altering the responsiveness of adipocytes to adenosine.[32] Adenosine limits lipolysis and ST may be acting to partially remove this limitation and thus increase lipolysis during early lactation.

The intracellular regulatory cascade controlling lipolysis is itself adaptable to different physiological states.[11,12,32,33,40–42] This is not only important in situations requiring an acute release of free fatty acids, but can also be controlled in a chronic manner to adapt to longer term changes in physiological or environmental states. One example of chronic regulation is that by ST during lactation as described above. Another is given by the function of the thyroid system. In hypothyroid rats, a given rate of lipolysis requires a higher concentration of cAMP compared with control, whereas in hyperthyroid animals the same amount of cyclic AMP stimulates a greater rate of lipolysis than in controls.[41]

These adaptations of lipolysis during lactation and the adaptability of the cyclic AMP cascade to differing physiological conditions, gave rise to the hypothesis that some portion of this intracellular cascade regulating hormone-sensitive lipase was altered by the lactational state. We demonstrated that the amount of lipolysis stimulated by the same concentration of cyclic AMP was 3 times greater per g of adipose tissue and 5 times greater per mg of cellular protein in dairy cattle in the first 2 months postpartum compared with the last month prepartum.[43] Thus, one of the mechanisms by which adipocytes increase sensitivity to lipolytic stimulation during lactation may be a tighter molecular connection between cyclic AMP, protein phosphorylation, and hormone-sensitive lipase activity. A similar increase in the tightness of connection of these pathways has been proposed for the other situations in which physiological state alters the relationships between β-adrenergic binding and lipolysis, such as different thyroid states, adaptation to different temperature or disease conditions.[40,41]

Another mechanism regulating the metabolism of glucose and fatty acids in the adipose tissues is the activity of the SNS.[42,44–46] When intake of energy is decreased, the release of norepinephrine (NE) from the SNS to brown and white adipose tissues is altered, theoretically to conserve energy.[42] In rats, reduction of NE synthesis in or release from the SNS causes an increase in the accretion of body fat.[44] In obese mice a greater accumulation of fat is associated with a reduction in SNS release of NE.[45] In rodents during pregnancy, there is a decreased oxidation of fat in brown adipose tissue, again theoretically to spare energy for fetal growth and lactation.[46,47] This reduction is also due to a decrease in NE from the SNS. The commonality of these adaptations to energy status suggests that metabolic regulation in adipose tissue during lactation is also controlled by the SNS.

We tested the hypotheses that the basal concentration of NE and the activity of the SNS in white adipose tissue was altered in pregnant or lactating animals. Rats at day 18 of pregnancy or days 7 and 21 of lactation were compared with age-matched virgin controls. The basal concentration of NE was measured in three adipose depots (retroperitoneal, perimetrial, and cardiac) and in heart muscle and liver. In pregnant animals, concentration of NE was reduced in perimetrial and retroperitoneal adipose tissues.[c] This is similar to the changes reported for brown adipose tissue and is consistent with the reduction in lipolysis and accumulation of body fat in pregnancy. Basal concentrations of NE increased in perimetrial adipose tissue at day 7 and day 21 of lactation, but in cardiac and retroperitoneal adipose tissue the increase in NE content did not occur until day 21 of lactation. This is the time at which milk production and adipose mobilization would be highest. This is consistent with the concept that the greater lipolysis during peak lactation is associated with more NE stimulation of lipolysis.

In a second study, the activity of the SNS was estimated by measuring the turnover of NE in these tissues after injection with α-methylparatyrosine (AMPT), a blocker of NE synthesis.[48] The turnover of NE in retroperitoneal and perimetrial adipose tissue of pregnant animals was the same or less than for controls, while in cardiac adipose tissue it was greater. At day 7, the increase in basal NE in perimetrial and retroperitoneal tissues was again noted,[c] but in no adipose tissue was the turnover different (*Table 1*). At day 21 of lactation, the basal concentration and turnover of NE was increased in perimetrial and retroperitoneal depots compared with virgin controls (*Table 1*). Tissue NE content and turnover of heart and liver tissues were not different across physiological states, indicating that the adaptation was unique to adipose tissues.

These studies suggest that the SNS also adapts to help regulate lipolysis during lactation. It is not clear whether this adaptation is important in early lactation; however, the rise in SNS release of NE later in lactation is consistent with the elevated rates of lipolysis at this time in the rat or cow.[5,8,10–12,15] In dairy cattle, rates of lipolysis remain increased after peak lactation, even though feed intake increases, insulin is increased, ST is decreased, energy balance is positive, and rates of lipogenesis are markedly increased.[10–12,32] This apparent "uncoupling" of regulation of lipogenesis and lipolysis allows for fine control of the system to meet both the needs of the lactating mammary gland and the need to increase adipose reserves after peak lactation. The low energy cost of recycling fatty acids through triglycerides (by constitutively high rates of esterification and lipolysis) is offset by the increased sensitivity and flexibility of serving both maternal needs. The adaptation of the SNS seems to be a secondary adaptation to extended lactation, and is probably acting in addition to the regulation by insulin and ST.

When one ponders all the reported descriptions of hor-

[c]McNamara, J.P., and Murray, C.E. (1994). Adaptations in sympathetic nervous system activity in white adipose tissue during pregnancy and lactation: role in regulating adipose tissue metabolism (abstract). *J. Anim. Sci.* **72,** (Suppl1) 226.

Table 1 Norepinephrine content and turnover in adipose tissue of virgin, pregnant, or lactating rats

Adipose tissue	Day 18 pregnancy		Day 21 laction	
	Basal	3 hours after AMPT	Basal	3 hours after AMPT
Perimetrial				
Virgin	31.8 ± 7.5	13.8 ± 1.4	19.6 ± 1.8	12.9 ± 3.4
Bred	16.2 ± 11.4*	11.1 ± 4.0**	38.5 ± 9.1*	24.6 ± 5.0**
Retroperitoneal				
Virgin	22.2 ± 2.5	12.7 ± 2.3	12.6 ± 3.3	15.3 ± 2.3
Bred	15.4 ± 3.7*	7.7 ± 1.7**	32.2 ± 13.5*	23.2 ± 3.3**

*Bred vs. virgin and pregnant vs. lactating, $P < 0.05$.
**Rate of turnover due to injection of α-methylparatyrosiine (AMPT) different between bred and virgin, $P < .05$.
Number of animals was 5 to 7 for each group.

monal and physiological mechanisms at face value, they demonstrate the existence of a complex and redundant control system acting on adipose tissue during lactation, a system which provides a high level of flexibility and efficiency to the animal. To improve research and application further, they also suggest that one cannot interpret the effects or mechanisms of a hormone without a serious consideration of multiple potential mechanisms. That is, the mechanism of action of a hormone, like its effect, may be different in different stages of lactation, or in fact one or more effect may be overidden by the actions of other hormones or the nervous system.

Biological scientists should not cease looking for universal concepts and mechanisms to help explain biological regulation. Rather, we need to define specific situations, so we should dampen somewhat our desire to construct simplistic and inclusive graphic descriptions of phenomena, and accept the facts as they are and use them to explain each specific situation. Each situation must be specifically defined and understood if we are to be able to predict responses to changes in dietary components. Simplifications and generalizations may easily be as wrong as they are quick. As more detailed molecular descriptions of these regulatory systems become available, it is important to interpret them within the framework of each clinical or production setting and to relate them realistically to the known kinetics of nutrient flux in that situation.

Relationship of metabolism in adipose tissue to reproduction during lactation

General relationships between energy intake and body fatness with reproductive status have been noted for centuries. These observations inspired research efforts designed simply to relate measures of nutrient intake and body fatness with reproductive variables, including age at first menarche, or sexual maturity, postpartum infertility, timing of return to estrus in lactation or after weaning.[49,50] One such theory was that there was a "set-point" of body fat or composition, above or below which the adipose mass somehow signaled to the central nervous system (estrogen release from adipose was one signal proposed) that the body was either ready or not for cycling and pregnancy.[50] This research effort demonstrated that there were some such relationships, however, there were as many situations in which body fat content related negatively with cycling as positively.[49] Further work delved into more mechanistic studies attempting to relate concentrations of nutrients and metabolites with reproductive status. Again, much was learned, but much more was ruled out than was proven as a causal link. Although many observations and epidemiological research substantiated some link between the short- or long-term status of energy availability and reproduction, a causal link had proven elusive.

Some exciting research findings now link directly the flux of glucose with release of gonadotropin-releasing hormone (GnRH).[49] All who are interested in this area of research should read the thorough review of Wade and Schneider.[49] A major premise of that treatise is that we have moved past the simplistic view of relating body fatness or circulating hormone levels (which can be viewed as endpoints of nutrient status and not necessarily regulatory factors in themselves) to variables of reproduction. We should now concentration on the question: "What are the biochemical and molecular components of the stimulus and sensor of metabolic fuel availability?"[49] I would like to point out some specific recent examples which argue strongly against the idea that the body composition itself has a direct link to the organs which regulate reproduction, but rather support the theory that "in a variety of species, the primary locus of dysfunction in the energetically challenged female appears to be neural control of GnRH secretion."[49] Unfortunately, the amount of information on this topic from lactating animals and women is severely limited.

A primary regulator of ovulation is the release of luteinizing hormone (LH), which is in turn regulated by GnRH. Several studies have now demonstrated that the normal pattern of GnRH and LH pulsatility is unrelated to the composition of the body.[49,51,52] This was recently demonstrated in pigs, in which energy deficit was used to delay reproductive maturity.[51] In those animals, decreased starch intake delayed initiation of cycling in a dose-response manner. However, the body fatness of the animals which ranged from less than 5% to more than 30% was not related to initiation of cycling.[51] In addition, glucose infusion can increase LH release in young cycling pigs.[53] In several

studies, the release of GnRH and LH was initiated within hours of consuming a meal after a long period of anovulation caused by depressed energy intake.[49] Insulin injections or infusions can cause a reduction in GnRH and LH release, but this reduction is prevented by simultaneous infusions of glucose.[49] It does not appear that circulating free fatty acids play a direct role in regulation of the pulse generator of GnRH, as high fatty acids concentrations do not prevent the anovulation nor the depressed LH release seen with glucose deficit, nor does an increase in fatty acids or ketones in the presence of adequate glucose cause a depression in LH release or ovulation.[49]

This new information does not deny that the amount of fat at parturition or the loss of body fat after parturition can be related to reproductive status in women and especially in domestic animals. However, as stated so elegantly by Wade and Schneider,[49] both the body fatness and changes in patterns of reproductive hormones are in fact the effects of changes in glucose availability and are not causally related. The evidence now demonstrates that release of GnRH and LH, and thus ovulatory activity, is affected directly by glucose supply. In this context, glucose supply means rate of supply to the central nervous system, which, during lactation, is a function of absorption from the gastrointestinal system, gluconeogenesis, and demand by the mammary gland. Thus it may be the pattern of intake of specific nutrients in early lactation, and not the total energy intake, which relates most closely with reproductive status in domestic animals.[49,52–56,d] The intake of amino acids does not seem to be related to reproductive status.[49,56] The altered glucose status in early lactation of dairy cattle and swine is consistent with this being a causal factor in the higher incidence of delayed ovulation in some of these animals. This relationship was just recently confirmed for lactating beef cattle, in which high rates of glucose absorption (by means of feeding a high energy diet) caused a greater responsiveness of LH release to infusions of GnRH.[56]

The amount of body fat is still important to lactation in two different senses. The first is that, because body fatness is an indicator of energy reserves and thus past nutritional and physiological history, it is useful in decision making for nutritional management (again, for either women or domestic animals). In the second sense, within the context of the overall metabolic biology of the body, the amount and rate of glucose flux in the adipose tissues does alter the mixture of metabolic fuels available to the rest of the body and cannot be considered apart from the overall chemical fluxes of the body. In this indirect but important manner, adipose tissue may be another factor which affects reproductive status. For example, in animals which have a reduced rate of glucose utilization in the adipose tissue, either due to genotype or increased intake of dietary fats, the glucose availability to the central nervous system may be different than that in animals of similar body composition, but having higher rates of glucose use in the adipose tissue. This may help to explain some of the as yet undefined variation in milk production and reproduction among animals of similar body composition or genotype. In addition, this could be one reason for the improvement in reproduction noted in some studies feeding increased-fat diets to lactating dairy cattle, if in fact the additional fat spares glucose for use by other tissues.[16,20] This does not argue that adipose tissue itself is a regulatory organ for reproduction, but that, in the range of metabolic situations possible in lactation, the amount of adipose tissue and the rate of the metabolic pathways therein may be a contributing factor to the variation seen in reproduction during lactation. This may be especially true in lactating dairy cattle and swine, which display wide variations in rate of glucose intake and use, amount of body fat and ovulatory behavior.

[d]Koketsu, Y., Dial, G.D., Pettigrew, J.E., and Marsh, W.E. (1994). Influence of feed intake patterns on LH secretion and concentrations of serum insulin and plasma glucose in lactating sows (abstract). *J. Anim. Sci.* **72** (Suppl. 1) 329.

Direction for future efforts

Where do we proceed with the increased knowledge we now possess? First, we need more precise information on the mechanisms coordinating lactational support and the outcomes of various nutritional and milk-demand situations. What are the causes of changes in cellular response to hormone signals? Within adipose tissues, what are the relationships between hormone binding, second messenger dynamics, and enzyme phosphorylation and activity during pregnancy and lactation? What are the adaptations in adipose tissue to different stages of lactation and different levels of nutritional input or milk demand? Which adaptations at each level of biology are critical to optimize lactation? Does stage of lactation, rate of milk production, or nutrient supply alter expression of mRNA for enzymes in adipose tissue? Additionally, we need studies in animal models more closely resembling the human metabolism than the dairy animal or rat. Toward this end, swine may be useful.

The importance of lactation to the well-being of the mother and child and the importance of domestic animal lactation to the world food supply dictates an integrated research approach. To devise effective future research plans, we need to know the key regulatory mechanisms. To better plan prevention and intervention strategies, we need to predict, via increased use of dynamic, kinetic, and mechanistic relationships, the consequences of genotype, nutrition, and medical intervention. The mechanisms and kinetic parameters should be integrated, using mechanistic models of metabolism to predict responses to management of the lactational state.

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