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Adipose tissue metabolism during lactation: where do we go from here?

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The adipose tissues play an integral role in the preparation for, and the establishment and completion of, lactation. Within the animal kingdom, only mammals have such a highly developed tissue for storage of energy over long periods of time (Pond, 1984). One benefit of this storage tissue is for adaptation to environmental and seasonal changes in nutrient supply. In addition, the adipose tissue allows for successful growth of the young *in utero* and feeding of the neonate. In several species, a significant portion of the adult life of a female may be spent in dependence on adipose tissue for energy. The adipose tissues play an essential role in survival of mammalian species, the supply of human food through domestic animals, and direct survival and health of the human neonate and lactating mother.

The adipose tissue takes up sugars, fatty acids (and in some cases, excess amino acids) and converts them to triacylglycerols. The fatty acids may be hydrolysed and released from the adipocytes at extremely high rates. There is a constant and precisely regulated recycling of fatty acids through triacylglycerols in adipocytes. Enzymes catalysing synthetic (acetyl-CoA carboxylase (*EC* 6.4.1.2.), fatty acid synthetase (*EC* 2.3.1.85), lipoprotein lipase (*EC* 3.1.1.34)) and lipolytic (hormone-sensitive lipase (*EC* 3.1.1.3)) reactions are regulated acutely by direct phosphorylation, ATP phosphorylation state, or both. This recycling coupled with a sensitive regulatory system allows a rapid response to short-term changes in nutrient demand at a relatively low energetic cost. The activities of these enzymes are controlled by insulin, insulin-like growth factors, glucagon, sympathetic neural regulation through noradrenaline, and acute adrenal regulation via adrenaline. In addition, during pregnancy and lactation, progesterone, oestrogen, prolactin, placental lactogens, and somatotropin all have direct regulatory effects on expression and activity of key enzymes in adipocytes.

The most rapid rates of lipolysis, and the most rapid rates of lipogenesis observed in *in vitro* or in *in vivo* systems have been measured in lactating animals (McNamara, 1994). The demands of pregnancy and lactation to supply large amounts of protein, lactose and fat to the young are often only met by using adipose tissue reserves. The domestic dairy cow can store and then release 100 kg fat over a span of 6–8 months in late gestation and early lactation. This can be as much as 20 % of the adult weight. In rodents, although food intake increases tremendously, adipose tissue is mobilized as lactation progresses and dams nursing large litters can lose a large proportion of body fat during lactation.

In humans, the more moderate demands of the young baby do not usually dictate such a heroic biochemical struggle by the mother. In the more 'well-fed' Western European and North American populations, the net transfer of energy from adipose tissue to the baby may not be significant, as the mother can supply the needs of lactation from the diet. Nevertheless, even moderately-well-fed women may lose body fat during lactation, because the demand for specific milk fatty acids and the hormonal stimuli common to the lactational state induce a rapid rate of lipolysis and may depress *de novo* lipogenesis out of proportion to energy intake alone. Observations of only well-fed populations (and a lack of data from many other situations) have sometimes led to the conclusion that adipose tissue is not important in human lactation (Rasmussen, 1992; Prentice *et al.* 1994). This narrow interpretation ignores the significant population of women, regardless of geographical location, who are not 'well-fed', and whose baby must count on either or both the transfer of fat from mother to itself, or the use of fat by the mother during pregnancy and lactation for her own maintenance needs as well as the needs of the baby (Institute of Medicine Subcommittee on Nutrition during Lactation, 1991; Martinez *et al.* 1994; Scholl *et al.* 1994). This also ignores some of the specific chemical contributions of adipose tissue apart from energy needs, discussed later (see pp. 158, 164)

Another potential role for the adipose tissue in lactation has been suggested with recent work on transfer of: (1) compounds such as specific fatty acids, including conjugated linoleic acid, from maternal stores to the baby through the milk (Fogerty *et al.* 1988; Lin *et al.* 1995; McGuire *et al.* 1996); (2) contaminants, such as polychlorinated biphenyls and other lipophilic xenobiotics, from maternal fat stores to the baby through the milk (Walsh & Neville, 1994). The increased rate of lipid recycling in the adipose tissues, even without a net loss of body fat, may cause an increased delivery of such compounds to the baby.

The present paper will concentrate on a few major points concerning the function of adipose tissue during lactation. It should serve a general purpose to introduce some readers to the area, to challenge some existing interpretations and to stimulate further integrated research. Although an impressive amount of detail will be cited, there are still serious challenges to our understanding of adipose tissue function in lactation in various environmental and nutritional states. A lack of information on basic areas impedes our progress, including regulation of expression of genes coding for critical regulatory and catalytic proteins, the mechanisms of signal transduction and the integrated effects of several hormonal control systems. In addition, we need data on the integration of C flux in the adipose tissue, liver and mammary glands in situations widely different in rates of milk production (neonatal demand) and dietary nutrient availability. Because of these gaps we have a limited ability to predict with precision milk production, milk composition and body composition of domestic animals or human subjects under various conditions. It is both an encouraging and disheartening experience to peruse through a thousand or more references cited directly or indirectly in the present review (encouraging to know so much information is available on adipose tissue function; disheartening because even with that we have only a sketchy picture of the actual quantitative flux and regulation of nutrient interconversions in adipose tissue). These data will help some critical needs in the growing effort to integrate knowledge on adipose tissue into research and predictive models describing the lactational state.

RATES OF CARBON FLUX DURING LACTATION

There is no other physiological or nutritional state reported in which there is such a large adaptation in rates of C flux through adipose tissue as there is in lactation. However, this

adaptation itself varies tremendously among species, and within a species depending on demand for milk and maternal nutritional situations. In the four species most studied (laboratory rats, domestic dairy cows, domestic pigs and human subjects), rates of adipose tissue metabolism show wide variation during lactation due to the stage of lactation and nutritional status (Rebuffe-Scrive *et al.* 1985; Bell & Bauman, 1994; McNamara, 1994; Neville *et al.* 1994; Williamson & Lund, 1994; Parmley & McNamara, 1996). In these species, entry into the lactational state generally causes a decrease in rates of lipogenesis, somewhat of a decrease or no change in esterification, and no change to an increase in rates of lipolysis. The net effect is to have a transient diminution of fatty acids stored in adipose tissue while increasing amounts are secreted in milk or oxidized by other maternal tissues.

Although the demand for mammary secretion of lactose and fat alters the C flux through adipose tissue even in the well-fed animal, it is when lactation ensues in suboptimal nutritional situations that the true role of adipose tissue is appreciated. The ratio lipolysis : lipogenesis in subcutaneous adipose tissue of the dairy cow (Fig. 1(a)) demonstrates the widest directly-measured range of adipose tissue C flux reported in any lactating animals, although aquatic mammals and bears may exceed these rates (Bell & Bauman, 1994). As milk production is increased by genetic selection, the adipose tissue demonstrates a massive increase in net lipolysis, while a decrease in energy intake lowers *de novo* lipogenesis to negligible rates for a period up to 6 weeks. When milk production is phenotypically high, even with maximal feed intakes, net lipolysis still ensues for several weeks. In animals phenotypically able to produce only a lower amount of milk, or when energy intake is high, then net body fat loss is lowest. The ratio lipolysis : esterification (Fig. 1(b)) is much lower than that for lipogenesis but still demonstrates that there is a period of net fatty acid loss dependent on demand for milk fat and supply of precursors from the diet. Comparing lipolysis : lipogenesis with lipolysis : esterification illustrates the high rates of fatty acid turnover during lactation.

In laboratory rats, a similar adaptation is seen, and increased milk production by a higher litter size or reduced feed intake greatly enhances the net flux of C from adipose tissue. A lower energy demand or a higher feed intake slows the net rate of body fat loss, but the adaptations still occur (Williamson & Lund, 1994).

Lactation is not just one physiological state, but in fact has distinct stages of initiation, establishment, maintenance, and either a rapid or slow involution. Thus, the rates of C flux in adipose tissue are not constant during lactation, but rather reflect the particular lactational stage. In laboratory rats and most domestic pigs, rates of milk production rise rapidly in an asymptotic fashion, and thus, become limiting to litter growth in about 10 d to 2 weeks postpartum. After another 1–2 weeks, weaning occurs abruptly to allow faster growth of the young (albeit the time of weaning is decided in one species by that which is doing the lactating, and in the other species, by those who 'manage' the lactation). Thus, lactation consists of at least 'early' lactation, a period of time in which rates of milk secretion and energy intake are increasing together, and 'peak' lactation, in which milk production is sustained, but voluntary intake usually continues to increase. During peak lactation, even during high rates of energy intake, body fat continues to be lost due to the demand of the mammary gland.

Both human subjects and the domestic dairy cow have a lactation which can be expanded for a long period of time (8–10 months is common, several years is not uncommon in both species, even with concurrent pregnancy; Institute of Medicine Subcommittee on Nutrition during Lactation, 1991; Bauman & Vernon, 1993). In dairy cattle there is: 'early' lactation, in which both milk production and feed intake increase, but in which energy balance is usually negative; 'peak' lactation, in which milk production

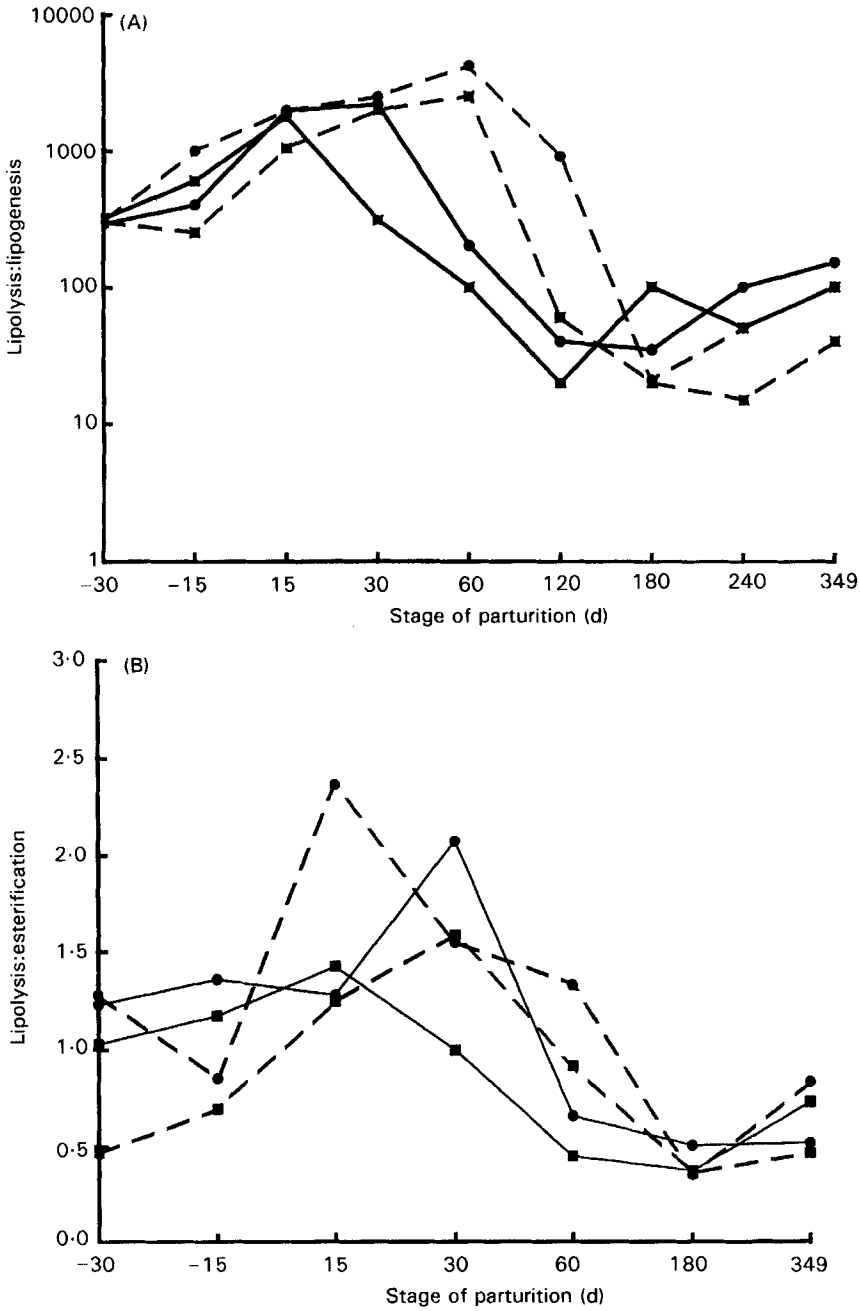

Fig. 1. The ratio, lipolysis : *de novo* lipogenesis in bovine adipose tissue *in vitro* during lactation. (A) Data are the rates of fatty acid release divided by the rates of fatty acid synthesis (nmol fatty acid synthesized/g tissue per 2 h) measured *in vitro* incubations at maximal substrate concentrations. (B) Rates of fatty acid release relative to the rates of fatty acid esterification in triacylglycerol *in vitro*, with the ratio normalized to a value of 1 at day −30 for the average genetic–high energy treatment group. (—), diets fed at 670 g concentrate and 330 g forage/kg; (– – –), diets fed at 380 g concentrate and 620 g forage/kg; (●), animals of superior genetic merit for milk production; (■), animals of average merit. (Data are taken from several experiments in our laboratory: McNamara, 1994; McNamara & Hillers, 1989).

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occurs at maximum rates, voluntary feed intake also reaches a maximum, and energy balance becomes less negative; 'middle' lactation, in which milk production may be sustained or drop slightly (10–30 % from peak) but voluntary energy intake remains high and energy balance becomes positive. Finally there is 'late' lactation, in which production of milk lactose may still exceed 2500 g/d and milk fat and protein each exceed 1700 g/d for several weeks, but feed intake also remains high and the adipose tissue restores its lost lipid.

The human subject invests as much time in lactation as the dairy cow, and in that way these two species are more similar to each other than either species is to the laboratory rat. As lactation progresses over time, the demand for milk lactose and fat and the developmental and involutionary processes of the mammary gland result in hormonal and neural signals common to each lactational stage. These signals alter rates of specific biochemical pathways in adipose tissues out of direct proportion to energy intake. Low-producing dairy cattle may actually produce milk energy at a rate (relative to maintenance needs) fairly close to that of human subjects. Thus, the cow may serve as an example of lactational metabolism applicable to several species including human subjects. The extended lactation of the cow allows for studies into critical interactions between various metabolic, endocrine and neural signals which alter intracellular regulation of enzyme activities and C flux. Information about the regulatory processes through the full lactational cycle will be needed even more as more women breast-feed, and breast-feed exclusively, for longer periods of time (Institute of Medicine Subcommittee on Nutrition during Lactation, 1991).

The domestic pig provides a unique model of lactation, in that, although a litter bearer, the relative energy demand of the litter, especially smaller ones, is much closer to that of the human subject than to that of either the dairy cow or the laboratory rat (Pond, 1986; Tumbleson, 1986; McNamara, 1995). In addition, the capacity of the sow to consume adequate energy is greater than in the dairy cow and rat, also more similar to the human situation. Rates of chemical interconversions in adipose tissue of lactating sows demonstrate a pattern similar to that of human subjects (Parmley & McNamara, 1996). When sows are fed during gestation a ration formulated to allow adequate fetal growth but not significant maternal fat accretion, the transition into lactation (and onto a ration now adequate in energy) results in increased rates of lipogenesis and esterification, and no change in rates of lipolysis (Parmley & McNamara, 1996). After 3 weeks of lactation on an energy-adequate ration the rates of lipogenesis decline markedly from early lactation and lipolysis does not change, resulting in some loss of body fat. When energy intake is restricted 33 %, lipogenesis rates drop 90 %, and esterification rates drop about 35 %, while lipolysis rates double (Parmley & McNamara, 1996). Thus, in these animals, there is a net efflux of fat from the adipose tissue to support mammary function, while a fairly high rate of fatty acid recycling is maintained.

This recycling of fatty acids is a critical aspect of adipose tissue function in lactation. The recycling occurs continuously, but has a relatively low energetic cost, amounting to only a few percent of the cost of storage of lipid energy. This recycling allows the animal a great range of responsiveness to physiological signals, generated by changes in the environment such as temperature or energy intake. The rate of recycling may also have a significant inheritance, as suggested by selection in dairy cattle and pigs. In contrast to the rat, in which the rate of recycling may drop in lactation (Williamson & Lund, 1994), the dairy cow and sow demonstrate simultaneously high rates of lipogenesis and lipolysis during a significant period of time (McNamara, 1995; Parmley & McNamara, 1996). This apparent contradiction is solved when one considers the full range of biochemical

pathways involved and their functions to the body other than just the provision of energy. For example, the mammary gland has a limited ability to make fatty acids *de novo* and, thus, must derive about 50% from the bloodstream directly. This amount does not appear to change more than about 10% over a wide range in intake and milk production rates (Palmquist, 1993). Thus, the cycling in the adipose tissue reflects the demand for fatty acids by the mammary gland and the primary importance of adipose tissue as the major site for lipogenesis in these animals. This cycling provides a useful model for basic research into the mechanisms of intracellular signalling and responses to complicated hormonal and neural messages; research which is applicable across a wide range of cellular biology.

A potentially-important species difference may be due to the fact that for the dairy cow and pig, the adipose tissue is usually the major site of *de novo* lipogenesis, while in the rat the liver contributes the major amount (Williamson & Lund, 1994). In the human subject it is still uncertain whether the adipose tissue or the liver provides most *de novo* fatty acids during lactation due to a critical lack of data. The importance of this process lies in the energetic cost of total body fatty acid recycling. If the majority of lipogenesis and recycling occurs in the adipose tissue, the cost is minimal and the benefits in adaptability great. However, it has been suggested for the human subject and perhaps rat that the energetic cost of interorgan fatty acid recycling may approach 20% of the total energy deposited (Williamson & Lund, 1994). This clearly is a substantial cost and, while the survival benefits are still important, the total cost may be amenable to decrease by altering the amount and composition of carbohydrates and fat consumed.

REGULATION OF METABOLISM DURING LACTATION

General homeostatic mechanisms

Maintenance of homeostasis is achieved by monitoring nutrient availability on a relatively short-term basis (minutes or hours). Rates of lipogenesis are controlled by the amount of substrate and by concentrations of insulin, glucagon, corticosteroids, noradrenaline and adrenaline. These hormones are basically a reflection of the immediate nutrient supply, and act to increase substrate uptake or alter pathway partitioning intracellularly, or both. There are probably genotypic and phenotypic differences in the efficiency of this control as shown by several genetic models of obesity and selected lines of animals which vary in body composition, adipose tissue metabolism and hormonal secretion rates (Bray, 1991*b*; Bell & Bauman, 1994; Williamson & Lund, 1994; McNamara, 1995). Esterification is also regulated by these hormones, but less tightly, and thus fatty acid is allowed to cycle continuously. As the other half of the recycling pathway, and to allow release of fatty acids to the body, lipolysis is also regulated by these agents, but most directly by noradrenaline (Lafontan & Berlan, 1993, 1995). Insulin also has longer-lasting effects on synthesis rates of several enzymes catalysing anabolic pathways, but these effects are still rather short-lived, lasting from 1 d to a few days after a fall in plasma insulin concentrations.

The normal chronic regulation of lipolysis by the release of noradrenaline by the sympathetic nervous system has not been well studied during lactation. Tonic release of noradrenaline is directly related to rates of lipolysis and the net amount of body fat accumulated in various models (Shimazu, 1981; Knehans & Romsos, 1983; Dulloo & Miller, 1985; Lafontan & Berlan, 1993, 1995). This regulation has been studied in depth for brown adipose tissue, but not for white adipose tissue (Trayhurn & Richard, 1985; Lafontan & Berlan, 1993, 1995) and the amount of fatty acid uptake and oxidation is a direct function of sympathetic activity in brown adipose tissue.

Environmental, hormonal or nutritional manipulations which increase sympathetic nervous activity decrease net fat accretion and those which decrease sympathetic activity increase fat accretion. In addition, sympathetic nervous system activity and intake of specific nutrients are related (Bray, 1991*a*) such that fasting usually decreases sympathetic activity. Dietary fat may directly change sympathetic activity (Young & Walgren, 1994). Thus, the dietary energy or fat–sympathetic nervous system couplet may alter adipose fatty acid metabolism in a manner similar to the glucose–insulin couplet; i.e. increasing the supply of a specific nutrient primes the system for feed–forward hormonal regulation to direct the metabolism of that nutrient. There are indirect indications that the sympathetic nervous system may play a role in lactation as well as direct indications (McNamara & Murray, 1994; McNamara, 1995).

General homeorhetic mechanisms during lactation

As the mother passes through various physiological states, regulatory systems operating at a 'higher level of organization' than the homeostatic systems come into play, these have been termed 'homeorhetic' systems (Bell & Bauman, 1994). The general effect of these higher systems is to attenuate or enhance as necessary homeostatic regulation. One major purpose of homeorhetic regulation is to direct various nutrients to different tissues to meet the demands of the physiological state, thus prolactin and somatotropin direct increases in lipogenesis in the mammary tissue and decreases in the adipose tissue. Hormones of reproduction including oestrogen, progesterone, prolactin, placental lactogen and somatotropin may alter the sensitivity of adipose tissue to homeostatic signals. However, several of these hormones also have direct effects on enzyme activity or gene expression which are separate from their effect on homeostatic hormone response. These effects may enhance the primary effect of the homeostatic regulator, or they may be 'permissive', allowing a specific effect to occur only when the reproductive hormone is present in sufficient amounts, this often appears to be the mechanism for steroids such as oestrogen and progesterone. With several hormones acting simultaneously, the effect of one hormone may be 'redundant' in nature, with the effect not readily seen until another system fails or is already operating at a maximal or minimal effect. This redundancy provides an additional measure of safety for the maternal body as a whole, but also an extremely difficult situation for the scientific community to define.

Often some hormonal effects are reported which may be unique only to very specific situations such as hypophysectomized animals and do not appear in 'normal' situations (Bauman & Vernon, 1993; Bell & Bauman, 1994). Several more 'normal' situations are usually characterized by unique combinations of stage of lactation, concurrent lactation and pregnancy, or in various interactions of lactation, nutritional status, season and environment (Bauman & Vernon, 1993; Bell & Bauman, 1994; McNamara, 1995). These situations add variation to 'the lactational state' and must be defined.

Specific regulation of lipogenesis and esterification during lactation

Lipogenic rates are directly regulated by the amount of substrate available (glucose or acetate or both), and further by the circulating concentrations of insulin. In high-producing or litter-bearing species, lipogenesis is decreased in adipose tissue as a combined function of lack of nutrients in relation to demand by the mammary gland (Bell & Bauman, 1994; Williamson & Lund, 1994). The mechanisms include a reduction in activity of most enzymes catalysing fatty acid synthesis. In rats this includes reduction of mRNA for acetyl-

CoA carboxylase (Pape *et al.* 1988; Ponce-Casteneda *et al.* 1991; Barber *et al.* 1992), this has not been reported yet for other species. During early lactation, the reduction in lipogenesis is a function both of reduced availability of substrates and a decrease in adipocyte responsiveness to insulin. This effect may be due to concentrations of somatotropin, or prolactin, which both increase in early lactation. Both these hormones have such effects in *in vivo* or *in vitro* systems, although the evidence is stronger for somatotropin (Barber *et al.* 1992; Bauman & Vernon, 1993; Bell & Bauman, 1994; Vernon *et al.* 1995*b*). Direct effects of prolactin are as yet unclear, as it has not been possible to demonstrate that adipocytes express prolactin receptors (Bell & Bauman, 1994).

These hormones may directly affect the enzymes involved, through an as-yet-undefined second messenger system. Somatotropin may reduce the insulin response at a point after receptor binding, which does not seem to be affected by somatotropin (Bauman & Vernon, 1993). Recent evidence suggests that one mechanism may be an inhibition of insulin processing by somatotropin or prolactin due to their ability to act as serine protease inhibitors (Marinchenko *et al.* 1992). However, the role of insulin processing in the mechanism of insulin action is not yet clear (Duckworth, 1988). This concept is also supported by the finding that polyamines may be involved in somatotropin action on signal transduction (Borland *et al.* 1993). When synthesis of polyamines is blocked by the addition of an inhibitor of ornithine decarboxylase (*EC* 4.1.1.17), growth hormone cannot inhibit lipogenesis, and replacement with spermidine restores this effect (Borland *et al.* 1993). Transcription of a second messenger may also be involved in this process, because when actinomycin D blocks ornithine decarboxylase activity and spermidine is added, the growth-hormone inhibition of lipogenesis is not restored (Borland *et al.* 1993). Polyamines are also protease inhibitors and may alter the proteolysis of insulin (McCroskey *et al.* 1989). A definition of the molecular mechanisms involved in the action of somatotropin, prolactin or the steroids in lactation should continue to be a major objective of research in lactation biology.

Uptake of fatty acids from circulating triacylglycerols and subsequent esterification into cellular triacylglycerols is catalysed by lipoprotein lipase. Activity of this enzyme is reduced in early lactation (Williamson & Lund, 1994). Insulin has a direct stimulatory effect on activity of this enzyme; thus, during lactation, activity may be regulated by the attenuation of insulin action by somatotropin. In late pregnancy, the concentration of mRNA for lipoprotein lipase in adipose tissue is reduced (Martin-Hidalgo *et al.* 1994), perhaps as a function of reduced action of insulin, but this has not yet been reported for lactation. This pathway accounts for a significant proportion of fatty acid flux through adipose tissue during lactation, and it is necessary that we meticulously define the quantitative effects of different hormonal states *in vitro* and *in vivo* on the activity of this enzyme. These studies should investigate both direct effects on enzyme activity as well as effects on transcription and translation of mRNA for lipoprotein lipase.

Specific regulation of lipolysis during lactation

Lipolysis is catalysed by the action of hormone-sensitive lipase, an intracellular enzyme acting at the interface of the cellular lipid droplet and cytosol (Belfrage *et al.* 1984; Lafontan & Berlan, 1993, 1995). This enzyme is under direct control by phosphorylation activation induced by the β-receptor and cAMP cascade system; activation and catalysis also involve physical translocation of the enzyme at the lipid–aqueous interface (Egan *et al.* 1992). Thus, the activity of this enzyme is a function of noradrenaline and insulin concentration. The reduced insulin sensitivity in early lactation regulates lipolysis during

this time (Bell & Bauman, 1994). Adrenaline may not play an important role beyond acute responses to environmental stimuli. The major mechanism of regulation is through the action of noradrenaline on β-adrenergic receptors, thus tonic and long-term lipolytic rates are a direct function of sympathetic nervous system activity and the amount and binding efficiency of β-adrenergic receptors.

The effect of lactational states on the intracellular responses of lipolysis to adrenergic stimulation has been studied in a number of different situations. In all species tested, with the exception of the domestic pig (Parmley & McNamara, 1996), the responsiveness of adipose tissue to β-adrenergic stimulation increases during early lactation (Vernon *et al.* 1991). As a summary of several studies, the onset of lactation results in the following adaptations in adipose tissue: the number of β-receptors stays the same or increases, the amount of adenylate cyclase (*EC* 4.6.1.1) activity increases, the concentration of cAMP increases, cAMP phosphodiesterase (*EC* 3.1.4.17) and total protein kinase A (*EC* 2.7.1.37) activity do not change, and hormone-sensitive lipase activity and the hormone-sensitive lipase activity : cAMP ratio increases (Vernon *et al.* 1991, 1995*b*; McNamara *et al.* 1992; Bell & Bauman, 1994; McNamara, 1994). In addition, adenosine attenuates the lipolytic cascade through a separate receptor and resultant reduction of cAMP concentration (Vernon *et al.* 1991), and this action is decreased in lactation, allowing faster rates of lipolysis. These studies demonstrate a well-coordinated effect to increase basal lipolysis and the response to lipolytic stimuli during early and middle lactation. The intracellular signal transduction system becomes more sensitive to adrenergic stimulation in adipocytes of lactating females.

As an important technical note critical to research in this area, several researchers have failed to obtain consistent differences in *in vitro* basal lipolysis during lactation, and interpret this as doubt of the involvement of this pathway. However, it is known that hormone-sensitive lipase is rapidly dephosphorylated when adrenergic stimulation is removed (Belfrage *et al.* 1984; Lafontan & Berlan, 1993). In our laboratory, we have been unable to correlate measures of *in vitro* enzyme activity (in broken-cell preparations) with rates in whole-tissue preparations because of this phenomomen. Thus, we consider whole-cell (tissue) preparations and descriptions of lipolysis under adrenergic stimulation more reflective of the *in vivo* state characterized by chronic noradrenaline release from the sympathetic nervous system.

Hormones which control this system include somatotropin, prolactin and oestrogen. Somatotropin is clearly involved, and may alter activity of the GTP-binding protein Gs to affect the cAMP response; however, the response is not always obtained in different situations (Bauman & Vernon, 1993; Vernon *et al.* 1995 *a,b*; Doris *et al.* 1996). Prolactin may have similar effects, but they have not been clearly demonstrated. There are depot differences as well: in the rat, the omental depots may be more responsive to lactation than the subcutaneous depots (Doris *et al.* 1996), although in larger species all depots lose significant amounts of fat.

A potentially important regulator of adipose tissue metabolism which has been relatively overlooked in lactation is the effect of oestrogen. Oestrogen has multiple effects on adipose tissue, the net effect of which is usually catabolic (Pasquier *et al.* 1988; Lazzarini & Wade, 1991; Lafontan & Berlan, 1995). Oestrogen directly affects the response of adipocytes to sympathetic nervous system activity through its own actions on adrenergic-receptor expression (Lafontan & Berlan, 1995) and on the intracellular signalling cascade (Lazzarini & Wade, 1991). Thus, the 'oestrogen–adrenergic control system' may be a complementary system to the 'somatotropin–adrenergic control system'. Alternatively, one system may be redundant to the other, with each taking primary control

in different nutritional, physiological or environmental situations. This function of oestrogen may have a role in maintaining the high rates of lipolysis in late lactation in dairy cattle after somatotropin concentrations decline.

The other mechanism of regulation which has not been well-studied by lactation biologists is the sympathetic nervous system. The activity of the sympathetic system in brown adipose tissue is changed during pregnancy and lactation of the rodent (Trayhurn & Richard, 1985), in both brown and white adipose tissue of obese animals (Knehans & Romsos, 1983) and in various energy balance states (Landsberg, 1990). We have demonstrated that the concentration of noradrenaline is increased in white adipose tissue during lactation in the rat, and sympathetic nervous system activity as measured by noradrenaline turnover may increase as well (McNamara & Murray, 1994; McNamara, 1995). This effect is not seen in late pregnancy, and the adaptation is much stronger in late lactation, after most of the lipid stores have been depleted.

Indirect evidence has also been collected from late lactation in lactating dairy cattle, in which the strict energy balance would suggest a low rate of lipolysis and a high rate of lipogenesis. However, in four different studies, under different dietary conditions, using *in vivo* and *in vitro* measures, we have found concomitant high rates of lipogenesis and lipolysis in dairy cattle during later lactation (McNamara & Hillers, 1989; McNamara, 1995; McNamara *et al.* 1995). In this period, substrate availability and insulin concentrations are high, and somatotropin and prolactin levels have decreased compared with early lactation. In addition, the animals are usually in the first or second trimester of pregnancy at this time, so progesterone would be expected to be high and oestrogen relatively low. However, the net demand for milk-fat precursor is still great, and the sympathetic nervous system may be the primary mechanism by which the maternal system meets, simultaneously, the needs of the mammary gland and her own need to increase lipid storage.

A molecular description of the intracellular mechanisms of hormone regulation of lipolysis is not yet complete, but there is much more known now than 10 years ago, and research is becoming more definitive. In the near future we should have a clear description of at least one intracellular mechanism by which a hormone of lactation (somatotropin) alters the responsiveness of a homeostatic regulator (insulin or noradrenaline). This will be a satisfying molecular proof of the integrative hypotheses of Barnard, Cannon and Hammond (see Bell & Bauman, 1994) and will allow meaningful manipulation of genetic expression to control metabolic pathways.

INTEGRATION OF ADIPOSE TISSUE WITH MATERNAL AND MAMMARY SYSTEMS

Supply of proper nutrient mixture to mammary gland

A major function of the adipose tissue is to provide fatty acids to other tissues. This becomes more important during times of a deficit of energy intake, but the fatty acid supply is a constant requirement of several other tissues regardless of energy supply. This is especially true for the mammary gland. At any point in time, the mammary gland has an exact requirement for approximately twenty amino acids, twenty or more fatty acids, and glucose (as well as vitamins and minerals). Regardless of the intake of the animal, the mammary gland must have the proper amount and mixture to operate at peak efficiency. For example, the mammary gland cannot synthesize more than about 60 % of milk fat from carbohydrates or volatile fatty acids; it must be supplied with fatty acids of medium and longer chain length. If either the quantity or the balance of supplied nutrients moves away from the exact requirement, then the total yield of milk, the composition of milk, or both, will change.

As the net flux of fatty acids from adipose tissue changes due to dietary supply of carbohydrate, fat or total energy, stage of lactation, stress, or in a larger frame, due to genetic selection, the nutrient supply mixture also changes. Two examples of the importance of the contribution of adipose tissue to milk production are the effects of dietary energy density (by altering forage : concentrate ratio) and by feeding different amounts and types of fatty acids.

Several studies have demonstrated a wide variety of effects on milk yield and composition of total milk fat, composition of milk fatty acids, total milk protein and composition of milk protein by manipulating dietary fat and carbohydrate supply (DePeters & Cant, 1992; Palmquist, 1993; Wu & Huber, 1994; Baldwin, 1995). There is ongoing a serious scientific discussion regarding the effects of feeding dietary fats to ruminants on the proportion of protein in milk, the total yield of milk protein and the type of milk protein synthesized. In over 100 studies, effects have been inconsistent and vary widely from negative to positive in relation to the control ration used. Confounding factors include the total composition of the diet, amount and fatty acid composition of the fat, whether or not the fat is protected from degradation in the rumen, total feed intake, intake of total protein and amount of rumen-soluble or -insoluble protein. Even after considering several of these factors, there remains a large variation in response of milk protein and fat to feeding different amounts and types of fats (Wu & Huber, 1994).

In one study, we fed different levels and types of fats for 285 d of lactation starting at day 17. We demonstrated specific differences in synthesis and net release of fatty acids from adipose tissue, as well as differences in milk-fat amount and composition and milk-protein yield and proportion (Harrison *et al.* 1995; McNamara *et al.* 1995). These effects varied according to genetic merit for milk production of the animal, stage of lactation, amount of fat fed and length of time of feeding fat. However, the major effect of feeding an additional 40 g ruminally-protected fat/kg diet was to decrease *de novo* lipogenesis in the adipose tissues, even when total energy intake was greater than control. There was a variation of over 1000 g fat/d supplied to the mammary gland in the 9 months of this study, within diets, demonstrating that the stage of lactation must be considered when interpreting effects of altering fat intake. Direct infusion of oils can also decrease lipogenesis and may increase lipolysis in adipose-tissue flux of lactating dairy cattle (Chilliard *et al.* 1991; Gagliostro & Chilliard, 1991). It is interesting to speculate that such effects may be partially mediated by an effect of dietary fat on activity of the sympathetic nervous system (Young & Walgren, 1994).

Integration of kinetic and physiological models

A better quantitative understanding of the regulation and fluxes of nutrient interconversions in adipose tissue, liver, muscle and mammary gland will improve our ability to undertake effective research and predict responses to various interventions. Thus, we must improve the total quantitative understanding of biological systems (quantitative in this sense means defining mathematical equations which describe causative relationships among hormones and nutrients in the animal as a whole system). Data on adipose-tissue C and N flux, rates of accumulation or loss, rates of hypertrophy and hyperplasia, sensitivity and response to hormones are all needed to improve our understanding of the adipose tissue and, thus, the whole animal. Studies investigating regulatory mechanisms must be undertaken within a larger objective to determine the quantitative effect on nutrient flux of the regulatory system being investigated.

There are a number of excellent examples of the integrative, quantitative approach to systems definition, in kinetic descriptions (see McNamara *et al.* 1991; Pettigrew *et al.* 1992; Baldwin, 1995), in physiological regulation (Cornish-Bowden & Cardenas, 1990) and in larger, practical applications (Black *et al.* 1986; Whittemore & Morgan, 1990; Mercer, 1992). These approaches recognize that optimal manipulation of the whole animal depends on a valid understanding of the subsystems in integration with the whole, that knowledge of isolated subsystems must eventually be validated against the behaviour of the whole system (subsystems rarely behave in isolation the same as when integrated with the whole) and that total understanding and, therefore, our ability to predict responses to nutritional, genetic or endocrine manipulation relies on a quantitative, mechanistic approach.

There is still a gap between 'nutritional' or 'kinetic' models and 'endocrine' or 'physiological' models. Part of this gap comes from the technological requirements of study in various areas and part from the complexity of the systems involved. The pertinent example to adipose tissue in lactation is the need to define direct and integrated effects of various hormones on the total kinetic flux through the adipose tissue and also on the resultant effects on the mammary gland and other maternal systems. Although these hormones have direct effects on various metabolic pathways, there is little information available which describes in mathematical terms the effects of these hormones on the maximal velocity or substrate sensitivity of a pathway, or even on total flux of C into and out of the adipose tissue.

The net effect of altering the status of several different hormones *in vivo* is integrated into the C and N input–output relationships among all tissues, accumulating at the animal level. However, experiments investigating hormone action are not often designed within such a framework, with notable exceptions (McGuire *et al.* 1995*a*,*b*; see also Pettigrew *et al.* 1992; Bell & Bauman, 1994). From a different point of reference, when such *in vivo* experiments are conducted, often the net result of changing the hormonal or nutritional status, even over a wide range, is not large when only strict input–output relations at the whole-animal level are investigated. Such a result may be obtained because there has been a fairly large change in function of two or more internal subsystems which act in relative opposition in an attempt to maintain homeostasis.

A general example of this complex relationship among subsystems is shown in an experiment in which a nutritional or hormonal treatment alters both nutrient intake and output of C or N in milk, which is often the case. The effect may be positive or negative on both input and output, or positive on one and negative on another. Also, the sensitivity of the effect on input and output may vary (for example, milk output goes up 20 % while nutrient intake goes down 5 %). Too often such experiments are interpreted, without recognizing the internal opposition among subsystems, as: 'there is no substantial effect of treatment X'. The other error is in interpreting a 'positive' effect (i.e. an effect desired by the experimenter) as only resulting from changes within one organ (such as the mammary gland) without benefit of strict consideration of all the internal subsystems involved. Thus, the true integrative effects are missed.

Often the experiments are imposed for a short period of time (short-term ration assignments in the grossly-over-used 'switchback' design in dairy cattle being an obvious example), and although 'significant effects' are obtained, the real effects of continuing such treatments for a longer period of time are not measured. To worsen the mistake, recommendations from such studies are shamelessly extrapolated to long periods of time and many different genetic, environmental or physiological situations.

Examples of experimental approaches which recognize the need for integration include: factorial assignments of nutritional or hormonal treatments over a practical or physiological range including expected minimal and maximal effects and at least two levels in between, and studies which extend through a sufficient period of time to measure the cumulative effect on all the organ systems involved. Studies which are designed to estimate values for kinetic variables such as maximal velocity, substrate sensitivity, hormonal binding (maximal and affinity) and the shape of the hormonal-response curve in various systems are extremely useful, providing both basic insights and applications to the whole system. When the importance of integration is recognized and improved technologies for estimating various functions are used, experiments testing complex hypotheses have yielded extremely powerful information.

The importance of such integration is provided by an examination of the equation forms shown in Fig. 2, taken from an experiment combining estimation of maximal rates of lipogenesis, esterification and lipolysis and energy intake and output (McNamara & Hillers, 1989) and the equations in Table 1, which describe pertinent chemical interconversions in biomathematical terms and are part of the research metabolic model designed and tested over the last three decades by Baldwin (1995) and colleagues.

Both sets of equations may be considered 'mechanistic' in that they describe processes at one level of biological organization (the whole animal) by means of processes at a lower, more fundamental subsystem level (tissue or organ). They are aggregates of biochemical reactions which describe only the major pathways of adipose tissue lipogenesis, esterification and lipolysis, total body gluconeogenesis, and mammary lipogenesis, esterification, lactose and protein synthesis. They are solidly based in biological principles and the form is validated by experimental data from dozens of laboratories. Neither the values of the variables nor the anatomical location of the pathways are relevant for this discussion. The equations recognize the contribution of maximal velocity, substrate sensitivity, hormonal effects, and sigmoidal responses to substrates and hormones. In addition, those in Fig. 2 demonstrate the extremely wide range of activity, and that very small changes in input–output relationships may result in very large changes in activity of one subsystem (the converse is also true, very large changes in one subsystem may occur within an inestimable difference in input–output). Even in this relatively simple description the absolute interconnection between these systems is evident.

Glucose availability is common to all these pathways, and it is through glucose that metabolism of fatty acids and amino acids is connected (this connection may occur in one or more organs). Glucose affects many pathways, and supply of glucose is a function of absorption and gluconeogenesis from propionate (not discussed here; for details, see Baldwin, 1995) and amino acids. For example, lipogenesis from acetate (AcTs) is linked directly to body triacylglycerol (Ts) and is a secondary function of glucose availability (the function KlAcTs/(Ahor1 × cGl), where K1 is substrate sensitivity, Ahor is anabolic hormone effects, C is concentration of substrate and Gl is glucose; see Table 1), in which glucose limits esterification. The inclusion of Ahor and Chor (catabolic hormone effects) demonstrate that these reactions are functions of anabolic hormones, which roughly translates to insulin, and/or catabolic hormones (noradrenaline) and both are a function of glucose availability. Thus, these equations represent reality, and lack of quantitative data limits inclusion of further detail for an animal level model (although more detail is included in tissue level submodels, see Baldwin, 1995). Milk-lactose synthesis is limited also by amino acid availability, which is a function of intake, muscle protein proteolysis and gluconeogenesis.

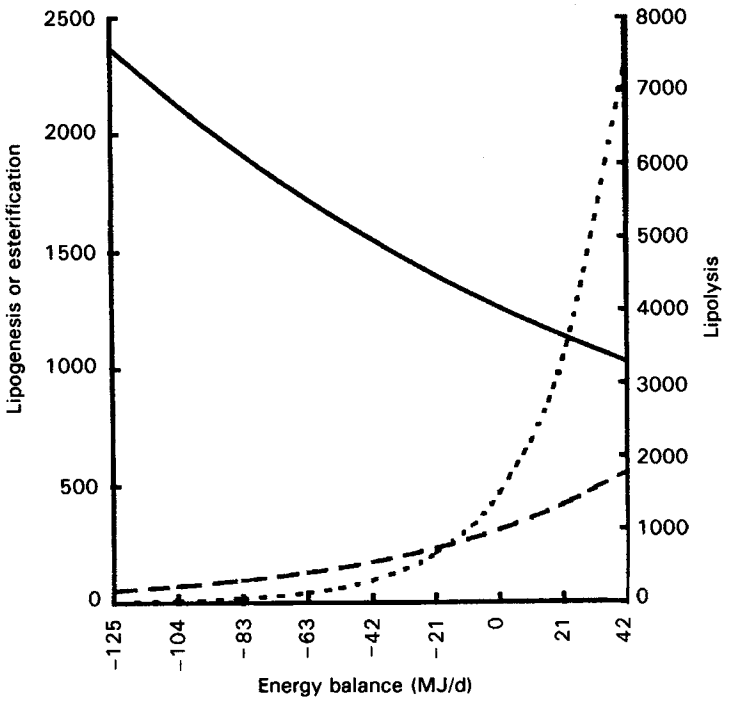

Fig. 2. Relationship between *in vivo* total energy balance and *in vitro* rates of lipogenesis (- - - -), esterification (– –) and lipolysis (—). Data are logarithmic curves of energy balance (metabolizable energy intake – milk energy secretion – maintenance energy; MJ/d) and rates of fatty acid metabolism (nmol/2 h per g tissue). (Data were taken from several experiments; for equation statistics, see McNamara & Hillers, 1989).

Often such models are criticized thus: 'That variable value cannot be correct, because we demonstrated a particular value'; that may be correct in one specific instance, but ignores the objective of such models. The research models cannot yet be absolute for each potential situation, only data from further experimentation will provide that. The modelling approach provides the logical framework for designing such experiments.

Often models are criticized as being 'too simplistic' because there may be available a piece of detail on some hormonal process. What is forgotten is that an absolute atomic description of some hormonal signal process may be available, but if an adequately-detailed kinetic description of the effect on C flux is not also available, there is no justification for including such descriptions in the model. This point if often lost by some who do not recognize the specific objective of a model being constructed. Overuse of variables in a model which cannot be validated serves no purpose (e.g. a model which has an objective to predict the time to reach the moon based on the weight of a rocket may not need to include a description of the maximal oxidative rate of a fuel, but a model which has an objective to define the most efficient fuel mixture for maximal rocket speed must).

In order to integrate knowledge on adipose function into the whole-animal system, questions of the following type need to be answered. What are the values for variables for maximal rates, substrate sensitivity, mass action constants and hormonal responsiveness on rate of turnover of fatty acids, including *de novo* lipogenesis, esterification, and lipolysis in different adipose tissue depots and the animal as a whole? This would include determining values for variables in situations of altered fatty acid intake (either in addition to, or in

Table 1. *Equations describing metabolic interconversions in lactating dairy cattle* (Adapted from Baldwin *et al.* 1987 and Baldwin, 1995)

Lipogenesis
$AcTs = VAcTs / (1{\cdot}0 + KAcTs/cAc + K1AcTs/(Ahor1 \times cG1))$,
$VAcTs = f(Ahor1 \times INS)$.

Esterification
$FaTs = VFaTs \times (EBW^{0.75} / (1{\cdot}0 + KFaTs/cFa + K1FaTs/(Ahor \times INS \times cG1))$.

Lipolysis
$TsFa = VTsFa \times (EBW^{0.75} \times Chor1 / (1{\cdot}0 + (cFa/K1TsFa)^{exp} + (KTsFa/cTs)^{exp})$,
where Ahor and Ahor1 = f(cGl/icGl) and Chor1 = f(icGl/cGl).

Proteolysis from lean body and viscera
$PbAa = kPbAa \times Pb$,
$PvAa = kPvAa \times Pv$.

Synthesis of lean body and viscera
$AaPb = VAaPb \times Bdna/(1{\cdot}0 + KAaPb/(Ahor \times cAa))$,
$AaPv = VAaPv \times Vdna/(1{\cdot}0 + KAaPv/(Ahor \times cAa))$.

Synthesis of milk protein
$AaPm = VAaPm \times Uenz \times Kminh/(1{\cdot}0 + KAaPm/cAa)$.

Gluconeogenesis from amino acids
$AaGl = VAaGl \times (EBW^{0.75})/(1{\cdot}0 + KAaGl/cAa)$,
$GlLmV = VGlLm \times Uenz/(1{\cdot}0 + KGlLm/cGl + KAaLm/cAa)$.

V, maximal velocity; K, substrate sensitivity (or mass action coefficient for proteolysis); c, concentration of substrate; Aa, Ac, Fa, Gl, Lm, Pb, Pv, Pm, Tm, Ts, amino acid, acetate, fatty acid, glucose, lactose, body protein, visceral protein, milk protein, milk fat and body triacylglycerol respectively; Ahor, anabolic hormone effects; Chor, catabolic hormone effects; INS, injected insulin; Uenz, total udder enzymes, which is a function of the lactation curve and can be altered by injection of bovine somatotropin; EBW, empty body weight; i, initial (reference); Bdna, total body (non-visceral) nuclear DNA content; Vdna, total content of nuclear DNA in viscera. Kminh, factor defining inhibition of milk synthesis by milk.

substitution for, starch or fibrous polysaccharides), in different stages of lactation, and in animals of varying levels of milk production. Levels of milk production may be altered by genetic selection or milking frequency in domestic cattle, by litter size in domestic pigs and laboratory animals, or by frequency or length of breast-feeding in women. What are the effects of direct or indirect hormonal action on adipose tissue, *in vivo* and *in vitro*, and in consideration of simultaneous direct and indirect effects of other hormones? Determination of these integrated effects is a strict requirement if we are to interpret hormonal effects *in vitro* or in specialized *in vivo* situations in relation to their true importance in the 'free-living' animal. The recent use of the 'hyperinsulinaemic-euglycaemic' clamp technique (Bell & Bauman, 1994; McGuire *et al.* 1995*a*,*b*) is one example which holds much promise toward elucidating such information.

Studies on the role of somatotropin, prolactin, insulin, insulin-like growth factors and their binding proteins, and steroid hormones need to focus both on the molecular mechanisms of these actions, and on the true quantitative effects (dose–response) of hormone actions. Thus, studies must by nature combine *in vitro* and *in vivo* approaches to elucidate meaningful information. There are several outstanding examples of studies of a fairly complex nature, involving detailed *in vitro* analyses within a framework of carefully-designed *in vivo* nutritional or hormonal regimens, which have helped to answer questions of both types (for details and further references, see Bauman & Vernon, 1993; Baldwin, 1995; McNamara, 1995).

The need to measure the quantitative effect of the hormone on the chemical interconversions of a nutrient in the system (animal) or subsystem (organ) for a model is

often misunderstood, and two equally wrong but different actions are taken: (1) it is concluded that 'models don't reflect reality because 'this hormone' is not explicitly included' and (2) some effect is included in a 'model' without sufficient justification but with unwarranted interpretations. In the first instance, sceptics claim the model fails because it does not include a particular process. In the second case, when a falsely-constructed model fails against critical validation, the entire 'quantitative' or 'modelling' approach is tarred with a broad brush. Both criticisms ignore the fact that the major purpose of modelling complex systems is to investigate, define and validate the contribution of each component in consideration of all the other components. When the model fails to reflect reality, we learn from that failure and are in an educated position to design the critical experiment to improve our knowledge. Too many successful examples of research and predictive models exist to criticize the correctness of the approach. To paraphrase the commercial community, if we can use this approach to put a man on the moon, we can use it to put milk on the table or in the baby.

Turnover and release of stored compounds

The last contribution of the adipose tissue in lactation we will consider is the turnover and release of specific fatty acids and other fat-soluble compounds in the adipose tissue. Because of the constant recycling, such compounds are continuously being released. Several different types of fatty acids are stored in the adipose tissue for long periods of time, thus composition of adipose tissue is a long-term reflection of previous exposure (Coppack *et al.* 1994; Raclot *et al.* 1995). Hydrolysis of stored triacylglycerols and release of fatty acids does not appear to be a random process in relation to fatty acid composition, but rather there may be some selectivity in hydrolysis depending on chain length and unsaturation characteristics (Raclot *et al.* 1995). Also, the adipose tissue stores several environmental lipophilic contaminants, e.g. polychlorinated biphenyls. Higher rates of lipolysis or extended periods of increased lipolysis would increase the release rate of such compounds. A lactating mammary gland becomes an exit point for such compounds from the body through the milk fat. Several suggestions have been made concerning whether or not to breast-feed if such contamination of the mother is suspected (for discussion, see Walsh & Neville, 1994). Balancing the demonstrated benefits of breast-feeding on the infant against the potential risks of entry of these compounds into the milk is a serious task, limited severely by quantitative data on the actual amount of the compounds in the adipose tissue, the rate of release and uptake and secretion by the mammary gland, in addition to determining the actual risk of consuming such compounds in different amounts. This is an area in which the study of rates of adipose tissue metabolism will be important to the safety of the food supply both from cows' milk or mother's milk.

Some fatty acids released by the adipose tissue are potentially specific regulators of gene expression, cell cycle, or immune function (Graber *et al.* 1994). Fatty acids can alter the timing and commitment to the cell cycle in a variety of cell types, and it has often been hypothesized, but never clearly tested, that increased amounts of specific unsaturated fatty acids (such as linoleic or arachidonic acid) may alter the expression of specific genes coding for milk-casein synthesis (DePeters & Cant, 1992) or even alter the amount of differentiation of active secretory cells. These hypotheses have a sound basis from studies in other systems, yet remain to be tested in lactating animals. The potential importance dictates that such studies be done.

Another emerging area is that of the potential role of conjugated linoleic acids in milk. These compounds have been shown to have specific effects on the growth and differentiation of cancerous cells in *in vitro* systems (Ip *et al.* 1991; Shultz *et al* 1992).

They are also present in milk of various species, including human milk (Fogerty *et al.* 1988; Lin *et al.* 1995; McGuire *et al.* 1996). At this time, the true role of these compounds has not been elucidated, but their potential importance demands attention. Suggestions have been made about breast-feeding, positive or negative, based on preliminary findings on such compounds with no demonstration of benefit or risk (for discussion, see Hachey, 1994). Regardless of the energy or fat balance of the mother, fatty acids of various types are constantly being released from the adipose tissue and supplied to the mammary gland. A better quantitative description of the amount and types of fatty acids released in such a manner will help guide informed application of knowledge concerning such potentially-important compounds.

Cooperation among scientists who work with different species, among scientists who work with different tissue systems, and among those who are 'basic', 'whole-animal', or 'applied' will continue to grow so that basic knowledge on adipose tissue processes is integrated into the real, practical and complex actions of the lactating female. Current views of society on research and subsequent funding decisions dictate such cooperation.

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