Review

Lipid metabolism in the lactating mammary gland

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1. Introduction

Fat is a major component of milk varying from about 2 g/l in rhinos to over 500 g/l in seals [1]. Over 95% of the fat is triacylglycerol, the remainder comprising small amounts of diacylglycerol, phospholipid, unesterified fatty acids, cholesterol and cholesterol esters [2]. The concentration of fat reflects
the needs of the young for energy and for water; it may also reflect water availability for the mother [3]. Production of milk fat represents a considerable investment in most mammals including those of domestic importance and its production imposes a considerable metabolic strain on an animal for at least part of lactation. For many animals there is a period during lactation when the nutrient requirements of the mammary gland exceed those of the rest of the animal [4–6]!

Attaining such high rates of metabolic activity in the mammary gland requires both a considerable increase in food intake and also a variety of adaptations elsewhere in the body to ensure the preferential use of nutrients by the mammary gland [7–9]. In addition there are usually periods when animals draw heavily on reserves of nutrients, particularly adipose tissue triacylglycerol [4–9]. The mammary gland can thus use fatty acids from a variety of sources: dietary fat, adipose tissue triacylglycerols, synthesis in the gland itself, the relative importance of each source depending on species and on the nutritional and physiological state of the animal [10]. One special feature of fatty acid synthesis in the mammary gland is that in most species most of the fatty acids produced have chain lengths of less than 16 carbons [2], hence analysis of the fatty acid composition of milk fat can provide some indication of the sources of the fatty acid precursors [10].

In this review we have, at one level, taken a holistic approach, presenting mammary lipid metabolism and its regulation in a whole body perspective, while at the molecular level we have focused on aspects where there has been recent progress or controversy.

2. Relationship to whole body metabolism during the lactation cycle

Milk production usually increases rapidly at parturition and then continues to increase more gradually to a peak before declining. In rats peak lactation is usually around 15 days post partum [11], while in cattle it is about 8 weeks [7]. In most species the decline in milk yield coincides with the gradual weaning of the young, but in dairy species milk production is sustained for abnormally long periods by milking. There is also a change in the concentration of milk fat, with a fall from a relatively high concentration at parturition in rats [12] and ruminants [13–15]. In rats the decrease is complete after about 5 days, following which the level remains relatively constant (Fig. 1a). In cows a nadir is reached around 8 weeks of lactation with a slight increase thereafter [13,15]. A very different pattern is found in macropod marsupials in which the young are born at a much earlier stage of development than in eutherian mammals. In such marsupials, milk production increases...
lipolysis is high. In rats there is a transient peak of about 60 ml from about 30 ml of milk production in rats suggest that it increases r

0.5 g/kg r tissue lipid [4,7]. For example, cows producing about 30 kg of milk/day at peak lactation (which is well within the normal range for dairy cows) were, during the first few weeks of lactation, secreting about 1.5 kg of milk fat/day and mobilising about 1 kg of adipose tissue lipid [4]; thus milk fat production was very much dependent on lipolysis in adipose tissue. However, while lipolysis can sustain high rates of milk fat production for a time, production is suboptimal in that milk fat concentration is decreased slightly during such periods [13]. Another consequence is that the fatty acid composition of milk has a much higher proportion of C₁₈ fatty acids when lipolysis is high [13]. In rats there is a transient decrease in food intake around parturition [5,6] when there is extensive mobilisation of adipose tissue lipid [16], but subsequently food intake seems to develop in line with milk production. Nevertheless there is some loss of adipose lipid even in rats fed ad libitum. The limited data available suggests a loss of about 0.5 g/day during early lactation rising to about 1.0 g/day around peak lactation [29,235–238]. Estimates of milk production in rats suggest that it increases from about 30 ml/day at 2 days post partum to a peak of about 60 ml/day around 14 days post partum [239]. These values, coupled with estimates of milk fat concentration (Fig. 1), suggest a rate of milk fat production of about 5–6 g/day over much of lactation. Adipose tissue lipid could thus contribute 10–20% of milk fat production. Previous estimates suggest a lower contribution (5–10%) [235,238], but in these, and in many other studies with rats, milk for compositional analysis was obtained following oxytocin treatment. This gives a markedly higher milk fat concentration (160–200 g/l) [235,238,240–243] than when milk is obtained in the absence of oxytocin (about 100 g/l) [12,244,245] as in the study described in Fig. 1. The discrepancy probably arises from oxytocin causing extrusion of alveolar milk which appears to have a higher lipid concentration [246,247]. Food restriction during lactation increases fat mobilisation from adipose tissue [29,235,237,238]; a rate of 4 g fat mobilised/day was found in rats fed to 30% of ad libitum intake [237]. As milk production falls when food intake is restricted [235,238,241], it follows that adipose tissue will make a much greater contribution to milk fat production in these than in ad libitum fed animals.

Well-fed humans differ from both rats and ruminants in that lactation does not normally require mobilisation of adipose tissue lipid [248–251]. One study reported no changes in the lipolytic system during lactation [252] but another reported enhanced response to catecholamines in femoral, but not abdominal, subcutaneous adipose tissue [253]. There may, therefore, be subtle changes occurring to support lactation, but they do not appear to be quantitatively important.

Although animals can move deep into negative energy balance during lactation, mobilising substantial amounts of adipose tissue lipid [4,7,235], fasting in most species results in a rapid cessation of milk production [10,19,20], but this is probably due to an inability to produce sufficient glucose and to provide amino acids for the mammary gland. Thus, seals, which normally fast during lactation, produce a high fat, lactose-free milk [21]. Transgenic mice which do not produce α-lactalbumin and hence cannot synthesise lactose also produce a milk with a very high fat content [22].

In addition to lipid mobilisation, fatty acid and lipid synthesis are diminished in adipose tissue during early lactation even if animals are not actually in negative energy balance [7–9]. As lactation progresses and milk yield declines, fatty acid and lipid synthesis increase in adipose tissue allowing restoration of lipid reserves [23]. The decrease in lipid

gradually over the first half of lactation when the young are continuously attached to the teat. Subsequently, there is a substantial rise in milk production over the second half of lactation [233,234]; in addition there is a change in milk composition, with a fall in carbohydrate and an increase in lipid concentration [233,234].

Around peak lactation nutrient demands of the mammary gland usually exceed those of the rest of the body [4–6]. This increased demand is met primarily by a massive increase in food intake. In rats for example food intake increases from about 17 g/day in adult virgin rats to over 50 g/day at peak lactation [5,6]. However, while food intake increases during early lactation in domestic ruminants, the increase follows the rise in milk yield, hence these animals experience a period of negative energy balance when a substantial proportion of milk production is achieved by mobilisation of body tissues, especially adipose tissue lipid [4,7]. For example, cows producing about 30 kg of milk/day at peak lactation (which is well within the normal range for dairy cows) were, during the first few weeks of lactation, secreting about 1.5 kg of milk fat/day and mobilising about 1 kg of adipose tissue lipid [4]; thus milk fat production was very much dependent on lipolysis in adipose tissue. However, while lipolysis can sustain high rates of milk fat production for a time, production is suboptimal in that milk fat concentration is decreased slightly during such periods [13]. Another consequence is that the fatty acid composition of milk has a much higher proportion of C₁₈ fatty acids when lipolysis is high [13]. In rats there is a transient decrease in food intake around parturition [5,6] when there is extensive mobilisation of adipose tissue lipid [16], but subsequently food intake seems to develop in line with milk production. Nevertheless there is some loss of adipose lipid even in rats fed ad libitum. The limited data available suggests a loss of about 0.5 g/day during early lactation rising to about 1.0 g/day around peak lactation [29,235–238]. Estimates of milk production in rats suggest that it increases from about 30 ml/day at 2 days post partum to a peak of about 60 ml/day around 14 days post partum [239]. These values, coupled with estimates of milk fat concentration (Fig. 1), suggest a rate of milk fat production of about 5–6 g/day over much of lactation. Adipose tissue lipid could thus contribute 10–20% of milk fat production. Previous estimates suggest a lower contribution (5–10%) [235,238], but in these, and in many other studies with rats, milk for compositional analysis was obtained following oxytocin treatment. This gives a markedly higher milk fat concentration (160–200 g/l) [235,238,240–243] than when milk is obtained in the absence of oxytocin (about 100 g/l) [12,244,245] as in the study described in Fig. 1. The discrepancy probably arises from oxytocin causing extrusion of alveolar milk which appears to have a higher lipid concentration [246,247]. Food restriction during lactation increases fat mobilisation from adipose tissue [29,235,237,238]; a rate of 4 g fat mobilised/day was found in rats fed to 30% of ad libitum intake [237]. As milk production falls when food intake is restricted [235,238,241], it follows that adipose tissue will make a much greater contribution to milk fat production in these than in ad libitum fed animals.

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synthetic capacity during early lactation is associated with decreased activities of LPL [8,14,24,253] and various enzymes of fatty acid synthesis including ACC and FAS [8,9,14] due both to decreased gene transcription of these enzymes [25] and changes in the systems involved in the acute control of fatty acid synthesis in the tissue [9]. While decreases in fatty acid and lipid synthesis and associated enzymes are found in both rodents and domestic ruminants, the magnitude of the decrease varies. Decreases are apparent in young rats, but in older rats, in which adipose tissue fatty acid synthesis is already low (activity decreases with age), the decrease may be negligible [26]. In contrast in ruminant animals, even in mature animals which have experienced several lactations, there is still a very substantial decrease in adipose tissue lipogenic capacity; around peak lactation the rate of fatty acid synthesis declines to about 2% of that of non-lactating animals of the same age [9,27]. The quantitative importance of this decline is indicated by an estimation of the rate of fatty acid synthesis of adipose tissue and mammary gland in non-lactating and lactating animals on a whole animal basis (Table 1). In ruminants the decrease in adipose tissue fatty acid synthesis has a quantitatively significant impact on the availability of lipogenic precursors to the mammary gland. In contrast, data for rats and mice (Table 1) suggest that the prime factor here is the massive increase in fatty acid synthesis in the mammary gland, with changes in adipose tissue having only a minor role in directing lipogenic precursors to milk fat synthesis. A concomitant of this is that fatty acid synthesis is increasing in the mammary gland at the same time as it decreases in adipose tissue, and as these changes are paralleled by changes in the amount and activity of key lipogenic enzymes, this means that transcription of these enzymes is under tissue-specific, reciprocal control. Resolving the factors and mechanisms of this has been a major challenge which has been greatly facilitated by the cloning and sequencing of several cDNAs, especially ACC (see Section 3.2.3).

The liver may also contribute to the fatty acid supply to the mammary gland in rats and mice as hepatic fatty acid synthesis is substantially increased during lactation [30,31]. The fate of this fatty acid is uncertain but it would seem likely that it is secreted and used by the mammary gland, facilitated by the hypoinsulinaemia of lactation coupled with a decreased ability of insulin to suppress hepatic triacylglycerol release [32]. An initial study suggested that the rate of triacylglycerol secretion into the blood was not enhanced during lactation [33], but more recent investigations suggest that this was an artefact of the procedures used to inhibit triacylglycerol utilisation in lactating rats [34].

Most studies with laboratory species have involved sampling tissues and blood in the morning. Rats, at least, show marked diurnal variation in food intake, consuming almost all at night when non-lactating and, while food consumption occurs during the day in lactating rats, food intake is still markedly higher at night [35,36]. Both milk production [254], and mammary fatty acid synthesis [35] are also maximum at night in rats and then decrease during daylight hours (i.e., when animals are usually sampled). In contrast, hepatic [35,254] and adipose tissue [254] fatty acid synthesis show no diurnal changes in lactating rats. It is possible that there is some lipid accumulation in adipose tissue at night due to LPL activity (this is less suppressed in adipose tissue during lactation than fatty acid synthesis) with release of fatty acids during

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<th>Table 1</th>
<th>Effect of lactation on adipose tissue and mammary gland fatty acid synthesis on a whole animal basis</th>
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<tr>
<td>Species</td>
<td>Rate of fatty acid synthesis ($\mu$mol/h per animal)</td>
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<tr>
<td></td>
<td>Non-lactating</td>
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<td>Adipose tissue</td>
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<td>Cow</td>
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<td>Rat</td>
<td>345</td>
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<td>Mouse</td>
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* Calculated from data of Refs. [28,29].
the day, but this has not been investigated. So while adipose tissue lipid reserves do not appear to make a major contribution to milk production over lactation as a whole in rats, they may still have some role diurnally. If diurnal changes occur in domestic ruminants, the pattern is likely to be different from rats as they usually receive cereals twice daily and any changes are likely to be small due to the buffering capacity of the rumen.

3. Fatty acid synthesis

In eutherian mammals the ability of the mammary gland to synthesise fatty acid increases markedly at parturition and subsequently follows the same pattern of changes as milk production, that is increasing to a peak and then declining [37]. In rats at least, the increase in fatty acid synthesis is slower than the increase in LPL (Fig. 1b); this may account for the lower proportion of medium chain fatty acids (i.e., those synthesised in the gland) in milk fat in rats during early lactation (Fig. 1a). In rabbits [38,39] and to a lesser extent, cows [40], there is some increase in mammary fatty acid synthesis during pregnancy, whereas in other species (e.g., rats, sheep, pigs) [37] the increase in activity only begins at parturition. The increase in fatty acid synthesis is paralleled by an increase in the activity of key lipogenic enzymes [37]. ACC has been investigated in greatest detail, and studies have shown that the increase in activity is paralleled by an increase in enzyme protein [41] and mRNA [42]. With the lipogenic enzymes pyruvate dehydrogenase [43,44], and ACC [45] which occur in active and inactive states, lactation induces an increase in the proportion in the active state in the mammary gland. Induction of the major lipogenic enzymes at parturition is related to the changing endocrine profile at this time, principally reduced serum progesterone and raised prolactin, and possibly suckling per se through removal of milk-borne factors [46].

Macropod marsupials show a more complex pattern of changes than eutherian mammals as the composition of milk is initially high in carbohydrate and low in fat, but about half way through lactation, when the young are no longer permanently attached to the teat, the concentration of carbohydrate in milk falls while the concentration of lipid rises [233,234].

3.1. Characteristics of mammary fatty acid synthesis

3.1.1. Medium-chain fatty acid synthesis

Milk fat from humans, ruminant and most other non-ruminant mammals contains high proportions of medium-chain fatty acids (C<sub>8.0</sub> - C<sub>12.0</sub>) [2]. A few species (e.g., guinea pigs) do not produce such medium-chain fatty acids, but the reason for this is not known [2]. Medium-chain fatty acids are synthesised de novo within the mammary epithelial cell and consequently the proportion of these fatty acids in milk reflects the contribution of mammary fatty acid synthesis to total milk fat content. Synthesis of medium-chain fatty acids in mammary gland during lactation occurs as a result of a tissue-specific modification of the universal FAS reaction. FAS in higher eukaryotes comprises 6 enzyme activities on a single polypeptide; functional FAS exists as a dimer in a 'head to tail' orientation. Recent advances in the molecular biology and enzymology of the FAS reaction have recently been documented in an excellent review by Smith [47] and thus will not be considered in depth in this article. However, some consideration of the FAS reaction is required to appreciate the mechanism for medium-chain fatty acid synthesis in lactating mammary gland. FAS catalyses the condensation of malonyl-CoA from the ACC reaction with acetyl-CoA (and also butyryl-CoA in the case of ruminants) eliminating CO<sub>2</sub> to form an intermediate ketoacyl moiety. This then undergoes a 3-step β-carbon reduction to give a saturated acyl moiety extended by two carbons. A further 6 cycles of condensation with malonyl-CoA and β-carbon reduction results in the formation of the saturated C<sub>16:0</sub> fatty acid, palmitic acid, the major product of the FAS reaction in the majority of eukaryotic cells. This product specificity is the result of a number of constraints. Firstly, the loading reaction that catalyses the reversible translocation of substrates between CoA-SH and the 4'-phosphopantetheine prosthetic group of FAS has a specificity for acetyl and butyryl moieties. Thus once the acyl-chain has extended beyond four carbons it cannot readily be released from FAS as a CoA ester. Secondly, the rate of condensation of the acyl-CoA chain increases with chain-length, with the
result that once condensation is initiated the reaction proceeds to long-chain-length fatty acids. Thirdly, acyl moieties with 16 or more carbons cannot readily be elongated and thus become susceptible to hydrolysis by the intrinsic thioesterase activity, termed thioesterase I, resulting in liberation of the free fatty acid. Additionally thioesterase I has very high specificity for C_{16:0} as opposed to C_{14:0} acyl-chains. Alteration of the specificity of the chain-termination reaction of FAS to produce medium-chain fatty acids in mammary tissue appears to be fundamentally different in ruminants and non-ruminants. In rodents, rabbits and humans alteration in the specificity of acyl-chain termination results from an interaction of FAS with a second thioesterase, thioesterase II, that is not part of FAS but is present in the cytosol as a discreet monomeric polypeptide of 29 kDa [48,49]. The thioesterase II enzyme has access to the elongating acyl-chain on the 4'-phosphopantetheine prosthetic group of FAS and can hydrolyse the thioester bond, resulting in the release of saturated fatty acids of chain-length C_{5:0} to C_{12:0}. Such a modification of product specificity is remarkable given that the thioesterase II is able to access the elongating acyl-chain which is already within interaction distance of the six intrinsic enzyme activities. Cleavage occurs without affecting the substrate turnover rate of FAS and promotes chain-termination of an acyl intermediate whose condensation rate is proceeding so rapidly that it does not readily accumulate on FAS. This implies a high degree of dynamic equilibrium between the domains of FAS rather than merely a static order of enzyme activities, suggesting that large conformational changes occur on catalysis. The dynamic model is further supported by the interaction in vitro of thioesterase II isolated from lactating rat mammary gland with yeast FAS. Although the constituent domains of the two non-identical subunits of this enzyme are arranged in a different order from those in animal FAS, thioesterase II nevertheless promotes a change in its product specificity to medium-chain fatty acids [50]. Interestingly a similar acyl-chain termination mechanism exists in the production of lipids by the uropygial glands of aquatic birds for waterproofing of feathers [51]. Cloning of the thioesterase II enzymes from the two tissues [52–54] has revealed a high degree of homology, centering on a conserved serine motif, Gly-x-Ser-x-Gly which is thought to make up part of the active site. These thioesterase II enzymes exhibit a low but significant level of homology with the intrinsic thioesterase I domain of FAS, which also has a serine active site, suggesting a distant evolutionary relationship between the thioesterases I and II [55].

The capacity to synthesise fatty acids in rat and rabbit mammary gland is maximally achieved during lactation and is associated with induction of ACC and FAS enzyme activities at parturition [39,41,56]. However, the ability to synthesise medium-chain fatty acids is attained in rat mammary gland prior to the observed increase in fatty acid synthesis at parturition and is even observed in mammary epithelium from virgin rats, where 90 mol% medium-chain fatty acids are synthesised, which is similar to that observed by the mammary gland as a whole at early lactation [57]. During peak lactation in the rat the proportion of medium-chain fatty acids synthesised actually falls, relative to early lactation, and this appears to be because levels of FAS enzyme rise relative to thioesterase II, thus reducing the potential for medium-chain fatty acid synthesis. In contrast amounts of medium-chain fatty acids secreted into rat milk increase from parturition to peak lactation (Fig. 1). This discrepancy between mammary-synthesised medium-chain fatty acid and the proportion present in rat milk is likely to be accounted for by a greater contribution of non-mammary derived fatty acids to milk fat at early lactation.

High levels of thioesterase II are detected in mammary gland during pregnancy prior to the observed increase in FAS activity and appear to be associated with the increasing contribution of mammary epithelium to the developing tissue [56]. Indeed thioesterase II appears to be associated with the mammary phenotype per se and is found in the lumen and alveolar structures of immature and mature virgin rats [58,59]. Thus thioesterase II expression may occur as a result of stem-cell progression to the mammary phenotype and is thus a mammary epithelial marker [59]. To date the thioesterase II gene has not been cloned; knowledge of the promoter would allow targeting of transgene expression to the mammary gland at a time other than lactation, which is conventionally achieved through the synthesis of a hybrid gene comprising the gene of interest linked to a milk protein gene promoter and other control elements [60,61]. Addition-
ally such an approach would enable mammary-specific cell lineage DNA elements and their corresponding transcription factors to be isolated. Clearly this is an issue for the future.

In contrast to rodent mammary gland and duck uropygial gland, ruminants do not require a thioesterase II to change the specificity of FAS to synthesise medium-chain fatty acids. In addition to being able to load acetyl-, malonyl- and butyryl-CoAs, ruminants possess a FAS that contains a loading acyltransferase whose substrate specificity extends to C<sub>6:0</sub>, C<sub>8:0</sub> and C<sub>10:0</sub>, with the result that it is capable of loading, and also releasing, medium-chain acyl-chains such that they can equilibrate between CoA-esters and enzyme-bound forms [62–65]. This altered specificity is intrinsic to the lactating ruminant mammary gland, as the product of FAS in other ruminant tissues is predominantly C<sub>14:0</sub> and C<sub>16:0</sub> [15]. The altered specificity does not appear to result from the expression of a second FAS gene in lactating mammary gland, as in cell free preparations the products of ruminant mammary gland FAS are also predominantly C<sub>14:0</sub> and C<sub>16:0</sub> [63]. Part of the reason for the altered specificity of ruminant FAS must reside in the structure of the acyltransferase domain relative to that of the corresponding domain of non-ruminant FAS; to date no ruminant FAS has been cloned. Termination of the growing acyl-chain at C<sub>6:0</sub> to C<sub>10:0</sub> from lactating goat mammary FAS is promoted in vitro by the addition of a CoA-ester removal system such as bovine serum albumin, microsomes or acyl-CoA binding proteins [63,66,67]. Such removal of the medium-chain fatty acids as CoA-esters is also believed to occur in this fashion in vivo and to be facilitated by the proximity of FAS in lactating ruminant mammary gland to the endoplasmic reticulum and the developing milk fat globule, possibly requiring the initial action of an acyl-CoA binding protein [66,67]. Recently FAS has been found associated as part of a lipoprotein complex with the milk fat globule membrane proteins, butyrophilin and xanthine oxidase [68,69] and a group of small GTP-binding proteins in bovine mammary cytosol; such a complex was found to interact with the endoplasmic reticulum and with lipid droplets, both in vitro in cell-free incubations and in sections of mammary tissue [70]. Clearly the relationship between these lipoprotein complexes and the mechanism for medium-acyl-chain termination in ruminant mammary gland remains to be determined, though it is likely that the specificity for a change in fatty-acyl chain termination is achieved through the action of co-factors as part of macromolecular structures.

### 3.1.2. Fatty acid desaturation

Monounsaturated fatty acids, primarily oleic acid C<sub>18:1</sub>, are a major component of milk fat in a number of species including humans [71], rodents [18] and ruminants [72]. The oleic acid present in milk is derived from several sources including the diet and desaturation of stearic acid C<sub>18:0</sub> by SCD in the mammary gland and other tissues, predominantly liver in the case of rodents and humans, and adipose tissue in the case of ruminants. The relative proportion of oleic acid originating from each of these sources depends on the species, diet and stage of lactation. Immediately after parturition SCD activity increases in the liver of lactating rats, but, perhaps surprisingly, decreases in the mammary gland (Fig. 1b) [73]; this implies that in this species the major source of oleic acid is plasma-derived. Whatever the cause, the ratio of oleic to stearic acid of rat milk decreases with lactation. Lactating ruminant mammary gland appears to lack the ability to elongate palmitic acid C<sub>16:0</sub> [74,75] and therefore all the C<sub>18</sub> fatty acid is plasma-derived, originating either from the diet or from adipose tissue lipolysis. SCD activity increases in bovine mammary microsomes immediately after parturition, consistent with the notion that a significant proportion of the oleic acid in bovine milk is synthesised from stearic acid within the mammary gland [76]. Consistent with this, expression of the gene for SCD is markedly increased in ovine mammary gland during lactation. Experiments with mammary explants from pregnant sheep have demonstrated that SCD mRNA is induced by culture with prolactin in the presence of insulin and hydrocortisone, suggesting that increased serum prolactin in part explains the induction of expression of the gene during lactation (M.C. Barber, R.J. Ward and M.T. Travers, unpublished results).

### 3.2. Regulation of mammary fatty acid synthesis

#### 3.2.1. Acute hormonal control of fatty acid synthesis

Several lines of evidence indicate acute control of mammary fatty acid synthesis. Mammary fatty acid
synthesis shows a diurnal rhythm in lactating rats which appears to be independent of any gross change in enzyme activity [35]. Fasting results in a rapid decrease in mammary fatty acid synthesis and, in most species, milk production in general [10,19,20,37]. In rats, detailed time course studies have shown that the rate of fatty acid synthesis falls to very low levels within 6 h of the start of a fast and precedes changes in enzyme concentration [77].

Fatty acid synthesis in other major lipogenic tissues (liver, adipose tissue) is subject to acute positive and negative control, the former primarily by insulin and the latter via agents (catecholamines, glucagon) which activate adenylate cyclase and hence protein kinase A, and by fatty acids themselves [78,79]. In the adipocyte, insulin promotes fatty acid synthesis by several mechanisms including increased glucose transport, activation of pyruvate dehydrogenase and ACC, and inhibition of lipolysis [78]. Insulin can also enhance hepatic fatty acid synthesis by activation of the above enzymes and also indirectly by inhibiting lipolysis in adipose tissue [79]. Catecholamines and glucagon can inhibit fatty acid synthesis directly by phosphorylation of ACC, possibly via activation of AMP-stimulated protein kinase [80,81] and indirectly by enhancing lipolysis.

The mechanism of the acute control of fatty acid synthesis in the mammary gland is still not fully resolved, and some aspects remain controversial. Mammary epithelial cells lack receptors for glucagon, hence one potential direct control is absent [82]. The mammary epithelial cells have β-adrenergic receptors [83,84], but incubation of isolated mammary acini [85] or perfusion [86] of mammary tissue with β-adrenergic agonists does not lead to inhibition of fatty acid synthesis and indeed causes activation of ACC. The reason for this is not fully resolved, but whereas the mammary gland produces cAMP and has protein kinase A, cAMP levels may be limited by relatively high levels of cAMP-phosphodiesterase [87]. Incubation of mammary acini with theophylline, a cAMP-phosphodiesterase inhibitor, did result in a decrease in the rate of fatty acid synthesis [82]. Fatty acids themselves, however, appear to have a key role, as increased availability of exogenous fatty acids is associated with decreased synthesis (see below). Therefore catecholamines and glucagon may alter mammary fatty acid synthesis indirectly by increasing lipolysis in adipose tissue. In this respect it is pertinent to note that fatty acyl-CoA-esters can activate AMP-stimulated protein kinase which can phosphorylate and inactivate ACC [80,81]. Fatty acids can also inhibit ACC by an allosteric mechanism [80].

Insulin appears to be the major, acute, stimulatory effector of mammary fatty acid synthesis. Mammary epithelial cells possess insulin receptors and their number per cell increases with the onset of lactation [88]. Incubation of mammary epithelial cells [82,85,89–94] or acini with insulin, or perfusion [86,95] of mammary gland with insulin, increases the rate of fatty acid synthesis although in a few studies effects of insulin were not significant in rats fed on standard chow [90,92,93,96]. In general effects of insulin were most marked when the rate of fatty acid synthesis was diminished by fasting [92,94], feeding high fat [85] or cafeteria [91] diets, or when an inhibitory agent such as acetocacetate [90,93] was included in the incubation medium. Insulin deficiency in vivo for even as little as 2 h decreased the rate of mammary fatty acid synthesis [94,97]. Insulin infusion [98] or use of the more rigorous hyperinsulinaemic, euglycaemic clamp technique [99,100] has shown that insulin increases the rate of fatty acid synthesis in the mammary gland in vivo. Again, effects of insulin were enhanced if the rate of fatty acid synthesis was decreased by fasting [98] or feeding fat-rich diets [85,91]. Insulin is also required for the restoration of fatty acid synthesis on re-feeding fasted rats [97].

The mechanisms whereby insulin enhances mammary fatty acid synthesis are not fully resolved but may involve both direct effects on the mammary cells and indirect effects, for example through inhibition of lipolysis in adipocytes. Within the mammary gland, insulin has been reported to activate phosphofructokinase [101], pyruvate dehydrogenase [102,103] and ACC [86,101] and there is additional indirect evidence to support control by insulin at the level of these enzymes [45,92,104,105]. However, in contrast to its effects on adipocytes, insulin does not appear to enhance glucose transport in mammary epithelial cells [104,106] (transport is by Glut-1 rather than Glut-4 translocases [107,108] plus some Na-dependent glucose transport) [109]. Nevertheless, insulin in vivo did stimulate 2-deoxyglucose uptake by the mammary gland in rats, [100] which, as it can be
metabolised only to 2-deoxyglucose-6-phosphate, implies an effect on either transport or hexokinase activity. The reason for this apparent discrepancy between in vivo and in vitro findings is uncertain, but the in vivo effect may be due to a lowering of the glucose-6-phosphate concentration [101] and hence increased activity of hexokinase [110].

Two aspects of insulin action on mammary epithelial cells are unusual. Firstly, the tissue appears to be very much more sensitive to insulin than adipose tissue or skeletal muscle [100]; this conclusion is based on the uptake of 2-deoxyglucose in vivo during hyperinsulinaemic, euglycaemic clamp studies. The molecular basis for this enhanced sensitivity to insulin relative to other tissues is uncertain, but would not appear to be due to a greater number of receptors per cell [37]. Secondly, the effects of insulin are sustained for much longer in mammary tissue than liver or adipose tissue; thus in the rat mammary gland perfused in situ the glucose uptake enhanced by insulin was maintained unchanged for a further 60 min at least after a 15 min infusion with insulin [95]; again the molecular basis of this is unknown. Adaptations such as these, coupled with the apparent absence of the usual counter-regulatory systems operating via cAMP (i.e., glucagon, catecholamines) probably account for the high rates of mammary fatty acid synthesis despite a relatively low serum insulin concentration.

Insulin may also enhance mammary fatty acid synthesis indirectly by decreasing lipolysis in adipose tissue. Indeed Neville and Piciano [10] have recently suggested that this may be the major mechanism whereby insulin modulates mammary fatty acid synthesis, pointing out that in the various in vivo studies where effects of insulin have been demonstrated in rats, the animals would have been in the post-prandial state and hence, while not measured, serum fatty acid levels would probably be high. While such a mechanism most likely contributes to, and may account for the greater stimulatory effects of insulin on fatty acid synthesis in vivo than in vitro, the various studies noted above indicate that insulin also has direct effects on the mammary epithelial cells, so both direct and indirect mechanisms probably operate. The contributions of these two types of mechanism are likely to depend on the state of the animal (in essence the current rate of lipolysis).

ACC can be phosphorylated by a number of protein kinases including protein kinase C and calmodulin-dependent protein kinase, as well as protein kinase A and AMP-stimulated protein kinase, but studies of the serine residues phosphorylated in intact cells [80,81,111] and site-specific mutagenesis studies [112] suggest that only the latter two protein kinases are important physiologically. Phosphorylation by these kinases decreases activity of the enzyme and increases the $A_{0.5}$ for citrate, an allosteric activator of the enzyme [80,111]. The mammary gland has a relatively high AMP-stimulated protein kinase activity [113] which may, amongst other actions, help to protect such a metabolically active tissue against ATP depletion. AMP-stimulated protein kinase phosphorylates serines 79, 1200 and 1215 of the rat 265 kDa isoform of ACC, while protein kinase A phosphorylates serines 77, 1200 and 1215 [80,81,111]. Both analysis of serines phosphorylated in intact cells [80,81,111] and site-specific mutagenesis studies [112] have shown that phosphorylation of serine 79 is responsible for the effect of AMP-stimulated protein kinase. However, for protein kinase A the mechanism is less clear. Studies with intact cells showed that serine 79 and not serine 77 was phosphorylated when protein kinase A was activated, suggesting that the effect of this kinase was indirect, involving activation by an unresolved mechanism of AMP-stimulated protein kinase [80,81,111]. In cells in which protein kinase A stimulates lipolysis, an increase in fatty acyl-CoA could mediate activation of AMP-stimulated protein kinase. More recent site-specific mutagenesis studies, however, show that serine 1200 is the key target of protein kinase A [112]; these authors suggest that the kinase may have a direct effect on ACC in vivo independent of mediation by AMP-stimulated protein kinase. This is particularly pertinent with respect to the mammary epithelial cell, for in this cell type the predominant isoform of ACC lacks 8 amino acid residues near to serine 1200 (Section 3.2.3.2); this isoform is more susceptible to phosphorylation of serine 1200 by protein kinase A with subsequent loss of activity [114], so it is surprising that, as noted above, perfusion of the rat mammary gland [86] or incubation of isolated mammary acini [85] with catecholamine activated, rather than inhibited, ACC; the basis of the activation is unknown.
Fasting results in a decrease in ACC activation status and affinity for citrate, with a concomitant increase in enzyme phosphorylation in mammary cells, and this is reversed by refeeding [115]. This effect of refeeding did not occur in streptozotocin-treated rats, indicating a requirement for insulin [115]. The factors which cause these changes in ACC activity are still not clear, but presumably activation either of AMP-stimulated protein kinase or of protein kinase A (or both) is involved. Curiously, although ACC activation status decreases with fasting, the change appears to follow rather than precede or parallel the decline in the rate of fatty acid synthesis [77] which is better matched by a decrease in phosphofructokinase and also pyruvate dehydrogenase activity [77]. However, fasting will induce an increase in plasma fatty acid concentration which may inhibit ACC, initially by an allosteric mechanism, followed more slowly by a change in phosphorylation status.

3.2.2. Modulation of mammary fatty acid synthesis by plasma-derived fatty acids

As the lipid concentration of milk within a species does not vary markedly except just after parturition, it is implicit that there is a reciprocal relationship between the use of fatty acids taken up from the blood and those provided by fatty acid synthesis in the gland itself. Many examples of this are available. In humans on high-fat diets (25–40% energy as lipid), only about 10–12% of milk fat comprises medium-chain fatty acids, reflecting a modest contribution of mammary gland fatty acid synthesis [116]. On low-fat, high-carbohydrate diets the proportion of medium-chain fatty acids in human milk increases (18–45%) [117]. Reductions in the contribution of medium-chain fatty acids to bovine [15,118] and rat [18] milk are seen when the fat content of the diet is increased. When cows are in negative energy balance and mobilising adipose tissue lipid for milk fat synthesis, the proportion of medium chain fatty acids of milk fat is decreased [15].

The variable contribution of mammary fatty acid synthesis to milk fat content suggests that plasma-derived fatty acids are able to modulate synthesis of fatty acids within the mammary gland. Feeding a diet high in fat suppresses the rate of fatty acid synthesis in both mammary gland and liver in rats during lactation [18,91], as does an oral load of triacylglycerols [119]. Similarly a high-fat diet decreased the rate of medium-chain fatty acid synthesis in human mammary gland in vivo [255]. The relatively high fat content of most Western diets is probably the reason why the rate of fatty acid synthesis is lower in mammary epithelial cells of humans than those of rats [256]. The ability of different fatty acids to inhibit fatty acid synthesis varies and is tissue-specific [120]. Thus mammary fatty acid synthesis is inhibited to the greatest extent by dietary polyunsaturated fatty acids; monounsaturated fatty acids are the next most effective inhibitors followed by saturated fatty acids [120]. Dietary medium-chain fatty acids also inhibit mammary fatty acid synthesis, but are less effective than long-chain fatty acids [120]. Diets rich in polyunsaturated fatty acids (but not saturated fatty acids) also inhibited hepatic fatty acid synthesis [120], but in contrast to the mammary fatty acid synthesis, feeding diets rich in medium-chain fatty acids increased hepatic fatty acid synthesis [120]. Furthermore dietary polyunsaturated fatty acids inhibit SCD activity in both mammary gland and liver, resulting in increased tissue ratios of C₁₆:₀:C₁₆:₁ and C₁₈:₀:C₁₈:₁ [121]. The suppression of the rate of fatty acid synthesis in lactating rats fed a high-fat diet during lactation was associated with a reduction in the total amount of ACC enzyme, but the proportion of the enzyme in the active state was unchanged [85].

The primary factor mediating the effects of dietary fat and also adipose tissue-derived fatty acids on mammary fatty acid synthesis is probably unesterified fatty acid or its acyl-CoA-ester [257]. While early studies on the effects of fatty acids on the activity of a variety of enzymes were shown to be due to non-specific detergent effects [122], further in vitro studies with intact adipocytes [123] and hepatocytes [124] using physiological concentrations of fatty acids bound to albumin showed that fatty acids could induce a rapid and reversible inhibition of fatty acid synthesis. In vitro palmitic, oleic, linoleic and linolenic acids were all equally effective, whereas stearic acid was a more potent inhibitor [123,124]. Studies with goat mammary epithelial cells revealed a similar pattern of effects except that palmitic acid stimulated fatty acid synthesis [125]; the reason for this is unclear but may relate to palmitic acid being the preferred fatty acid for the initial acylation of glycerol 3-phosphate by mammary epithelial cells
[125]. Oleic acid inhibited, while palmitic acid stimulated, fatty acid synthesis in bovine mammary epithelial cells [126]. With rats, oleic acid inhibited mammary fatty acid synthesis in vitro in one study [127] but not in another [128]; differences may relate to the concentration of albumin used. There is some information suggesting inhibition by medium-chain fatty acids in vitro in rat mammary gland [127,129]. However, in one of these studies, interpretation of the findings is complicated by methodology: measurement was made of the rate of incorporation of labelled glucose into total lipid rather than into fatty acid [129]. The reason for inhibition by medium-chain fatty acids, which appears to be a peculiarity of mammary epithelial cells [129], is not clear but could act as a signal to suppress fatty acid synthesis if milk begins to accumulate in the gland [130], perhaps supplementing the effect of the recently discovered peptide inhibitor of milk secretion [131]. Possible mechanisms whereby fatty acids may inhibit fatty acid synthesis acutely are considered in the previous section.

The findings described in the present and preceding sections suggest that insulin and fatty acids, or really their concentration ratio in the blood, are probably the major acute regulators of mammary fatty acid synthesis. In well-fed lactating animals consuming diets rich in carbohydrates and low in fat, serum insulin and fatty acid levels will be relatively high and low respectively for this state, and mammary fatty acid synthesis will be elevated. A change to a diet rich in fat will lower serum insulin and raise serum fatty acid levels and result in a decrease in mammary fatty acid synthesis. Furthermore, situations (e.g., dietary restriction or inadequate appetite) which lead to enhanced lipolysis, in part through a decrease in serum insulin, will also lead to a decrease in mammary fatty acid synthesis.

When mammary acini from lactating rats fed a high-fat diet were incubated with insulin, the impairment in fatty acid synthesis induced by the high-fat diet was reversed, partially through an increase in ACC in the active-state, and also through an increase in the total amount of the enzyme [85]. That high-fat diets suppress the total amount of ACC enzyme [85] suggests that they may also exert chronic control, possibly transcriptionally or post-transcriptionally. High-fat diets suppress the induction of lipogenic enzymes in the adipose tissue and liver of weaned rats; this effect is primarily at the transcriptional level [132–134]. Polyunsaturated fatty acids have been demonstrated to suppress the transcription of a number of lipogenic enzyme genes including SCD and FAS [135–139] both in vivo and in cultured cells. The mechanism for the transcriptional repression by polyunsaturated fatty acids or perhaps their acyl-CoA-esters [257] is unknown (see also Section 4.2).

3.2.3. Transcriptional control of mammary fatty acid synthesis

The cDNAs and genes for several of the principal enzymes involved in milk fat synthesis, ACC [140–142], FAS [143,144], LPL [145] and SCD [146,147], have been cloned from a number of species. Expression of the genes for these enzymes is ubiquitous, though elevated expression is observed in the lipogenic tissues. Developmental changes during pregnancy result in mammary gland acquiring a greatly enhanced lipogenic potential. Greatest insight into the chronic regulation of this metabolic adaptation has come from consideration of the ACC gene.

3.2.3.1. Hormonal regulation. The cloning of rat ACC from lactating mammary gland [140] showed that although ACC enzyme activity is acutely regulated by reversible phosphorylation [148], in the longer term changes in total ACC activity were paralleled by changes in the level of ACC mRNA in the mammary gland [149]; ACC mRNA increased from barely detectable levels just prior to parturition to approximately 30- to 40-fold higher just post-partum, and remained high after 7 days of lactation. This pattern loosely follows the temporal expression of the milk protein genes, although casein gene expression is apparent much earlier during pregnancy [149,150], indicating that expression of genes for milk protein and fat synthesis are not under identical control during mammary cell differentiation.

The induction in lipogenic potential in mammary gland during lactation has been principally attributed to increased prolactin responsiveness by the mammary epithelial cells. Serum prolactin levels increase at parturition in a number of species [46] and prolactin in the presence of insulin plus a glucocorticoid has been demonstrated to increase the rate of fatty acid synthesis, and the activities of ACC and FAS, in
cultures of mammary explants from mid-pregnant animals [38,151–153]. Also in lactating rats treatment with bromocriptine for 48 h, which lowers serum prolactin, reduced both mammary ACC mRNA abundance and total ACC activity [154,155]. Treatment with bromocriptine for 24 h also lowered total ACC activity in one study [45] but not in another [104], and decreased the rate of fatty acid synthesis in vitro [94,156] and in vivo [28], although again in one study no effect was observed [157]. As lowering serum prolactin for 24 h or more has no effect on the proportion of ACC in the active state in most studies [45,104,55], it would seem that a loss of ACC protein may be a major factor responsible for the fall in the rate of fatty acid synthesis. However, in one study, the fall in total ACC activity was counterbalanced by an increase in the proportion of ACC in the active state such that the effective activity of the enzyme was unchanged [155]. Lowering serum prolactin usually, but not always [28,97,153,157–159] results in an increase in serum insulin, which could perhaps account for the increase in activation status of ACC observed [155]. However, lowering serum prolactin also causes a decrease in the number of insulin receptors on mammary epithelial cells [158]. The reason for this varied response of ACC to lowering serum prolactin is thus uncertain, but our own studies suggest there may be a seasonal effect (independent of day length) [155]. The decrease in ACC mRNA and enzyme induced by bromocriptine treatment could be prevented by concurrent administration of prolactin, suggesting a role for prolactin in the regulation of this gene in vivo [154,155]. Further experiments using both bromocriptine and anti-rat growth hormone antisera to ablate serum prolactin and growth hormone, followed by adding back these hormones, showed that although double ablation had a similar effect to bromocriptine treatment alone, adding back prolactin only partially restored ACC expression. Full restoration required both growth hormone and prolactin [160], implying a need for growth hormone, possibly via induction or activation of other factors, in facilitating a full response to prolactin. Curiously in this system, ablation of prolactin, or of prolactin and growth hormone, had little effect on casein mRNA levels. This could be attributable to differences in the half-lives of ACC and casein mRNAs under these conditions rather than to differences in the transcription of their genes. However, the half-life of casein mRNA has been shown to decrease drastically in mammary explant cultures in the absence of prolactin [161] and therefore this is more likely to represent further evidence of independent regulation of milk protein and ACC gene expression. Milking frequency also regulates expression of the ACC and FAS genes and enzyme activities in goat mammary gland, suggesting that factors in milk feedback on the cell machinery to modulate lipogenic potential independently of endocrine factors [131,162]. In such a manner the synthesis of milk is determined by the demand of the young in both the short-term, through the rate of milk removal, and the long-term, through chronic endocrine changes and eventual involution.

3.2.3.2. ACC gene structure. Regulation of ACC gene expression in the mammary gland is complicated by the demonstration of multiple transcripts for this enzyme in this tissue differing in the sequence of their 5′UTRs [163], and of yet other ACC mRNAs with a different 5′ leader sequence in adipose tissue and liver of starved–refed rats [164]. Transcripts with this second type of leader sequence were not found in mammary gland [164]. Characterisation of the 5′ end of the ACC gene in rat showed that all of the mRNA variants could be obtained by use of a dual promoter system and alternative splicing of the primary transcripts [141] (Fig. 2).

The significance of the diversity of ACC mRNAs is unclear, especially as they all appear to give rise to the same protein. 5′UTRs have been implicated in regulation of translation [165]. Using in vitro translation of transcripts run off from plasmids constructed using various 5′UTRs, it was found that class 1 transcripts (containing exons 1, 4 and 5) were translated approximately 6-fold more efficiently than class 2 transcripts (containing exons 2, 4 and 5) [166]. The presence or absence of exon 3 had no effect on these rates. However, the presence or absence of exon 4, which has been found in adipose and mammary gland and which, although short, is very well conserved between mammalian species [142], was not tested in this system. Removal of most of the 5′UTR from class 2 transcripts increased their translation rate but not to the levels observed for class 1 transcripts. Although transcripts from promoter 1 (PI) may be more efficiently translated in vivo than those from
promoter 2 (PII), the function of the additional diversity generated by the presence of exons 3 and 4 is still unknown.

Class 1 transcripts were only found in adipose tissue under normal conditions but were induced in both adipose tissue and liver under conditions of starvation followed by re-feeding [164]. Class 2 transcripts were found in all tissues except adipose tissue, but were only inducible in mammary gland [42]. Interestingly the increase in class 2 transcripts in the mammary gland occurs at the same time as the decrease in class 1 transcripts in adipose tissue, but were only inducible in mammary gland [42].

As the two ACC promoters, PI and PII, appear to be regulated in different ways in different tissues by the same physiological stimuli, comparison of the structure of these promoters may shed some light on how this differential expression occurs. PI, the inducible promoter in adipose and liver, has both a TATA and CAAT box near the transcription initiation start site, a potential insulin response element (IRE), and a repressor element consisting of 28CA repeats [25]. Suppression by this element can be relieved by binding of CAAT enhancer-binding protein (C/EBP) to the CAAT box, implying some interaction between these sequences and/or the proteins that bind to them [167]. The other promoter, PII, which is constitutive in most tissues but inducible in mammary gland, has the basic characteristics of a housekeeping gene, namely no CAAT or TATA boxes, a high frequency of CpG dinucleotides and numerous sites for binding of the transcription factor Sp1 near the transcription start site [25]. PII also contains a number of other cis-acting sequences, identified by sequence comparisons and transfection of chimeric genes into cell lines; the possible relevance of these to the induction of ACC in lactation is discussed below.

Sequences in PII which bind transcription factors have been identified using a number of non-mammary systems; the relevance of these sequences to mammary ACC expression is therefore unclear. However, these studies may highlight regions which may also be important in a mammary gland context. A positive regulatory element in PII was identified by deletion analysis. The enhancer sequence was contained in a 115bp fragment [168] containing a core sequence of three symmetrical 13-mers. Removal of this sequence from the promoter decreased expression 94% in transfected cell lines, even though the five putative Sp1 sites remained. This sequence was also shown to function in an orientation- and promoter-independent fashion [168]. The fact that this promoter was active and that the effect of deleting the enhancer was similar in a preadipocyte, fibroblast and hepatoma cell line, demonstrated the housekeeping function of this promoter. The promoter with or without the enhancer has not been transfected into a prolactin-responsive mammary cell line, or DNase I footprinted with mammary nuclear extract, so it is not known if this sequence is involved in the induction of PII in lactation.

Other regions in ACC PII were identified as being potential regulatory sequences by comparison with...
known regulatory elements from other genes; for example two cAMP response element (CRE)-like [169] and two IRE [170] sequences have been found. In mouse 30A5 preadipocytes prior treatment with cAMP is necessary before insulin treatment can induce differentiation and expression of ACC [171]. As these cells express class 2 transcripts and few or no class 1 transcripts, even when differentiated, these cells provide a model system for delineating the importance of these putative cis-acting sequences. Using deletion and point mutation of these sequences, together with transfection of the chimeric genes into these cells, it was found that an insulin response could only be obtained when both IREs were present, and importantly only when the CRE-like sequences were also present. Thus induction of ACC by insulin in this system requires cooperative interaction between the cAMP and insulin response elements. The consensus sequence for IREs is fairly poorly defined, and Kim et al. suggest that one of the reasons for this may be the involvement of other cis-acting sequences, not necessarily CREs, in the generation of the insulin response.

The two CRE-like sequences are slightly different, one being a CRE and the other an AP-2 element. The latter has been implicated in mediating both cAMP and phorbol ester induction of gene transcription and to function as a basal enhancer [169]. Mutation of these sequences showed that it is the AP-2 element that is required for the insulin response, and that activation requires the AP-2 to be phosphorylated, probably by protein kinase A [172]. This is somewhat paradoxical as cAMP activation of protein kinase A also results in phosphorylation, and therefore inactivation, of ACC enzyme. The effects of cAMP in this system vary depending on the stage of differentiation, and on the concentration and the duration of exposure. Therefore it is possible that different combinations of these conditions may result in the activation of different pools of protein kinase A and in the varied responses observed. It is difficult to extrapolate from results in this system to the mammary gland. Levels of cAMP are higher in mammary gland in pregnancy than in lactation [87] and insulin levels also decrease at parturition [4,8]. In explant cultures of mammary gland insulin has been found to be necessary, but not sufficient, to cause an increase in lipogenesis or milk protein gene expression [38,161].

Therefore interaction between the IREs and factors responding to signals generated by one of the other hormones essential for a full response (e.g., prolactin or glucocorticoid) may be necessary in this system as well.

Glucose has been shown to activate ACC gene expression in a pancreatic β-cell line, INS-1, maximally at concentrations of greater than 20 mM [173]. This high glucose concentration was found to activate ACC PII in mouse 30A5 preadipocytes at day two of differentiation [174]. This effect was enhanced in the presence of insulin, but insulin on its own had no effect. PII contains MLTF/USF (Myc-Like Transcription Factor/Upstream Stimulatory Factor)-like binding sites found in other glucose responsive genes, for example L-type pyruvate kinase [175], but these did not appear to be the regions responsible for the glucose effect in this instance. In this promoter the response appears to be mediated via two Sp1 sites in the upstream GC rich region [176]. This is curious as Sp1 is a ubiquitous transcription factor present in all cells. Binding of Sp1 and subsequent activation of transcription were found to be dependent on the dephosphorylation of Sp1, possibly via a glucose-induced increase in protein phosphatase 1 (PP1) [176]. Thus the induction of ACC transcription by glucose may be an indirect effect via a glucose response element in the PP1 gene, possibly of the more usual MLTF/USF type though this remains to be determined. Again the relevance of these studies to the induction of ACC in the mammary gland is difficult to determine. It is unlikely that mammary cells will be exposed to glucose concentrations as high as were used here; however, it is possible that other factors could utilise the same pathway to activate ACC transcription, that is activation of the PP1 gene up-regulating ACC transcription via increased binding of Sp1.

A negatively regulating sequence has also been identified in ACC PII. Tumour necrosis factor-α (TNF-α) treatment of transfected 30A5 preadipocytes results in binding of a protein to a region 359-389bp upstream of the transcription start site which results in a decreased transcription of ACC and prevents differentiation of these cells [177]. The possible function of TNF-α in mammary gland expression of ACC is unknown.

The PII promoter therefore contains a number of
cis-acting sequences which have been demonstrated to be functional in an in vitro model system, but which remain to be proven significant either in vitro or in vivo in mammary cells. In whatever fashion these sequences interact to result in the expression of mammary gland ACC, the expression of this gene nevertheless does correlate with that of the milk protein genes. The promoters for the milk protein genes, however, contain a different complement of cis-acting sequences including C/EBP and nuclear factor-1 (NF-1) binding sites, glucocorticoid response elements, and binding sites for a factor termed Stat5 which, although not mammary gland-specific, is necessary for the induction of the milk protein genes by prolactin and which may act by displacing a repressor of transcription, YY1 [178,179]. As no Stat5 or YY1 sites are present in the ACC PI1 sequence, a Stat5-based mechanism is probably not involved in the induction of ACC in the mammary gland. The studies described above suggest that control of ACC expression in the mammary gland probably involves a number of cis-acting sequences and the factors that bind to them in response to a number of hormones and metabolites, though the precise interactions are at present unknown.

As stated previously (Section 3.2.1) ACC is regulated by reversible phosphorylation. A variant ACC mRNA has been identified in lactating rat mammary gland which has a 24 nucleotide deletion in the coding region and gives rise potentially to a protein lacking 8 amino acids upstream of the ser-1200 phosphorylation site; the absence of these 8 amino acids increases the extent of phosphorylation at this site in vitro by protein kinase A [114]. If this species occurs in vivo it would have a more decreased enzyme activity and an increased $A_{450}$ for citrate than the longer species. Both the long and short forms of ACC mRNA have been found in all 3 major lipogenic tissues, implying that it is not simply the product of use of one or other promoter. Also the ratio of the long to the short forms is unchanged under lipogenic conditions in the liver, whereas in adipose the shorter form is increased to a greater extent. Recently it has been shown that the increase in ACC mRNA in mammary gland at lactation is mainly due to an increase in the amount of the short form. Further, lactating mammary tissue is the only tissue in which the short form is more abundant than the long [180] (Fig. 3). The mechanism of the generation of this shorter mRNA is unknown but we may speculate that it will involve differential splicing of exons using tissue-specific or hormone-induced factors. The need, in lactating mammary gland, for an ACC that is phosphorylation-prone and more citrate-dependent than in other tissues is unclear.

4. Lipoprotein lipase and the uptake of plasma-borne fatty acids

4.1. Lipoprotein lipase

The ability of tissues to utilize fatty acids from circulating triacylglycerol-rich lipoproteins (chylomicrons and VLDL) is determined by the activity of the enzyme LPL. LPL catalyses the hydrolysis of lipoprotein triacylglycerol, selectively cleaving-off fatty acids at the $sn$ 1(3) position; this is the rate-determining reaction in the utilisation of fatty acid from these sources. A number of immunohistochemical and biochemical studies have indicated that LPL is located, within tissues, both on (or near) the surface of the major cell-type of the tissue in question (e.g., adipocytes in adipose tissue, myocytes in muscle) as well as on the luminal surface of vascular endothelial cells. Since chylomicrons and VLDL are too large to
migrate between the circulation and the extravascular interstitial fluid, the action of LPL in hydrolysing their triacylglycerols must be brought about by those LPL molecules on the luminal aspect of the vascular endothelium.

4.1.1. Site of production

Mammary tissue contains various cell types in addition to parenchymal secretory epithelial cells. These include adipocytes, in varying proportion to epithelial cells depending on the developmental status of the mammary gland. Because parenchymal cells account for most of the cellularity and most of the lipoprotein triacylglycerol fatty acid utilisation during lactation, it has been assumed that they are the site of synthesis of mammary LPL. The use of collagenase to isolate acini from mammary tissue of both pregnant and lactating rats both inactivates extracellular and exo-cellular LPL initially present in the tissue and yields an acinar preparation free from adipocytes. The finding that LPL activity was present in membranes [181] and extracts prepared from such acini [87] apparently supported this assumption. An immunohistological study in guinea pig mammary gland [182] further confirmed the origin of mammary LPL in secretory epithelial cells. Nevertheless, subsequent work on mouse mammary LPL by Neville and co-workers cast doubt on this conclusion. Reasons for the discrepancies in the histological localisation studies of LPL between guinea pig and mouse mammary tissue are not known with certainty; it may be significant that the guinea pig study used cryosections of fresh tissue, whereas tissue was fixed, dehydrated and embedded for the mouse study. Neville’s group found that the localisation of immunoreactive LPL, in histological sections of mouse mammary tissue, was not coincident with the distribution of secretory epithelial cells. Furthermore, in situ hybridisation experiments with an LPL riboprobe showed signal to be concentrated in some of the interstitial cells between alveolar structures and more diffusely distributed within epithelial cells [183]. These workers proposed that mammary LPL originates in mammary adipocytes and is subsequently secreted and transported, by cellular uptake and transcytosis, both to its final site of action on the capillary endothelial cell and through the secretory epithelial cell into milk [10,183]. As will be discussed below, there is abundant evidence indicating that mRNA levels are not well correlated with active LPL expression — indeed, an inverse relationship is suggested by several studies — and that immunoreactive LPL cannot be equated with enzymatically active molecules of this protein. The latter point is emphasised in the work of Jensen et al. [183] by the lack of prominence of capillary endothelial LPL in their anti-LPL immuno-stained sections of mouse mammary gland. Neville and colleagues aduce further evidence in support of their hypothesis from the similarities which they claim between the nutritional regulatory characteristics of mammary LPL and those of adipose tissue; these are examined in the following sections. Regardless of the validity of this claim, their overall postulate requires that the mammary interstitial cells in which they have shown concentration of LPL protein and mRNA are lipid-depleted adipocytes or adipocyte precursors. No experimental evidence supports this identification. In contrast, there is evidence that fibroblasts expressing the LDL receptor will take up and accumulate LPL [184] and that the imposition of a block on LPL secretory traffic leads to an intracellular accumulation of inactive enzyme within LPL-secreting cells (see e.g., [185]). Thus the findings of Neville’s group might equally result from scavenging uptake of LPL or from regulatory blockage of LPL secretion by mammary adipocytes, reflecting the ‘global’ down-regulation of adipose tissue LPL during lactation [10,183].

4.1.2. Acute control of mammary lipoprotein lipase

Several layers of regulation govern the activity of LPL in tissues expressing this protein. The general details of this regulation, including the contribution of molecular biological knowledge to our understanding of them, are considered fully in several recent reviews [186–189]. Briefly, evidence to date suggests that physiological regulation can be exerted at one or more of the maturation stages undergone by LPL according to the following scheme. Newly-synthesised protein is glycosylated within the endoplasmic reticulum; next, various trimming reactions involving selective removal of glucose and mannose residues also occur in the endoplasmic reticulum. The still-inactive glycoprotein is then transported through the Golgi complex where further trimming of mannose
residues occurs and conversion of LPL oligosaccharide chains into the complex types typical of mature LPL takes place. At an ill-defined subsequent point along the secretory pathway, LPL dimerizes, acquires a high affinity for heparin and becomes catalytically active [190]. Phosphorylation/dephosphorylation of LPL influences catalytic activity and targeting towards secretion or intracellular degradation; isoprenaline-induced phosphorylation of adipocyte LPL has recently been shown to diminish activity of cell-associated enzyme and to enhance its degradation [191]. Once secreted from the cells in which it is synthesized, the processes of LPL extracellular transport, transcytosis and binding to the capillary endothelium may all be subject to modulation resulting in alterations in the effective concentration of catalytically active enzyme on the capillary endothelium. The mechanisms underlying these dynamic processes are ill-understood. Apolipoproteins (or related molecules) have been implicated in several aspects of the binding of LPL to cells and extracellular matrix (for example, [184,192,193]). The role of heparin sulphate components of extracellular glycosaminoglycans in the immobilization of LPL on the cell-surface of vascular endothelial cells is also well-characterised [194].

It has long been known that LPL undergoes reciprocal regulation in adipose tissue and heart in response to fasting and re-feeding: adipose tissue LPL declines during fasting, whereas most studies find an increase in heart (and other muscle) LPL [187]. Analysis of LPL cDNAs in various tissues has given no evidence for heterogeneity of coding sequence ([195] and refs. therein). Similarly, at the level of expressed protein, it is likely that the only differences between tissues are ones attributable to post-translational modifications [196]. A decline in the specific activity of LPL accompanies the fasting-induced reduction of enzyme activity in adipose tissue. Activity is affected more markedly in the combined pools of extracellular and exo-cellular enzyme (which can be released from the tissue by incubation in heparin-containing buffers) than in the intracellular LPL pool [197,198]. It has recently been shown that changes in LPL specific activity in adipose tissue result from the alteration of an equilibrium between inactive (possibly monomeric) enzyme and its active counterpart, which can be physically separated from one another on heparin-agarose. Fasting shifts this equilibrium far in favour of the inactive form [199]. No such molecular heterogeneity of catalytic efficiency was found in muscle, where the effect of fasting was to enhance the secretion of fully active enzyme, thereby increasing its proportion in the heparin-releasable pool. The underlying mechanistic basis for this tissue-specific regulation is not understood, although these molecular descriptions of the changes affecting LPL, particularly in adipose tissue and cell culture models of adipocyte physiology and development, represent a major advance towards that understanding.

Such molecular descriptions are not yet available for the nutritional responses of mammary LPL: indeed, in this tissue even the basic details of cell physiology are in dispute. Relatively few studies have been carried-out on the effects of fasting/re-feeding on mammary LPL. In the rat, mammary tissue was unresponsive to fasting in unmated animals and during early pregnancy [200]. At these stages of mammary gland development, the tissue mass consists almost entirely of adipocytes; it is therefore of interest that other adipose tissue depots in the early pregnant rat responded conventionally to fasting with a major reduction in LPL [200]. From mid-pregnancy onwards until close to parturition, this pattern of responsiveness was reversed, with adipose tissue becoming resistant to fasting-induced decreases in LPL and mammary gland, at least in this study, assuming sensitivity to this regulation. The resistance of adipose tissue to fasting-induced down-regulation of LPL persists at least until peak lactation in the rat [201]. In part, this may result from the already depressed level of total activity in the tissue throughout lactation. Superimposed on these changes in responsiveness of LPL during the mammary development/involution cycle, there is an increase in the absolute level of mammary LPL activity in the tissue of the fed animal throughout pregnancy, mirrored by a decline in the level in adipose tissue LPL [24,200]. Changes in the characteristics of mammary LPL parallel the proportion of the tissue mass which is contributed by alveolar epithelial cells. To account for these changes in terms of an origin for mammary LPL in adipocytes within the mammary tissue mass [183] it would be necessary to postulate additional elements to the model proposed [202], providing a mechanism for the mammary epithelial cells to influ-
ence the activity of mammary adipocytes in their synthesis and secretion of LPL. For animals at peak lactation, reports vary over the response of mammary LPL to fasting. LPL decreased in mice with the most marked effect being on the heparin-releasable component [202]. In rats, Williamson’s group found, after 24 h of starvation, a decrease in total (i.e., intracellular plus extra- and exo-cellular) mammary LPL when this was expressed as a function of tissue weight [201]; 2 h of re-feeding was sufficient to restore normal fed levels of LPL. Expressed on a comparable basis, the same authors found a slight (statistically insignificant) increase in lipid uptake by mammary tissue in the same study. These results suggest that the physiologically relevant pool of mammary LPL, in contrast to the total activity measured, was not depressed during 24 h starvation. Available evidence (summarised in [203]) suggests that this effect of fasting on mammary LPL is mediated by insulin. Clegg [87] reported no change in total (i.e., intracellular plus extra- and exo-cellular) mammary tissue LPL activity in lactating rats starved for 24 h, and a decrease in intracellular LPL (both expressed as a function of tissue weight), implying an increase in the activity of the extra- and exo-cellular pool, although this was not directly measured. The activity of LPL in the milk of lactating humans was measured as an indirect index of mammary LPL activity [204]: after an overnight fast, milk LPL levels rose in response to infusion of glucose and to insulin infusion in glucose clamp experiments. However, in grey seals which fast immediately before and during lactation, post-heparin plasma LPL levels were strongly correlated with milk fat output throughout the brief lactation, implying that mammary LPL levels were not depressed by fasting [205]. These discrepant findings highlight a clear need to undertake more detailed molecular characterisation of mammary LPL in a lactating animal model. Some progress towards this objective has been made by Neville’s group [202]; for future studies it is desirable that the choice of an appropriate model should take account of the level of LPL secreted into milk, which varies greatly between species [204]. Where this level is high, there is a likelihood that interpretation of results will be complicated and may be confounded. The important aspects to define include distribution within mammary tissue (intracellular vs. extra- and exo-cellular) and sub-cellular localisation. Within each compartment, specific catalytic activity (requiring immunological assessment of protein mass), post-translational modifications and aggregational status must be defined as for the well-characterised non-mammary models discussed above.

The ability of catecholamines to down-regulate adipocyte LPL at a post-transcriptional level is well-documented (see e.g., [206]); translational control by isoprenaline in 3T3 L1 adipocytes has also recently been demonstrated [207]. Ailhaud and co-workers have suggested that these effects of beta agonists may be a special case of a general inverse correlation between fatty acid levels and adipocyte LPL [208] and they have demonstrated that long-chain fatty acids regulate adipocyte LPL within a few hours by influencing its post-translational processing. The physiology of the mammary gland during lactation leads to the expectation that LPL in this tissue should not be inversely correlated to serum non-esterified fatty acid levels. Although there are no reports of direct effects of plasma non-esterified fatty acid on mammary LPL several observations point to a role for fatty acids in the nutritionally-induced modulation of fatty acid synthesis in mammary gland [183] (see also Section 3.2.2). Although the possibility clearly exists for plasma non-esterified long-chain fatty acids to regulate LPL activity at the capillary endothelium by product inhibition, the physiological significance of this and other direct effects of non-esterified fatty acid on LPL [208,209] is not proven. An emerging field of study addresses the ability of fatty acids to act as transcriptional regulators; this ability is firmly established (see Section 4.2 below) for a number of lipid-metabolising enzymes in adipose tissue [210], and invites the speculation that related controls may operate on key enzymes of lipid biosynthesis and accretion in mammary tissue.

4.1.3. Long-term control of mammary lipoprotein lipase

Reference has already been made to the reciprocal responses of mammary and adipose tissue total LPL to the onset and maintenance of lactation (Section 3). A number of studies in which serum prolactin was manipulated either by hypophysectomy [211] or by use of bromocriptine [157,159,212,213] have indicated a key role for prolactin in the increase of
mammary LPL at parturition and the subsequent maintenance of LPL activity in the mammary gland during lactation [157,159,211,213]. In essence, lowering serum prolactin decreased LPL activity in the mammary gland and increased activity in adipose tissue. Lowering serum prolactin with bromocriptine also decreased the uptake of 14C-triolein fatty acids by the mammary gland in vivo [157,203,213]. However, another study found no effect of lowering serum prolactin with bromocriptine on mammary LPL activity [154], and while milk yield fell, the concentration of milk fat increased such that the yield of milk fat appeared to be unaltered by treatment [214]. However, the use of oxytocin to facilitate milking of the rats could have led to an overestimation of milk fat concentration, especially in the bromocriptine-treated rats, due to release of alveolar milk (see Section 2). Lowering serum growth hormone as well as prolactin in this study did, however, induce a fall in mammary LPL activity (lowering growth hormone by itself had no effect) [154]. The reasons for the apparent differences between this latter study and earlier ones in response of LPL to prolactin are not clear. It is possible, however, that prolactin effects on LPL are indirect, perhaps through changes in fat (fatty acid) accumulation in the gland. Preventing milk removal by teat sealing led to a fall in mammary LPL without any changes in serum prolactin levels [157]. Williamson and colleagues, although showing that lowering serum prolactin with bromocriptine decreased mammary LPL, also concluded that the effect of prolactin was indirect, probably through lipid accumulation in the gland through impairment of milk fat secretion [203]. In the studies by Barber et al. [154] the dose of bromocriptine used (1.5 mg/kg body weight per injection twice daily) decreased milk secretion by about 50% and complete suppression of milk secretion required ablation of both serum prolactin and growth hormone. In the studies by Williamson and colleagues [203,213], a much higher dose of bromocriptine (10 mg/kg body weight per injection twice daily) was used. There is some evidence that ergot alkaloids can, in addition to their effect on serum prolactin, also inhibit milk secretion themselves [215]; so secretion may have been reduced by both the lower level of serum prolactin plus a direct effect of the bromocriptine itself. In support of this, prolactin replacement therapy did not completely reverse the effect of this high dose of bromocriptine on milk fat secretion [203]. Thus the apparent discrepancies with respect to the effects of bromocriptine on mammary LPL once lactation is initiated could arise from the mechanism being indirect, via an accumulation of lipid in the gland, through an impairment of milk secretion. To trigger this mechanism, milk secretion seemingly has to fall to very low levels which may not be achieved by lowering prolactin alone. Hypophysectomy also appeared to reduce milk yield to a very low level (growth hormone, as well as prolactin, would have been reduced in this study and the animals were not eating normally) [211] and so again the effect attributed to prolactin on LPL could have been indirect. It is also possible that effects of prolactin on fatty acid synthesis and ACC, once lactation is established could be indirect too, operating via a similar mechanism to that proposed for LPL. If so, then ACC must be much more sensitive to a build-up of lipid in the gland than LPL, for a marked decrease in its activity and in the abundance of its mRNA was found on a low-dose bromocriptine regime when no changes in LPL activity or mRNA were apparent [154], and milk fat yield appeared to be unimpaired [214].

In addition to insulin and presumably to fatty acids, tri-iodothyronine may also modulate mammary LPL activity; whether this is a direct or indirect effect is not known [216].

To facilitate comparison of the regulation of mammary LPL with that of fatty acid synthesis, we have followed the convention of distinguishing between acute and long-term aspects of its control. Typically, long-term control concerns regulation at transcriptional and translational levels, whereas acute enzyme control involves post-translational modification and ligand-mediated modulation of activity. However, it is likely that this distinction is not entirely valid for mammary LPL. Just as for its nutritionally-induced modulation (see Section 4.1.2), the longer-term changes in its activity associated with lactogenesis and involution (on which the nutritionally-induced changes are superimposed) have not been fully characterised with respect to those key aspects listed in the previous section. Furthermore, models of long-term, developmentally-programmed LPL regulation in other cells and tissues indicate that the coordination of transcriptional control with regulation at the
translational and post-translational level does not conform to an intuitive pattern – for instance, LPL mRNA may be up-regulated while activity of an unchanged protein mass is down-regulated [198,208]. Such observations suggest that the possible occurrence of transcriptional regulation of mammary LPL merits exploration not only in connection with long-term modulations of its activity but equally during those events arbitrarily defined here as acute.

4.2. Uptake of fatty acids into mammary epithelial cells

Fatty acid binding proteins located in the plasma membrane have been implicated in the transmembrane movement of fatty acid [217]. There is indirect and circumstantial evidence which links one of these to mammary epithelial cells. Initially identified on the surface of adipocytes by affinity-labelling procedures, the 88 kDa protein now known as FAT (fatty-acid translocator) has been cloned and sequenced [218]. Recombinant expression of FAT in fibroblasts enhanced their ability to take-up exogenous long-chain fatty acids [219]. FAT is also expressed coordinately with soluble tissue-specific fatty acid binding proteins during differentiation of preadipocytes [203] and in myocyte development [220]. A soluble fatty acid binding protein produced by mammary cells was shown by Grosse’s group to inhibit mammary cellular proliferation and was hence named MDGI – mammary derived growth inhibitor [221]. The expression of MDGI was also shown to be developmentally regulated, coinciding with the onset of lactogenic capacity in mammary cells and tissue [222,223]. Two other groups independently isolated and characterised mammary fatty acid binding proteins and found them to be similar to the heart-type analogue – H-FABP [224,225]; Grosse and colleagues carried out the most detailed characterisation of MDGI and concluded that it was similar to H-FABP but with differences significant to justify their assertion that it was a distinct mammary-specific protein. This claim has been the subject of a recent thorough reappraisal [226] – it now seems likely that MDGI it identical to H-FABP.

The expression of FAT in mammary cells was not reported in a study where several rat tissues were screened by Northern blot analysis for FAT mRNA [218]; however, in the light of the above, we postulate that FAT is expressed in mammary epithelial cells and that its expression will be co-ordinate with that of MDGI/H-FABP. Such co-ordinate expression in mammary cells would lend strong support to the proposal that FAT plays a key role in fatty acid utilisation in these and other cells. The close homology between FAT and CD36 [218] – a cell surface glycoprotein found on endothelial cells and lactating (but not non-lactating) mammary cells [227] – while currently of uncertain significance, encourages these speculations.

In the differentiation of preadipocytes to adipocytes, expression of several proteins including FAT and the adipocyte-specific FABP can be transcriptionally regulated by long-chain fatty acids acting via a recently described ligand-dependent transcription factor FAAR [210]; transcriptional activation was also achieved by the non-metabolisable fatty acid analogue 2-bromo-palmitate. FAAR is related to the peroxisomal proliferator-activated receptors (PPARs) but has distinctive selectivity for long-chain fatty acids [210,228] and is expressed earlier in adipocyte differentiation [210]. The role of PPARs in the transcriptional regulation of LPL in several cells and tissues is well-established [228]; it remains to be demonstrated if FAAR also participates in transcriptional regulation of LPL and if these regulatory mechanisms influence the expression of lipid-metabolising enzymes in mammary tissue.

5. Lipid secretion

Triacylglycerol-rich lipid droplets are secreted from the apical surface of mammary epithelial cells by an exocytotic process, resulting in the encapsulation of each lipid droplet by a membrane – the milk fat globule membrane – at least partly derived from the apical plasma membrane [68]. The mechanism of lipid-droplet assembly has been investigated in Keenan’s laboratory using a cell-free model derived from lactating mammary gland [229]. Several proteins, both from cytosol and from the endoplasmic reticulum lumen [230], are required for maturation of small cytoplasmic lipid droplets into mature milk lipid globules; association of ADP-ribosylation factors with putative lipid assembly complexes (see Section 3.1.1) suggests their involvement in the se-
cretion of the newly-synthesised lipids. Butyrophyllin is a major component of the milk fat globule membrane; although it has been thoroughly characterised, its presumed role in secretion of the milk fat globule is unknown [68]. The study of milk fat secretion is hampered by the lack of a suitable and robust experimental model; that described by Zeisel and co-workers [231], using cultured primary mammary cells from rats, has yet to gain wide acceptance. The model described by Williamson and colleagues [232], based on acute preparations explanted from rat mammary tissue, readily enables comparisons between tissues at different stages of development and from animals which have been subject to pharmacological manipulation. Rates of lipid secretion measured using this model match those attained by the tissue in vivo, and greatly exceed those supported by mammary cells in primary culture [231]. The latter model, however, much more readily lends itself to the kinds of in vitro experimentation, such as the transient or stable expression of recombinant proteins, which may ultimately unravel the mechanisms underlying the regulation of milk lipid secretion and its coordination with other biosynthetic and secretory events in mammary epithelial cells.

6. Key areas for future research

While much is known about the biochemistry of lipid synthesis in the mammary gland, there is still much to be learnt about the regulatory systems, especially the intracellular signalling systems. The basis of the novel effects of insulin and signalling systems controlling lipogenic gene expression by prolactin and other hormones are obvious targets. The latter of course needs more information about the promoter structures of these lipogenic enzyme genes. More information of gene structure should also help clarify the roles, and mechanisms of action, of fatty acids in the control of gene expression in the gland.

The key areas in relation to mammary LPL research are those that address deficits in our understanding of the biochemical and cell biological events involved in maturation, intracellular transport, secretion and indeed cellular origin of the enzyme in the mammary gland. Valuable pointers as to the types of questions to be asked and the experimental strategies to be adopted are to be found in the investigations into these areas in other cell types, which are discussed above (Section 4.1.2). A similar analysis of future prospects applies to the uptake of fatty acids by mammary epithelial cells: clarification of the putative involvement of the translocator FAT in this process is required.

Little is known about the mechanisms involved in lipid secretion in the mammary cell, in part reflecting the lack, until recently, of a suitable system for tackling this problem. Of particular interest in this respect is how the secretion of lipids and other milk constituents are coordinated. Another key aspect is the role and mechanism of action of FIL, the peptide Feedback Inhibitor of Lactation [131] in the control of mammary lipid secretion.

The mammary gland is, of course, a complex organ comprising a number of cell types. There is growing evidence for an important role of local, intercellular mechanisms regulating mammary development and function. Understanding of the control of lipid metabolism in the mammary gland will thus require not only a detailed knowledge of molecular structure and interactions of key components, but also clarification of the role of probable intercellular interactions influencing the process.

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References

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