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# Lipid metabolism during lactation: a review of adipose tissue-liver interactions and the development of fatty liver

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## Introduction

Fatty acids are the major source of energy for most tissues during periods of negative energy balance; however, fatty acids can, in some circumstances, have pathological effects. Fatty acids are stored as triacylglycerols (TAG), mostly in the various adipose tissue depots of the body. However, if blood unesterified fatty acid (NEFA) levels are elevated for prolonged periods, as may occur during lactation or obesity, TAG can accumulate in other tissues including liver and muscle cells (myocytes), and this can have pathological consequences such as the development of ketosis (Grummer, 1993; Drackley et al. 2001) or type 2 diabetes (Boden & Shulman, 2002; McGarry, 2002).

Adipose tissues, however, are not just stores of fatty acids, or to be precise TAG; they are now known to secrete a wide variety of substances, some of which have endocrine functions, acting on the hypothalamus to influence appetite and modulating the metabolism of various tissues including the liver and muscle (Arner, 2003; Vernon, 2003; Havel, 2004; Kershaw & Flier, 2004; Pittas et al. 2004; Steppan & Lazar, 2004; Tomas et al. 2004). It is now thought that some of these hormones, collectively called adipocytokines, secreted by adipose tissue are involved in the development of fatty liver, which occurs in conditions such as obesity and type 2 diabetes. The possibility of adipocytokines being involved in the development of fatty liver during lactation is considered in this review. Adipocytokines do not act in isolation, but function against a background of classical hormones such as insulin, catecholamines and growth hormone, which have major roles in lipid metabolism.

## Major pathways of fatty acid metabolism and their regulation

Liver, adipose tissue and, during lactation, the mammary gland, are the major sites of fatty acid metabolism. All three tissues can synthesize fatty acids *de novo* and esterify them to TAG (Vernon, 2002). Key enzymes of synthesis *de novo* are acetyl CoA carboxylase and fatty acid synthase, while the initial step of fatty acid esterification is catalysed by glycerol-3-phosphate acyl transferase (GPAT). In ruminants, acetate is the major substrate for synthesis *de novo*, although the mammary gland also uses appreciable amounts of β-hydroxybutyrate. However, glucose is required to generate some NADPH needed for synthesis *de novo* and is also the major precursor of the glycerol-3 phosphate needed for esterifying the fatty acids to TAG. In addition to synthesis *de novo*, adipose and mammary tissues secrete lipoprotein lipase (LPL). This allows these tissues to obtain fatty acids from TAG of chylomicrons and very low density lipoproteins (VLDL) (secreted by intestinal and liver cells respectively) in the blood.

Adipocytes are adapted to store large amounts of TAG – quantities that would cause toxic damage to other cell types (Unger & Orci, 2001; Boden & Shulman, 2002). It probably is not the TAG themselves that are injurious, but rather the concomitant increase in fatty acids and derivatives such as ceramides in the cell due to turnover of the TAG. In adipocytes, the TAG droplet is surrounded by a network of proteins such as perilipin, which restrict hydrolysis and so keep the cellular concentration of fatty acids themselves low (Brasaemle et al. 2000). In addition, adipocytes have high levels of fatty acid binding protein, which reduce the amount of free fatty acid in the cell (Smith et al. 1985; Kaikus et al. 1990). During periods of negative energy balance and during stress, adipocytes release NEFA into the blood; these NEFA are produced by hydrolysis of TAG by the action of hormone-sensitive lipase. During such periods, adipose tissue secretes vasoactive factors (e.g. adenosine, prostacyclin, prostaglandin E), which help remove fatty acids from the tissue (Vernon, 2003); this is probably particularly important during periods of stress when very high rates of lipolysis may occur.

The liver has a more complex role in lipid metabolism than adipose or mammary tissue, taking up NEFA from the blood and either oxidizing them to $CO_2$ or ketones (acetoacetate and β-hydroxybutyrate), which are released into the blood for use elsewhere in the body, or esterifying

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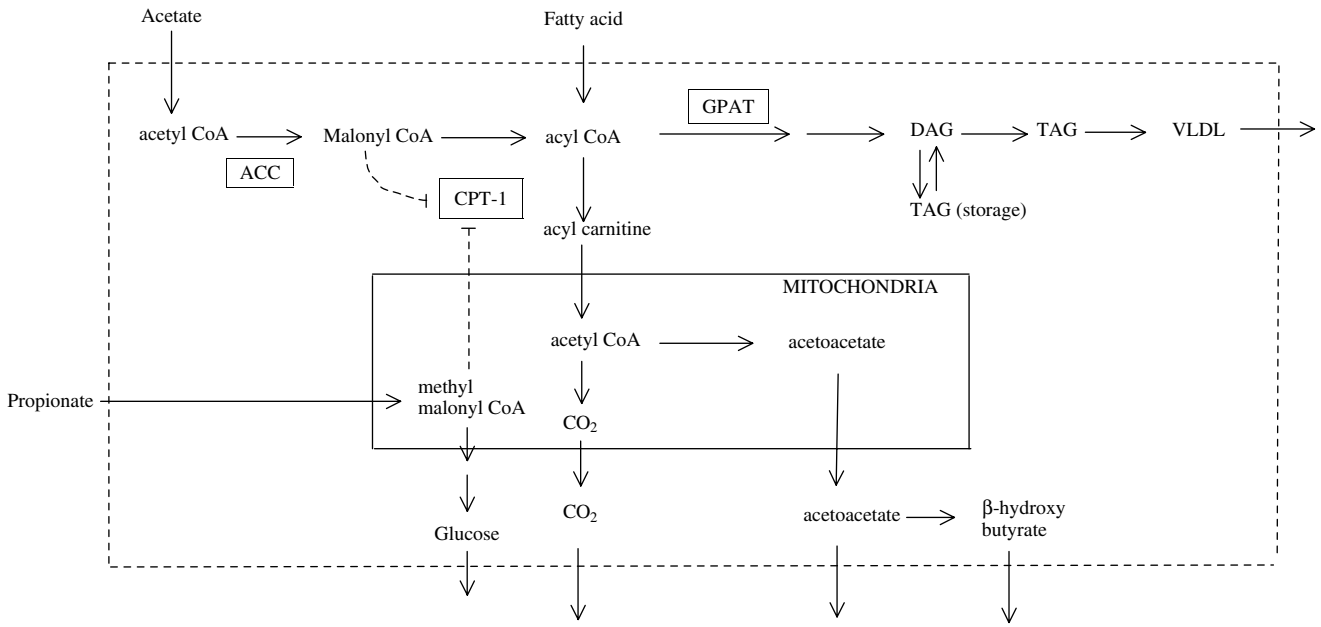


**Fig. 1.** Hepatic fatty acid metabolism. ACC, acetyl CoA carboxylase; GPAT, glycerol-3 phosphate dehydrogenase; CPT 1, carnitine palmitoyl transferase 1; DAG, diacylglycerol; TAG, triacylglycerol. Solid lines indicate metabolic pathways; dashed lines indicate allosteric inhibition.

fatty acids to TAG and phospholipids (Fig. 1) (Zammit, 1990; Vernon, 2002). These lipids are then secreted into the blood as lipoproteins including VLDL. Whether fatty acids are esterified or oxidized depends largely on the activites of GPAT and carnitine palmitoyl-transferase-1 (CPT 1). GPAT catalyses the initial step of fatty acid esterification, whereas CPT 1 is an enzyme located in the outer membrane of the mitochondria, which catalyses the conversion of long-chain fatty acid acyl CoA esters to their acyl carnitine derivatives. This allows their transport into the mitochondria for fatty acid β-oxidation.

By contrast to many monogastric species, the rate of fatty acid synthesis *de novo* is very low in the liver of ruminants (reflecting the need for gluconeogenesis), and is probably regulatory in function (Zammit, 1990). This is because the product of acetyl CoA carboxylase, malonyl CoA, modulates fatty acid oxidation by inhibiting the activity of CPT 1 (Zammit, 1999). Importantly, the concentration of malonyl CoA in the liver is determined primarily by the relative activities of acetyl CoA carboxylase and fatty acid synthase, rather than the lipogenic flux (Zammit, 1990). Thus the malonyl CoA concentration of ovine hepatocytes is similar to that of the rat, despite major differences in the rate of hepatic synthesis of fatty acids *de novo* in the two species (Zammit, 1990). Furthermore, propionate decreases hepatic fatty acid oxidation in ruminants, and in these species CPT 1 is inhibited by methylmalonyl CoA, an intermediate of propionate metabolism in the mitochondria (Zammit, 1990). Hence in the fed state, in which the liver is receiving substantial amounts of propionate and acetate from the diet, CPT 1 activity will be suppressed, and fatty acids will be directed to lipid synthesis rather than to oxidation. During fasting, glucose is synthesized from lactate, pyruvate, alanine, other amino acids and glycerol rather than propionate, hence inhibition of CPT 1 by methylmalonyl CoA is relieved and fatty acid oxidation increased (Zammit, 1990).

Ruminants also differ from monogastrics in that there is very little β-hydroxybutyrate dehydrogenase in the mitochondria, essentially all activity residing in the cytosol (Zammit, 1990; Drackley et al. 2001). This enzyme catalyses the conversion of acetoacetate (the product of ketogenesis in the mitochondria) to β-hydroxybutyrate. Consequently acetoacetate is transported as such into the cytosol, where it partly equilibrates with β-hydroxybutyrate (Zammit, 1990; Drackley et al. 2001). The reason for this species difference is not clear. One consequence is that ruminant mitochondria have a higher NADH:NAD ratio than do mitochondria from monogastrics and as result there will be a lower ratio of mitochondrial oxaloacetate to malate. A low mitochondrial oxaloacetate concentration has long been considered a key factor promoting ketogenesis, but the low concentration has been attributed to a high rate of gluconeogenesis, whereas a high NADH : NAD ratio may be the real cause (Zammit, 1990).

## Endocrine, paracrine and autocrine control

Fatty acid metabolism is subject to complex acute and chronic control by hormones and other factors. Insulin is the key anabolic hormone of adipose tissue, increasing

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**Table 1.** Some substances secreted by adipose tissue

*Metabolic modulators*
- Lipoprotein lipase
- Acylation-stimulating protein
- Apoprotein E
- Fatty acids
- Prostaglandin E

*Vasoactive factors*
- Prostacyclin
- Monobutyrin
- Angiotensin
- Atrial natrinuretic peptide

*Hormones*
- Oestrone
- Oestradiol
- Testosterone
- IGF-1

*Adipocytokines*
- Leptin
- Resistin
- Tumour necrosis factor α
- Interleukin-6
- Adiponectin (AdipoQ, Acrp30)

*Complement system*
- Factor B
- Factor C
- Factor D (adipsin)

*Binding Proteins*
- IGF-binding protein
- Retinol-binding protein
- Cholesterol ester transfer protein

lipogenesis and LPL activity and inhibiting lipolysis, whereas catecholamines, released by sympathetic nervous activity, inhibit lipogenesis and stimulate lipolysis. Effects of insulin and catecholamines are modulated by autocrine and paracrine factors (e.g. acylation-stimulating protein, adenosine, prostaglandins) produced within adipose tissue (Vernon, 2003). Furthermore, adipose tissue is under chronic, homeorhetic control by factors such as growth hormone (GH), which attenuates the effects of insulin on lipogenesis and enhances the lipolytic effects of catecholamines (primarily by diminishing the anti-lipoytic effects of adenosine and prostaglandins) (Bauman, 2000; Vernon, 2003). Adipose tissue is also subject to autonomic control (see below).

The complex, multifactorial control of fatty acid metabolism in adipocytes is in sharp contrast to the almost rudimentary and mostly vicarious endocrine control that occurs in the mammary gland. Prolactin is required for the initiation of lipid synthesis in the gland at the beginning of lactation in both ruminants and non-ruminants, but its role once lactation is established is uncertain (Barber et al. 1997). Insulin and catecholamines do not appear to have direct effects on mammary lipid metabolism in lactating ruminants, but both can have indirect effects by altering lipolysis in adipose tissue and hence NEFA availablity (fatty acids are allosteric inhibitors of acetyl CoA carboxylase) (Neville & Picciano, 1997). GH has galactopoetic effects in ruminants, which include increased output of milk fat (Bauman & Vernon, 1993), but the mechanism is still not fully resolved. GH enhances nutrient partitioning in favour of the mammary gland, but this would not appear to account for all the effects of the hormone on the mammary gland. The much more complex control of fatty acid metabolism in adipocytes compared with the mammary gland probably reflects the fact that adipose tissue is the major energy reserve of the body, and so the size of its TAG pool needs to be controlled carefully (see below), whereas TAG synthesized in the mammary gland are secreted.

Insulin increases fatty acid synthesis and esterification and decreases fatty acid oxidation in the liver; these effects of insulin are attenuated by glucagon (Zammit, 1990; 1999). These are direct effects of the hormones on hepatocytes, and are mediated through changes in enzyme activity; in addition, insulin can also modulate liver metabolism indirectly by inhibiting lipolysis in adipose tissue and so decreasing the supply of NEFA to the tissue. GH stimulates gluconeogenesis in the liver, but by contrast to its effects on adipose tissue, GH does not appear to have direct effects on hepatic lipid metabolism (Bauman & Vernon, 1993).

## Adipose tissue as an endocrine tissue

Adipose tissue secretes a wide variety of substances (Table 1). Some are primarily autocrine or paracrine, acting within the tissue to modulate metabolism and blood flow (important in the handling of fatty acids within the tissue) (Vernon & Houseknecht, 2000; Vernon, 2003). Others, such as the sex steroids, are thought to have endocrine roles. Of particular interest, in the present context, are a group of peptide hormones termed adipocytokines (leptin, resistin, interleukin-6, tumour necrosis factor α and adiponectin) (Arner, 2003; Vernon, 2003; Havel, 2004; Kershaw & Flier, 2004; Steppan & Lazar, 2004). Adipocytokines are thought to have a role in regulating adipose tissue mass as their rate of secretion varies with the degree of adiposity, but in addition they can also modulate the metabolism of other tissues including liver. While adipose tissue is the sole or at least major source of leptin, resistin and adiponectin, interleukin-6 and tumour necrosis factor α (TNFα) are also secreted by cells of the immune system (Coppack, 2001; Pittas et al. 2004). The relative contributions of adipose tissue and cells of the immune system to serum interleukin-6 and TNFα concentrations are unknown, and probably depend on the nutritional and pathological state of the animal. There may also be species differences. In man there is a significant release of interleukin-6, but there appears to be little release of TNFα from adipose tissue from non-obese individuals *in vivo* (Mohammed-Ali et al. 1997); however, it seems probable that adipose tissue is an important source of serum TNFα in obese rodents. Even if TNFα

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is not normally released by adipose tissue, it can have indirect effects on other tissues as TNFα stimulates secretion of interleukin-6 and inhibits secretion of leptin and adiponectin by adipose tissue (Coppack, 2001; Arner, 2003; Fasshauer & Paschke, 2003).

Adipose tissue differs from other tissues in that its mass can vary considerably in the adult, depending on nutritional status. While having a large amount of adipose tissue will protect an animal from death by starvation, it can render an animal more susceptible to predation; hence there are additional, 'autonomic' controls, to regulate the mass of adipose tissue depending on need (Vernon et al. 2001; Vernon, 2003). Adipocytes secrete leptin, which can act on the hypothalamus to decrease food intake and increase energy expenditure; secretion is increased by obesity and decreased by fasting in man and rodents (Ahima & Flier, 2000; Havel, 2004; Kershaw & Flier, 2004) and in ruminants (Vernon et al. 2001). Obesity also increases secretion of interleukin-6 and TNFα and, in some species, resistin by adipose tissue (Arner, 2003; Fasshauer & Paschke, 2003; Warne, 2003; Kershaw & Flier, 2004; Steppan & Lazar, 2004). Interleukin-6 and TNFα can also decrease appetite, while all three can act on adipocytes to decrease response to insulin (Arner, 2003; Fasshauer & Paschke, 2003; Warne, 2003; Steppan & Lazar, 2004). Serum TNFα concentration is also increased by obesity in sheep (Daniels et al. 2003), and administration of TNFα induces insulin resistance in cattle (Kushibiki et al. 2000; 2001). Thus adipose tissue secretes factors that diminish the supply of nutrients to the tissue and also limit the ability of insulin to stimulate lipid synthesis in the tissue.

Importantly in the present context, adipocytokines also modulate the metabolism of other tissues including liver. Thus leptin is thought to limit lipid accumulation in muscle and liver, in part at least by increasing fatty acid oxidation (Unger, 2000; Tomas et al. 2004). Resistin, interleukin-6 and TNFα may also cause insulin resistance in other tissues including liver (Arner, 2003; Fasshauer & Paschke, 2003;Warne, 2003; Havel, 2004; Kershaw & Flier, 2004; Steppan & Lazar, 2004; Tomas et al. 2004). In contrast to the other adipocytokines, obesity decreases secretion of adiponectin by adipose tissue (Fasshauer & Paschke, 2003; Heilbronn et al. 2003; Havel, 2004; Kershaw & Flier, 2004). Serum adiponectin levels are also reduced in type 2 diabetes, and treatments that increase serum adiponectin concentration improve whole-body insulin responsiveness (Fasshauer & Paschke, 2003; Heilbronn et al. 2003; Havel, 2004; Pittas et al. 2004). Indeed, treatment with adiponectin plus leptin completely reversed the marked insulin resistance of lipoatrophic mice, whereas neither agent alone was completely effective (Yamauchi et al. 2001). Adiponectin enhances the ability of insulin to decrease hepatic gluconeogenesis, and decreases TAG accumulation in muscle and liver (Fasshauer & Paschke, 2003; Heilbronn et al. 2003; Havel, 2004; Pittas et al. 2004; Tomas et al. 2004). Curiously, adiponectin appears to decrease TAG levels by increasing fatty acid oxidation in muscle, but by decreasing fatty acid transport into liver cells (Yamauchi et al. 2001). As TAG accumulation in myocytes is associated with insulin resistance (Boden & Shulman, 2002; McGarry, 2002), a decrease in TAG concentration induced by adiponectin should increase insulin sensitivity of these cells. Recently, two adiponectin receptors have been identified, one highly expressed in liver and the other in muscle cells (Yamauchi et al. 2003), which might account for the different effects of adiponectin on fatty acid uptake and metabolism in the two tissues.

**Table 2.** Some serum hormone changes during early lactation in ruminants

| *Increased* | *Decreased* | *Unchanged* |
|---|---|---|
| Prolactin | Insulin | Glucagon |
| Growth hormone | Thyroxine | |
| Glucocorticoids | Triiodothyronine | |
| Resistin* | Leptin | |

* Expression in adipose tissue increased.

## Adaptations to early lactation

Early lactation in dairy cows is very often a period of negative energy balance as appetite usually increases more slowly than the increase in nutrient output arising from milk production (Ingvartsen & Andersen, 2000; Vernon et al. 2002). The period is associated with a number of changes in serum hormone (Bauman, 2000; Ingvartsen & Andersen, 2000) and adipocytokine concentrations (Table 2). Serum leptin concentration and leptin gene expression in adipose tissue are both decreased during lactation in rodents, and the nocturnal rise in serum leptin concentration seen in fed, non-lactating rodents is markedly attenuated (Vernon et al. 2002). The negative energy balance of lactation appears to be an important factor responsible for the hypoleptinaemia in rodents (Vernon et al. 2002). In addition there is also evidence for increased leptin clearance from serum during lactation (Vernon et al. 2002), while the suckling stimulus is at least partly responsible for the attenuation of the nocturnal rise in serum leptin during lactation (Denis et al. 2003). Serum leptin concentrations fall around parturition in dairy cows, primarily owing to animals moving into negative energy balance (Bloch et al. 2001; Holtenius et al. 2003; Liefers et al. 2003; Reist et al. 2003). The hypoinsulinaemia of early lactation probably mediates the effect of negative energy balance on leptin secretion (Bloch et al. 2003; Leury et al. 2003), but in addition the ability of insulin to stimulate leptin secretion also appears to be attenuated during early lactation in cows (Leury et al. 2003). Serum leptin concentrations were markedly decreased relative to levels in non-lactating, non-pregnant ewes, during early lactation in ewes that were suckling two or three lambs, and were in negative energy balance (Sorensen et al.

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2002). However, in another study, in which ewes were suckling single lambs, lactation had no effect on energy balance and there was no effect of lactation on serum leptin concentration (Ehrhardt et al. 2001). Thus in a variety of species, the negative energy balance of lactation results in a fall in serum leptin concentrations, but other factors may also contribute to the hypoleptinaemia.

Resistin gene expression in adipose tissue appears to be increased by lactation in cows (Komatsu et al. 2003), but not in mice (Bing et al. 2002), while serum adiponectin concentration is decreased by lactation in mice (Combs et al. 2003). While a fall in serum leptin concentration during early lactation is consistent with the negative energy balance that usually occurs in this state, the changes in resistin gene expression and serum adiponectin concentration are contrary to what might be expected from the negative energy balance. Chronic undernutrition is reported to cause an increase in serum adiponectin in mice (Combs et al. 2003), and weight loss in man is associated with increased serum adiponectin levels (Havel, 2004; Wolfe et al. 2004). However, prolactin treatment of lactating mice decreased serum adiponectin concentration (Combs et al. 2003), suggesting that prolactin has a dominant effect over energy balance in lactating mice. Resistin gene expression is increased by obesity (Fasshauer & Paschke, 2003; Steppan & Lazar, 2004), and serum resistin and adipocyte resistin gene expression decrease on fasting in rodents (Rajala et al. 2004). Thus, some additional factor other than energy balance must be responsible for the increased resistin gene expression found in lactating cows.

The hypoleptinaemia of lactation should contribute to the increase in appetite and may also be responsible for other adaptations such as the hypothyroidism (Vernon et al. 2002). Why lactating animals do not respond fully to the hypoleptinaemia, and increase their appetite more so that they achieve energy balance is not known. In rats, where again lactation results in negative energy balance and hypoleptinaemia, failure to achieve energy balance does not appear to be due to a physical constraint (Vernon et al. 2002), and this is arguably the case for cattle too (Sorensen et al. 2002). However, other factors in addition to leptin are likely to be involved in regulating appetite during lactation, as peripheral administration of recombinant leptin to lactating goats had no apparent effect on food intake (A Sorensen, A Gertler and RG Vernon, unpublished observations), while exogenous leptin reduced food intake by less than 20 percent in lactating rats (Vernon et al. 2002). Other, recent studies in rats, also suggest that leptin does not have a major role in the hyperphagia of lactation in this species (Denis et al. 2004).

In early lactation, adipose tissue metabolism is usually in catabolic mode; fatty acid synthesis is markedly reduced and there is also a decrease in LPL activity and fatty acid esterification (McNamara, 1997; Bauman, 2000; Vernon, 2002). By contrast, these same processes are markedly increased in the mammary gland (Barber et al. 1997). The overall effect is to ensure the preferential utilization of precursors for TAG synthesis in the mammary gland. The hypoinsulinaemia and the rise in serum GH are likely to contribute to the decreased lipogenic capacity in adipose tissue, and in addition, adipocytes become transiently less responsive to insulin (McNamara, 1997; Bauman, 2000; Vernon, 2002). The basis of this insulin resistance is not yet known, but the recent finding that resistin expression is increased during lactation in cows, provides a possible explanation (Komatsu et al. 2003). In contrast to lipogenesis, the rate of maximum catecholamine-stimulated lipolysis is increased in both cattle and sheep (McNamara, 1997; Bauman, 2000; Vernon, 2002). Increased serum concentrations of GH and glucocorticoid hormones may account for these changes in the responsiveness of the lipolytic system. Paradoxically, lactation also increases response to the antilipolytic factor, adenosine, in sheep adipose tissue (Bauman, 2000; Vernon, 2002); the factors responsible are unknown, but glucocorticoids could be involved (Vernon, 2002).

As well as an increased response to catecholamines in adipocytes, it is likely that the lipolytic signal is also enhanced during lactation. There is no direct evidence for this in ruminants; however, there is some evidence for increased sympathetic activity in white adipose tissue in lactating rats (McNamara & Murray, 2001). The hypoinsulinaemia of lactation should also favour lipolysis. Curiously, whereas the ability of insulin to stimulate lipogenesis in adipose tissue is diminished during lactation, the antilipolytic effect of insulin is maintained (Vernon, 2002). Whatever, early lactation is usually a period of high lipolytic activity and hence elevated plasma NEFA concentrations.

Skeletal muscle can use glucose and fatty acids as energy sources. During lactation the ability of insulin to stimulate glucose uptake by the sheep hind limb is diminished (Vernon et al. 1990); this is probably a partitioning mechanism to favour glucose utilization by the mammary gland. Hypoleptinaemia (Unger, 2000), decreased serum adiponectin concentration (Combs et al. 2003) and elevated plasma NEFA levels (Boden & Shulman, 2002; McGarry, 2002) can all cause increased TAG levels in muscle, which can result in a diminished response to insulin (Boden & Shulman, 2002; McGarry, 2002). Interestingly, there is evidence of increased lipid in muscle during lactation in sheep (Smith et al. 1981), probably due to the combination of elevated NEFA, hypoleptinaemia and perhaps changes in other adipocytokines, which could contribute to the insulin resistance of the tissue.

Lactation has profound effects on liver metabolism (Drackley et al. 2001; Vernon, 2002). Gluconeogenesis is usually increased 2-fold or more to meet the demands of the mammary gland for lactose synthesis (Drackley et al. 2001). The increased energy needs of the liver for gluconeogenesis are met primarily by increased fatty acid oxidation (Drackley et al. 2001), which is facilitated by the

enhanced lipolysis in adipose tissue and increased blood flow to the liver (Reynolds et al. 2003). Several intracellular mechanisms promote the oxidation of fatty acids in the liver during lactation. A fall in serum insulin and rises in glucagon and also in NEFA concentrations will all act to decrease acetyl CoA carboxylase activity; this will lower malonyl CoA concentration, leading to increased CPT 1 activity and hence fatty acid oxidation. In addition, the amount of CPT 1 (Dann et al. 2000) and the concentration of carnitine in the hepatocyte (Grum et al. 1996) both increase. What happens to methylmalonyl CoA concentration during lactation is not known, and is not easy to predict. In mice at least, serum adiponectin levels decrease (Combs et al. 2003), which may diminish hepatic response to insulin. Within the mitochondria complete oxidation of fatty acids to $CO_2$ is limited by the need for ATP in the cell, hence surplus acetyl CoA is converted to acetoacetyl CoA and transported into the cytosol, where it is partly converted to β-hydroxybutyrate prior to release into the blood (Zammit, 1990; Drackley et al. 2001). Secretion of ketone bodies is advantageous to the mammary gland, as they are readily used by the tissue for fatty acid synthesis *de novo*.

Despite the fall in the serum insulin:glucagon ratio and other mechanisms that operate to promote fatty acid oxidation, fatty acid esterification is also increased in the liver during lactation. This is due primarily to the substantial increase in NEFA uptake, but in addition, activities of some enzymes of esterification are also increased (Van den Top et al. 1995). By contrast VLDL secretion is not thought to change and may even decrease, possibly owing to a decrease in apoprotein B production (Bauchard et al. 1996; Drackley et al. 2001). As a consequence, synthesis of TAG often exceeds secretion via VLDL, and so there is usually accretion of lipid droplets in the cell. The hypoleptinaemia, and possibly changes in resistin and adiponectin during lactation may also promote the TAG accumulation. It is curious that the liver does not increase its output of VLDL; accessing TAG fatty acids of VLDL by a tissue requires LPL activity, and so can be subject to tight homeorhetic control, directing use of fatty acids to the mammary gland.

The physiological purpose of the accumulation of TAG in the liver is not clear, and may be a default of a system whereby NEFA uptake by the liver is determined by supply rather than need, reflecting the liver's role in regulating the nutrient composition of the blood. In lower vertebrates the liver is a major site of lipid storage, but this has been replaced by adipose tissue in mammals (Vernon, 2003). The lipid droplets normally clear later in lactation or when lactation terminates. However, in excess they are associated with a move to a pathological situation.

## Fatty liver

Accumulation of TAG in the liver during early lactation is usual in cows and, in moderation, does not appear to impair function. However, when the ratio of stored lipid to glycogen exceeds about 2:1, pathological problems, including decreased gluconeogenesis and ureogenesis, and ketosis begin to develop (Grummer, 1993; Drackley et al. 2001). The reason for the pathological effects of high levels of TAG in liver is unclear, but could be due to prolonged elevated levels of NEFA and/or their CoA esters in the cell. In muscle at least, a key factor seems to be a concomitant increase in ceramide production (Boden & Shulman, 2002; McGarry, 2002).

The accumulation of high, deleterious amounts of hepatic TAG is not associated with any apparent increase in the activities of enzymes involved in TAG synthesis; indeed a small decrease in GPAT activity was found in cows with fatty liver (van den Top et al. 1996). Activities of both hepatic acetyl CoA carboxylase and fatty acid synthase are decreased in cows with fatty liver (Murondoti et al. 2004a), but whether such changes result in a change in malonyl CoA concentration, and hence CPT 1 activity, is unclear. Cows with fatty liver have a lower hepatic propionyl CoA carboxylase activity (Murondoti et al. 2004b), which might result in a decrease in methylmalonyl CoA concentration, and thus an increase in CPT 1 activity. However, the activity of 3-hydroxy-acyl CoA dehydrogenase, an enzyme of fatty acid β-oxidation, was slightly lower in livers of cows with fatty liver (Murondoti et al. 2004b). Thus the development of *post-partum* fatty liver in cows would not appear to be due to any marked change in activities of enzymes of fatty acid metabolism in liver.

Thus, while fatty liver and ketosis are manifest in the liver, and can be attributed to a failure to balance TAG synthesis with secretion, the primary cause is sustained high NEFA concentrations in the blood due to high rates of lipolysis in adipose tissue, coupled with NEFA uptake by liver being determined by supply rather than by need (Grummer, 1993; Drackley et al. 2001). Increased lipolysis in adipose tissue in turn arises from an imbalance between mammary gland requirements for milk production and food intake. The mammary gland must send signals of some sort to the hypothalamus to adjust appetite to need, but these signals have not been elucidated (Vernon et al. 2002). For example, food intake varies with suckling stimulus in rodents, while lactating rats, in which milk production was reduced to low levels by treatment with bromocriptine, continued to have high food intake providing the pups were still present to suckle (Denis et al. 2003). In addition there will be signals from elsewhere in the body including adipose tissue (e.g. leptin), which will also influence appetite. Some degree of negative energy balance is advantageous during early lactation as it increases metabolic efficiency and favours nutrient partitioning towards the mammary gland, but the problem is in ensuring that the animal does not descend so far into negative energy balance that hepatic function becomes impaired.

At present we do not know the signals that determine appetite relative to mammary demand and hence energy

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balance. However, it is well established that cows that are fat at parturition or over-fed prior to parturition, are particularly at risk to considerable imbalance; such animals tend to eat less relative to need and so mobilize more fat than thinner animals (Grummer, 1993). The reason for the poorer appetite in fat cows is not known. Adipose tissue from such cows has an enhanced lipolytic response to catecholamines (Rukkwamsuk et al. 1998), probably in part because the rate of catecholamine-stimulated lipolysis varies with adipocyte size (Vernon et al. 1995). Serum insulin levels during early lactation do not appear to be changed by prior over-feeding (Rukkwamsuk et al. 1998, 1999; Holtenius et al. 2003). Thus fatter animals may well have a greater response to a lipolytic stimulus, and hence higher blood NEFA concentrations; whether this leads to a diminished appetite is unresolved. An inverse relationship between plasma NEFA concentration and feed intake has been observed in lactating cows (Ingvartsen & Andersen, 2000). Prolonged intravenous infusion of fatty acid or lipid into ruminants and rodents decreases appetite (Ingvartsen & Andersen, 2000), as does infusion of fatty acid into the third ventricle of the brain (Obici & Rossetti, 2003). Such studies are consistent with increased hypothalamic fatty acid oxidation resulting in decreased appetite. By contrast, pharmacological inhibition of hypothalamic CPT 1 (Obici et al. 2003), or intracerebroventricular infusion of inhibitors of fatty acid synthase, which should increase hypothalamic malonyl CoA levels and so inhibit CPT 1 activity, decreases appetite (Hu et al. 2003; Ruderman et al. 2003). Decreasing hypothalamic CPT 1 activity should decrease fatty acid oxidation (Obici & Rossetti, 2003). Thus some studies suggest that increased fatty oxidation decreases appetite, whereas other studies suggest that decreased fatty acid oxidation decreases appetite! A possible explanation for these conflicting conclusions is that it is not fatty acid oxidation, but increased levels of fatty acid, or their CoA esters, or perhaps some derivative such as ceramide, in the hypothalamus which depresses appetite (Obici & Rossetti, 2003).

Other factors may also modulate appetite during early lactation. Over-feeding during the *prepartum* period increases serum leptin in cows, but *post-partum* serum leptin fell to similar levels in both over-fed and under-fed animals (Holtenius et al. 2003). Despite similar leptin levels, the previously over-fed cows ate less than did the under-fed animals in the *post-partum* period; that is, feed intake varied independently of serum leptin concentration. We have found that in lactating rats suckling different numbers of young, food intake and milk production varied with litter size, whereas serum leptin was decreased to a similar extent in all animals independent of litter size (Denis et al. 2003). Leptin, by itself, thus does not provide an explanation for *post-partum* differences in intake in previously over-fed and under-fed animals. Increased adiposity increases the serum concentrations of TNFα and interleukin-6 in rodents, both of which can diminish appetite (Steppan & Lazar, 2002; Fasshauer & Paschke, 2003). Increased serum TNFα was also seen in obese sheep (Daniels et al. 2003), and a correlation between serum TNFα, insulin resistance and hepatic TAG accumulation has been noted in cows (Ohtsuka et al. 2001). Administration of TNFα also increased serum NEFA concentration in cattle (Kushibiki et al. 2000, 2001, 2002). Increased cytokine production by adipose tissue from over-fed cows could thus contribute to their lower feed intake during early lactation.

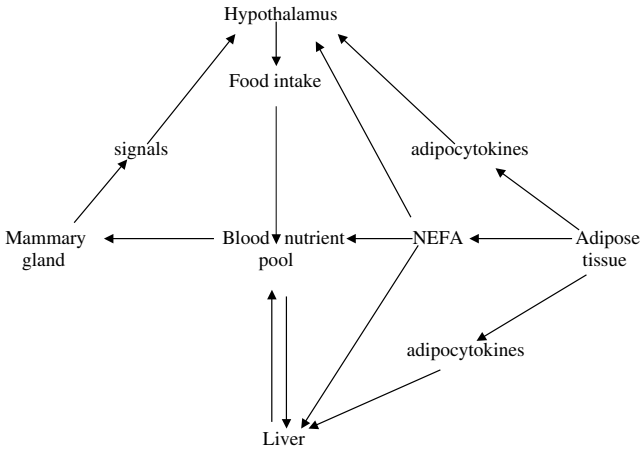


**Fig. 2.** Tissue interrelationships in the regulation of food intake and liver metabolism during early lactation.

While the primary causes of fatty liver are due to events outwith the liver, obesity does alter the secretion of hormones that can affect liver function. As already noted, the hypoleptinaemia of lactation may promote TAG accumulation in liver. Obesity also results in increased secretion of resistin and interleukin-6 and decreased secretion of adiponectin by adipose tissue in rodents (Fasshauer & Paschke, 2003; Heilbronn et al. 2003; Steppan & Lazar, 2004). Both resistin and interleukin-6 have been reported to decrease insulin sensitivity of liver (Fasshauer & Paschke, 2003; Steppan & Lazar, 2004). By contrast, adiponectin increases insulin sensitivity of liver, and resembles leptin in that it can decrease TAG accumulation in muscle and liver (Fasshauer & Paschke, 2003; Heilbronn et al. 2003; Havel, 2004; Tomas et al. 2004). What is of potential interest in the present context is that adiponectin treatment decreased both alcohol-induced and obesity-induced fatty liver in mice (Xu et al. 2003). Furthermore, thiozolidinedione drugs, such as rosiglitazone and pioglitazone, which activate the peroxisome proliferator activator receptor-γ (PPARγ), and increase whole body insulin sensitivity, also increase plasma adiponectin levels (Arner, 2003; Ferre, 2004). Treatment of type 2 diabetics with pioglitazone both increased plasma adiponectin and decreased hepatic fat content (Bajaj et al. 2004; Tiikkainen et al. 2004). Thiozolidinedione drugs also partially reversed the insulin resistance induce by TNFα injection in cattle (Kusibiki et al. 2001), but it is not known whether this involved a

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change in adiponectin levels. Whether such treatment would alleviate fatty liver in lactating cows has not been investigated, but would seem to be an interesting possibility.

Over-feeding or obesity in the *prepartum* period may thus predispose animals to excess accumulation of hepatic TAG *post partum* by several mechanisms: increased production of adipocytokines such as leptin, TNFα and interleukin-6 by adipose tissue may depress appetite; the larger size of adipocytes will make them more responsive to lipolytic stimuli increasing serum NEFA concentration; increased secretion of TNFα may enhance lipolysis; decreased secretion of adiponectin, along with the hypoleptinaemia of lactation and perhaps increased secretion of other adipocytokines, may also act directly increase hepatic TAG accumulation (Fig 2).

## Implications

Adipose tissue has a critical role in the development of fatty liver and ketosis, as these changes in liver composition and metabolism arise both from excessive lipolysis in adipose tissue and altered secretion of adipose tissue-derived hormones, which modulate hepatic metabolism. Treatments of fatty liver and ketosis have focused mostly on decreasing lipolysis. Interestingly, glucagon infusion can prevent fatty liver, reflecting that in ruminants it is glucogenic but not lipolytic (Hippen et al. 1999). However, studies in man suggest that thiazolidenedione drugs, probably through altered secretion of adipocytokines, especially adiponectin, can also be used to alleviate fatty liver. Whether such drugs will have beneficial effects in lactating cows has still to be investigated. Whatever, a better understanding of the factors and mechanisms regulating appetite during early lactation in general, and the roles of adipocytokines in the modulation of appetite and liver metabolism, may help avoid the development of fatty liver and its pathological consequences.

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