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Symposium on 'Lipogenesis in farm animals'

Present and future studies on lipogenesis in animals and human subjects

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Lipogenesis occurs in all vertebrate species and has a critical role in energy balance, providing a means whereby excess energy can be stored as a fat. The metabolic pathways involved and their tissue distribution in different species, including man, are well known. The responses of lipogenesis to diet and to physiological and pathological states have been the subject of many studies. At a molecular level the major rate-controlling enzymes have been identified and their acute, and to a lesser extent chronic, control by hormones have been investigated extensively. However, there is no reason to suppose that all factors regarding lipogenesis have been identified (e.g. the recent discovery of acylation-stimulating protein). Little is known about the movement of newly-synthesized triacylglycerols in cells, either for secretion or storage. The production of leptin and tumour necrosis factor α by adipocytes provides a novel means of feedback control of triacylglycerol production, leptin by decreasing appetite and tumour necrosis factor α by inducing insulin resistance. The synthesis of these peptides appears to vary with the amount of triacylglycerol in adipocytes, but the molecular basis of this process is unknown. Elucidation of the signalling systems involved in the acute and chronic regulation of lipogenesis is also important, both with respect to some homeorhetic adaptations and also in some pathological conditions (e.g. non-insulin-dependent diabetes). Finally, molecular biology is revealing unexpected complexities, such as multiple promoters and different isoforms of enzymes (e.g. acetyl-CoA carboxylase; *EC* 6.4.1.2) exhibiting tissue specificity. Molecular biology, through transgenesis, also offers novel and powerful means of manipulating lipogenesis.

Lipogenesis: Triacylglycerol: Adipocyte

Lipogenesis, which we define as synthesis of triacylglycerols and also their constituent fatty acids, has played a key role in vertebrate evolution. Triacylglycerol provides a very efficient storage form of energy, for not only is it energy-rich, it is also hydrophobic. As a consequence, 1 g stored triacylglycerol contains less than 100 mg water, whereas glycogen not only has a lower energy value, it is also heavily hydrated. Vertebrates not only have the capacity to produce triacylglycerols, but they have also evolved a specialist tissue, adipose tissue, to store them. This capacity has allowed vertebrates to inhabit difficult environments (e.g. deserts and the Arctic) where food supply is uncertain. A store of energy has also facilitated migration from one area to another to take advantage of changing food supplies. Habitation of such environments has also been achieved by some invertebrate groups, but in vertebrates the possession of well-organized energy stores has allowed the evolution of homeothermy (Pond, 1986, 1992) which, while freeing animals from constraints of environmental temperature, increases massively the energy requirements. While poikilotherms have some mesenteric adipose tissue, they also store lipid in liver and muscle (Sheridan, 1994). In contrast, mammals have at least sixteen well-defined adipose tissue depots at various sites in the body; interestingly, the pattern of distribution must have appeared very early in mammalian evolution as it is found in

Abbreviations: ACC, acetyl-CoA carboxylase; TNF-α, tumour necrosis factor α.
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marsupials, and has been retained in eutherian species (Pond, 1984).

A capacity to synthesize and store triacylglycerol also has an important role in mammalian reproduction. Lipid is accumulated during the earlier stages of pregnancy to help meet the needs of the fetus. In some species mothers stop eating for a period around parturition, relying on adipose tissue lipid for their energy needs (e.g. rodents will remain in the nest for a period around parturition; Vernon & Pond, 1997). In many species the demands of the mammary gland for nutrients exceeds the ability of the mother to eat, hence there is again some use of adipose tissue lipid stores (Barber *et al*. 1997). In a few species (e.g. some bears and seals) mothers do not eat for several weeks during the early stages of lactation, and so are totally dependent on their body reserves to meet both their own and their offspring's requirements (Oftedahl, 1992).

Lipogenesis and the ability to store its product has thus had a key role in vertebrate evolution. Recently for human subjects, at least those with access to abundant food, excess lipogenesis and the concomitant obesity have become a major problem, but this problem is mostly a matter of behaviour rather than physiology.

Metabolic pathways, tissue sites and precursors

The metabolic pathways involved in the synthesis of fatty acids and their subsequent esterification to form triacylglycerols are well established; regulatory enzymes (e.g. acetyl-CoA carboxylase (*EC* 6.4.1.2; ACC) and lipoprotein lipase (*EC* 3.1.1.34); Fig. 1) have been identified and their properties studied in considerable detail (Saggerson, 1985). Key regulatory enzymes are subject to both acute and chronic control. ACC activity, for example, is controlled acutely by serine phosphorylation–dephosphorylation and also by allosteric mechanisms (e.g. inhibition by fatty acids), while the amount of enzyme protein varies, and is regulated by a number of hormones including insulin, growth hormone and prolactin (Hardie, 1989; Barber *et al*. 1997). However, while fatty acid synthesis has been studied in great detail, rather less is known about the mechanisms regulating esterification, and even less is known about mechanisms controlling the movement of triacylglycerols, once synthesized, to their destination in the cell. Some progress is being made with respect to their incorporation into lipoproteins in the liver (Zammit, 1996), while a protein (adipocyte differentiation-related protein) has been identified in adipocytes (Jiang & Serrero, 1992; Brasaemle *et al*. 1997) and also mammary epithelial cells (Heid *et al*. 1996), which is involved in the movement of triacylglycerol into lipid droplets for storage or secretion, depending on tissue. This aspect of lipogenesis is not easy (technically) to study, and we suspect much more remains to be discovered.

The major sites of lipogenesis are the intestinal mucosal cells, the liver, adipose tissue and, in lactating mammals, the mammary gland. Each tissue has a distinct function in this respect. The intestinal mucosal cells handle fatty acids absorbed from the diet, while the liver has a central clearing role, taking up and esterifying plasma fatty acids and synthesizing fatty acids *de novo* from acetyl-CoA derived from the catabolism of carbohydrates and their metabolites and, to a lesser extent, amino acids. The triacylglycerols thus synthesized are normally secreted as either chylomicrons (intestinal mucosa) or VLDL (liver) for use elsewhere in the body. Adipose and mammary tissues can obtain fatty acids through the action of lipoprotein lipase which they synthesize and secrete, and which hydrolyses triacylglycerols secreted by the liver and intestinal mucosal cells. These tissues, like liver, can also synthesize fatty acids *de novo*.

The nature of the precursors used for fatty acid and also triacylglycerol synthesis vary with diet and species. For animals consuming 'high-fat' diets, there is often little *de novo* synthesis of fatty acids, and fatty acids of dietary origin are used by the liver, and then, through the action of lipoprotein lipase, adipose and mammary tissue. This process pertains for human subjects consuming 'high-fat' Western diets (Frayn *et al*. 1996). For animals consuming

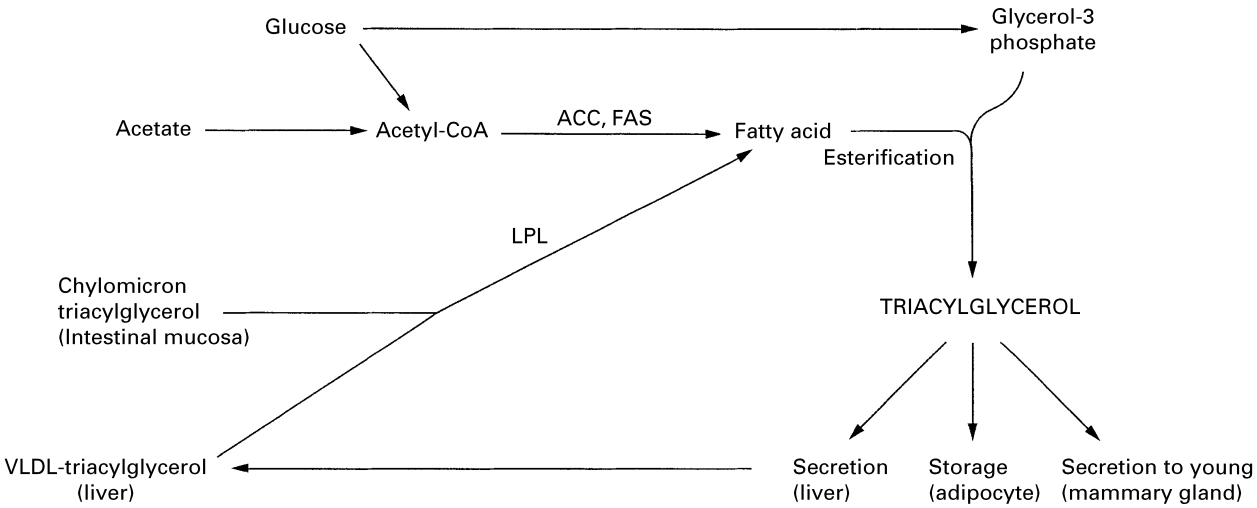

Fig. 1. Pathways of triacylglycerol synthesis. LPL, lipoprotein lipase (*EC* 3.1.1.34); ACC, acetyl-CoA carboxylase (*EC* 6.4.1.2); FAS, fatty acid synthase (*EC* 2.3.1.85).

carbohydrate-rich diets surplus glucose is used to synthesize fatty acids. The major site of fatty acid synthesis varies, with liver being predominant in birds and also human subjects, whereas adipose tissue is more important in pigs; both tissues are important in rats (Vernon, 1980). In ruminants adipose tissue is the major site of fatty acid synthesis, because most dietary carbohydrate is fermented to acetate, propionate and butyrate in the rumen, so liver metabolism is dominated by glucose synthesis, and acetate is the major lipogenic precursor in both adipose and mammary tissue (Vernon, 1980).

Regulation

We thus have considerable understanding of many aspects of lipogenesis. Nevertheless, new factors and complications are still being found. As mentioned previously, we have a poor understanding of the mechanisms involved in the movement of newly-synthesized triacylglycerols to lipoproteins for secretion onto the lipid droplet of adipocytes for storage. In addition, a number of novel mechanisms involved in the regulation of triacylglycerol synthesis, especially in adipocytes, are beginning to be unravelled.

Novel regulatory peptides

Adipocytes not only store fat, they also produce and secrete a number of interesting proteins. Studies with differentiating 3T3-L1 pre-adipocytes showed that they secreted three proteins, adipsin (factor D), factor B and factor C3 of the complement system (Choy *et al*. 1992); these three proteins were subsequently shown to be produced by human adipocytes (Cianflone *et al*. 1994). In the presence of chylomicrons and factor B, adipsin, which is a serine protease, cleaves a portion of factor C3 to produce factor C3a; this protein is further cleaved by carboxypeptidase to produce C3a desarginine (Cianflone, 1997; Fig. 2). This protein is identical to a small basic protein previously isolated from human plasma, which is termed acylation-stimulating protein as it stimulates fatty acid esterification and also glucose transport in adipocytes (Cianflone, 1997). Thus, by a rather convoluted mechanism, adipocytes produce an autocrine factor which acts on the cell to promote triacylglycerol synthesis. Details of the molecular mechanisms have still not been resolved, but they appear to involve protein kinase C (Baldo *et al*. 1995). Plasma acylation-stimulating protein levels are increased in gynoid obese human subjects, and dysfunction of the acylation-stimulating protein system has been found in hyperapolipoprotein B individuals, a dyslipoproteinaemia associated with CHD (Cianflone, 1997).

Many metabolic pathways are subjected to feedback control. Fatty acids, for example, inhibit ACC activity and hence fatty acid synthesis (Hardie, 1989). While adipocytes have a remarkable capacity to accumulate triacylglycerol, in most animals there is a need to carefully regulate the size of these stores of lipid, accumulating enough to meet possible needs, but not so much that they compromise mobility and increase the vulnerability to predation (Witter & Cuthill, 1993). The balance depends very much on circumstance; for example, animals faced with an Arctic winter accumulate a lot of fat since starvation rather than predation is the greatest threat to survival. The basis of the feedback control of triacylglycerol production in adipocytes has long remained elusive, but the recent discovery of leptin (Zhang *et al*. 1994) has provided at least a partial answer to this problem. Leptin is a peptide produced and secreted by adipocytes which interacts with the neuropeptide Y system of the hypothalamus to modulate appetite, high levels of leptin suppressing appetite (Campfield *et al*. 1996; Caro *et al*. 1996; Houseknecht *et al*. 1998). Leptin production and secretion are under complex hormonal (insulin, glucocorticoids and growth hormone) and sympathetic control, but importantly are also influenced by the amount of triacylglycerol stored in adipocytes, by an as yet undefined mechanism (Campfield *et al*. 1996; Caro *et al*. 1996; Houseknecht *et al*. 1998). The consequence of this process is that the serum concentration of leptin is proportional to the amount of adipose tissue (and hence adipocyte triacylglycerol) in the body. Leptin also increases energy expenditure by enhancing both thermogenic activity of brown adipose tissue and fatty acid oxidation in liver and other tissues (Flier, 1997; Zhou *et al*. 1997). Leptin then, by modulating energy balance through changes in appetite and energy expenditure, can act as an indirect feed-back inhibitor of triacylglycerol synthesis in adipocytes. In addition, there is now evidence for leptin having a direct autocrine effect on adipocytes, decreasing insulin stimulation of glucose uptake (Muller *et al*. 1997), which should lead to a decreased triacylglycerol synthesis.

While most interest in leptin has arisen from its potential role as an appetite modulator for treatment of obesity, its major physiological role may be as a signal of inadequate reserves of triacylglycerol in adipose tissue. Leptin stimulates secretion of several pituitary hormones, including gonadotrophins and thyrotropin; leptin administration reverses the diminished secretion of these hormones which

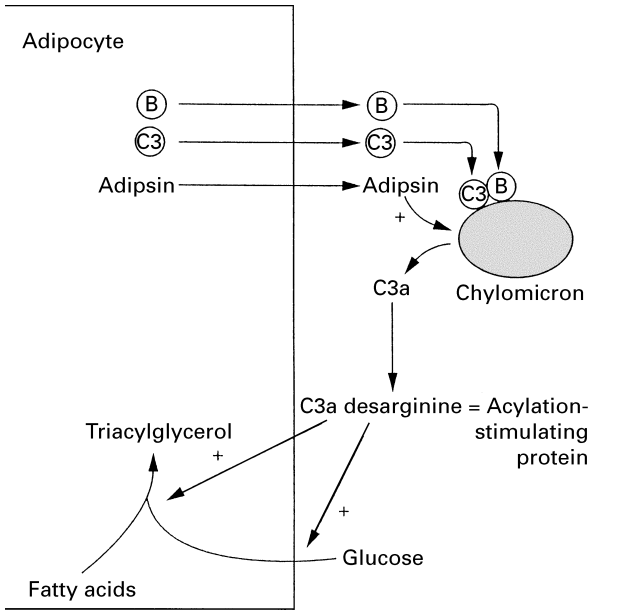

Fig. 2. Synthesis and actions of acylation-stimulating protein. +, Stimulates.

occurs during undernutrition (Yu *et al*. 1997). Low levels of adiposity are known to result in a loss of reproductive activity in female mammals, and leptin appears to be a key mediator in this process (Houseknecht *et al*. 1998; Rosenbaum & Leibel, 1998). As adipose tissue lipid reserves are of importance for pregnancy and lactation, this role of leptin helps ensure that female mammals do not embark on something as energetically demanding as reproduction without adequate reserves of energy.

Another peptide, which may act as a feedback inhibitor of triacylglycerol synthesis, is the cytokine tumour necrosis factor α (TNF-α). Adipocytes produce several cytokines, including TNF-α and interleukin 6 (Hotamisligil & Spiegelman, 1994). However, arterio–venous difference measurements in human subjects show that whereas interleukin 6 is secreted, TNF-α is not, suggesting a paracrine or autocrine role (Mohamed-Ali *et al*. 1997). TNF-α has a number of effects on adipocytes, including a decrease in lipoprotein lipase activity, glucose transport and adipsin secretion (Hotamisligil & Spiegelman, 1994). Interest in adipocyte TNF-α has arisen because levels increase during obesity and induce insulin resistance in adipocytes, at least in part by impairing activation of insulin receptor kinase (*EC* 2.7.1.112) activity (Hotamisligil & Spiegelman, 1994). Thus, TNF-α has been implicated in the non-insulin-dependent diabetes which develops in obesity, although this idea has been challenged recently (Schreyer *et al*. 1998). However, since TNF-α production increases as adipocytes enlarge and impairs various systems involved in triacylglycerol synthesis in adipocytes, it is arguably acting as a feedback inhibitor of triacylglycerol synthesis, thus having a physiological rather than a pathological role. Thus, while leptin may act as a primary modulator at normal levels of adiposity, TNF-α may act as an additional constraint on triacylglycerol accumulation in adipocytes as animals begin to become obese (Fig. 3). For both TNF-α and leptin there is still a need to determine the mechanism by which changes in the amount of triacylglycerol in adipocytes result in changes in the production of these key regulating peptides.

Homeorhesis

Lipogenesis is subject to chronic homeorhetic control (Baumann & Currie, 1980); i.e. lipogenic activity of different tissues is modulated to meet the changing needs of different physiological states. Lipogenesis in adipocytes is especially subject to this type of control. In young growing animals skeletal muscle development takes precedence, and accumulation of lipid in adipose tissue is restrained; as muscle growth diminishes, animals divert nutrients into lipogenesis in adipose tissue and so fatten (Bergen, 1974). The mechanisms regulating this partitioning of nutrients in growing animals are not well understood. The reproductive cycle also has considerable impact on lipogenesis in various tissues, and lactation provides what is probably the best of all examples of homeorhesis, with a massive rise in lipogenesis in the mammary gland and a concomitant fall in lipogenesis in adipose tissue (Vernon, 1996; Barber *et al*. 1997). These adaptations arise through tissue-specific changes in the expression of major lipogenic enzyme genes, and also changes in activation states of enzymes such as acetyl-CoA carboxylase. The factors and mechanisms responsible have been only partly resolved and involve tissue-specific differences in responsiveness to key hormones. Thus, prolactin increases lipogenesis in mammary tissue (but has no effect on adipocytes, which lack prolactin receptors), whereas growth hormone decreases lipogenesis in adipocytes (but has no effect on mammary epithelial cells, which lack meaningful numbers of growth hormone receptors; Vernon, 1996; Barber *et al*. 1997). The molecular mechanisms whereby these two very similar hormones exert their diametrically opposite effects on lipogenesis remain to

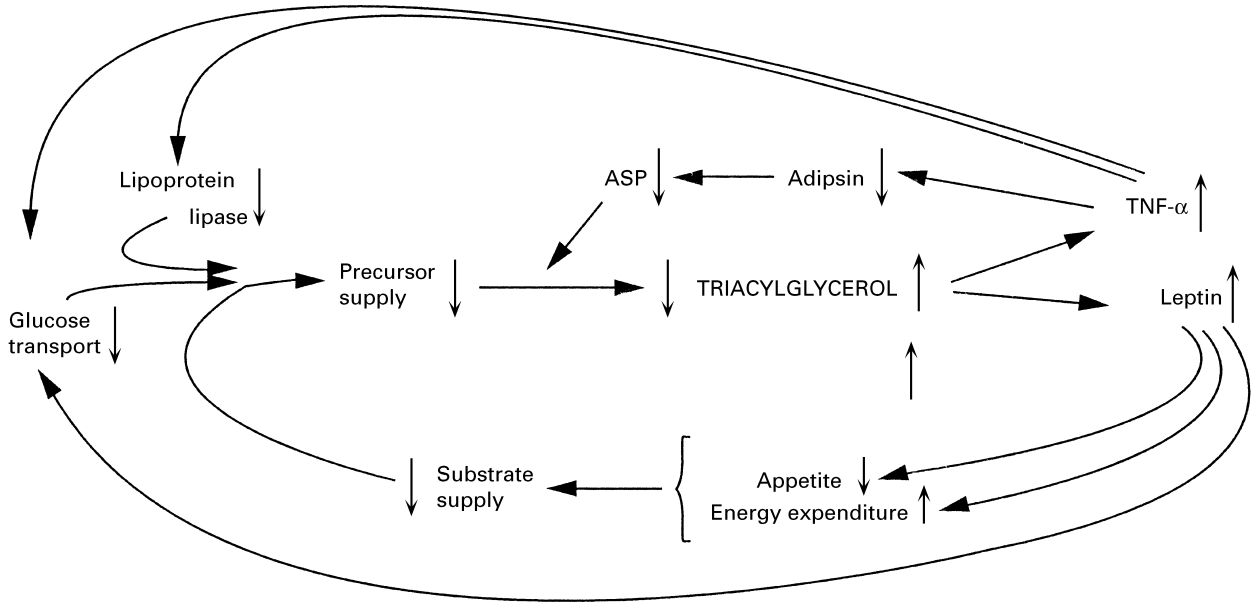

Fig. 3. Mechanisms whereby leptin and tumour necrosis factor α (TNF-α) can act to decrease triacylglycerol synthesis. ASP, acylation-stimulating protein. ↑, ↓, process or substance increased or decreased respectively; lipoprotein lipase, *EC* 3.1.1.34.

be resolved, but appear, in part at least, to involve changes in insulin action. Insulin is a major stimulant of adipose tissue lipogenesis, and in rodents, but probably not ruminants, also increases lipogenesis in mammary epithelial cells (Vernon, 1996; Barber *et al*. 1997). During lactation adipocytes become insulin resistant due to an as yet unidentified impairment of the insulin signal transduction system, downstream of the insulin receptor (Vernon, 1996). In contrast, in rodents the lactating mammary gland appears to be very sensitive to insulin (Burnol *et al*. 1987). Resolving the various molecular mechanisms involved in these homeorhetic adaptations of lipogenesis during lactation is thus another major challenge for future studies in lipogenesis.

There are also seemingly subtle homeorhetic adaptations amongst adipose tissue depots. During fetal development in lambs lipid is preferentially accumulated in perirenal adipocytes rather than subcutaneous adipocytes; indeed, the latter may lose lipid (Alexander, 1978). This process reflects the subsequent role of the perirenal adipose tissue as brown adipose tissue in the neonatal period, but the factors and mechanisms regulating this inter-adipose tissue partitioning of lipid during fetal development have not been resolved. The fetal lamb is perhaps a special case, but as animals fatten more lipid is accumulated in adipocytes of some depots than others. As a result, abdominal adipocytes are usually larger than carcass (subcutaneous, inter- and intramuscular) adipocytes, while pericardial adipocytes are usually relatively small (Pond, 1992; Vernon, 1992). This difference is at least partly due to variations in the expression of lipogenic enzyme genes in different adipose tissue depots (Cousin *et al*. 1993). Recent observations with sheep adipocytes isolated from seven different depots, with adipocytes varying markedly in size, have shown a significant correlation between lipoprotein lipase and ACC mRNA per cell and per adipocyte mean cell volume, independent of depot of origin (MT Travers, MC Barber and RG Vernon, unpublished results). The factors promoting differential gene expression in different depots are not well understood, but glucocorticoids and sex steroid hormones are implicated (Bjorntorp, 1991; Abate & Garg, 1995). In addition, other factors, e.g. differences in blood flow and hence nutrient supply, may also contribute (Vernon, 1992).

Molecular diversity

Developments in molecular biology have allowed detailed studies of enzymes and their genes, which have revealed novel levels of complexity with respect to the regulation of lipogenesis. ACC has been studied in most detail and is described here as an example.

ACC is the major regulatory enzyme of fatty acid biosynthesis (Fig. 1), and is also pivotal in that the product of this reaction malonyl-CoA, as well as being a substrate for fatty acid synthase (*EC* 2.3.1.85), modulates transport of fatty acids into mitochondria for β-oxidation through inhibition of carnitine palmitoyltransferase-I (*EC* 2.3.1.21) (Zammit, 1996; Brown & McGarry, 1997). Thus, ACC represents a point of metabolic control that signals 'conditions of plenty' with the synthesis of fatty acids, and 'conditions of austerity', i.e. starvation, due principally to decreasing flux through ACC and the resulting fall in malonyl-CoA concentration with oxidation of fatty acids that are released predominately from peripheral adipose tissue stores. This duality of function is particularly important for metabolic integration and fuel selection in liver, muscle and the pancreatic β-cell, and derangements of this system are implicated in the development of obesity and type-II diabetes (Prentki & Corkey, 1996). Thus, factors regulating the expression of ACC have provided an important focus, and will do so increasingly in future studies.

ACC enzyme activity results from transcription from two related genes, ACC-α and ACC-β, giving rise to protein products of 265 and 280 kDa respectively. ACC-α is ubiquitous, but its expression is highly inducible in adipose tissue, mammary gland and liver; tissues that account for the majority of whole-body lipogenesis. ACC-α is transcribed from three promoters in a tissue-specific fashion (Fig. 4; Luo *et al*. 1989; Kim & Tae, 1994; Barber & Travers, 1998); promoter 1 upstream of exon 1 and promoter 2 upstream of exon 2 give rise to multiple transcripts due to complex alternate splicing in the 5′ untranslated region that results in the translation of the same ACC-α isozyme; exon 5 is the first coding exon. In rats, sheep and probably human subjects promoter 1 is chiefly restricted to adipose tissue where it is present as the major class of transcript; promoter 2-derived transcripts demonstrate an ubiquitous distribution. Recently a third promoter activity, promoter 3, has been demonstrated in ovine mammary gland (Barber & Travers, 1998), where it comprises 30 % of total ACC-α transcripts and gives rise to a putative ACC-α with an alternate N terminus due to transcription being initiated downstream of exon 5. In promoter 3-derived transcripts, exon 5A is the primary transcribed and coding exon and is spliced onto exon 6, the first common exon with promoter 1- and promoter 2-derived transcripts. It is not yet known whether promoter 3 transcripts are present in species other than sheep.

ACC-β, on the other hand, is expressed predominately in tissues that utilize fatty acids as an energy source, i.e. skeletal muscle and heart. The major difference between ACC-α and ACC-β is in the N terminus (Ha *et al*. 1996; Abu-Elheiga *et al*. 1997). The seventy-four amino acid N-terminal region of ACC-α encoded by exon 5 is replaced by a 218 amino acid domain in ACC-β that accounts for the difference in size between the two ACC. The physiological role of ACC-β is not entirely clear at present. However, the expression of ACC-β in heart and muscle as the principal form of ACC has led to the proposal that ACC-β and its product malonyl-CoA are specifically involved in the regulation of fatty acid oxidation, and thus metabolic fuel selection, in these tissues (Ha *et al*. 1996; Abu-Elheiga *et al*. 1997).

A key question is how the many physiological roles of ACC are related to the observed transcript and isozyme diversity. At present the answer to this question is far from clear. Indeed, the relative functions of ACC-α and ACC-β described previously are merely speculative, especially with regard to the absolute association of ACC-β with the regulation of fatty acid oxidation. On this latter point there is considerable evidence to the contrary. First, there is no apparent increase in the relative abundance of ACC-β compared with ACC-α in the regions of the liver proposed

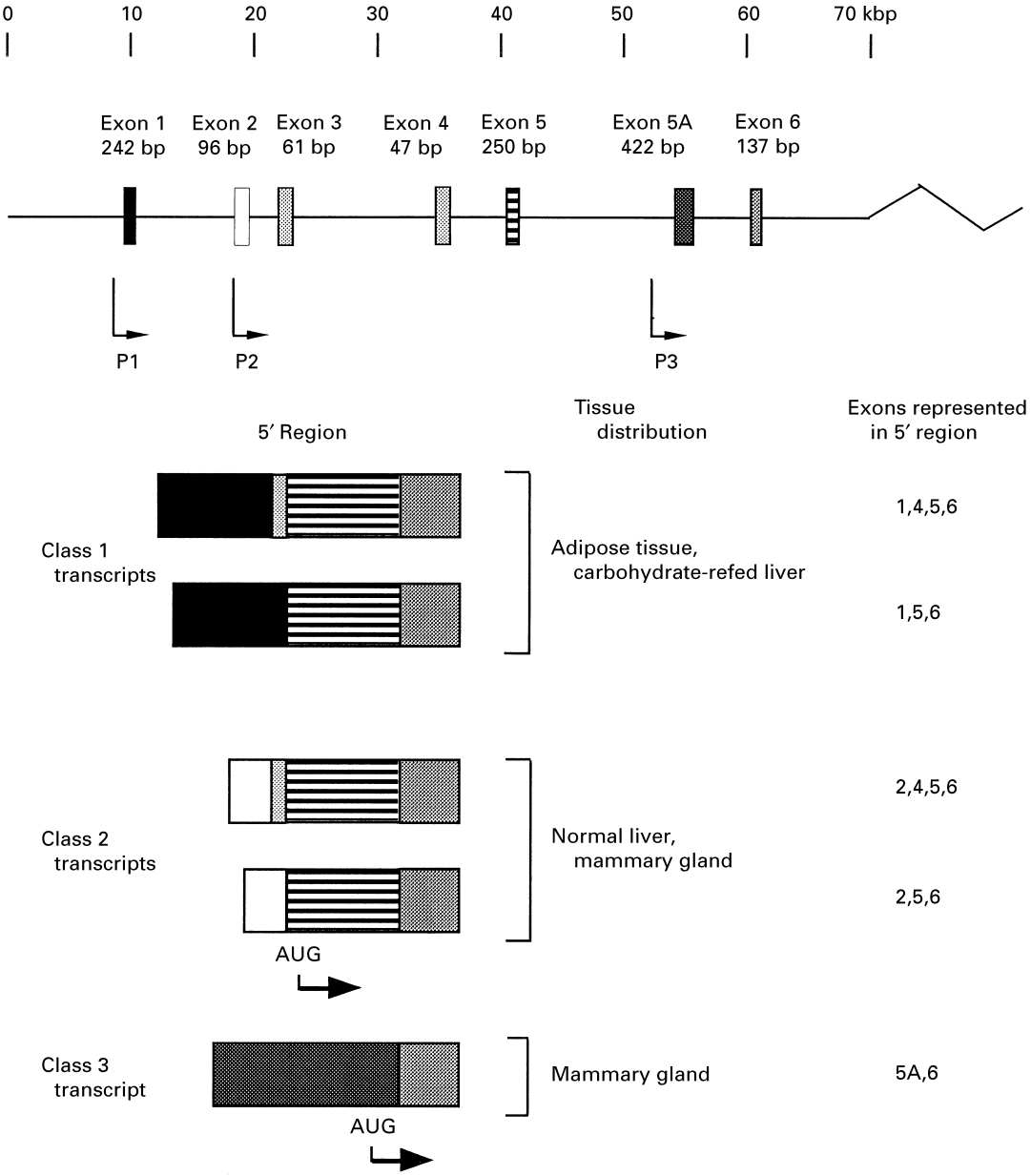

Fig. 4. Structure of the regulatory region of the acetyl-CoA carboxylase α (*EC* 6.4.1.2) gene. P, promoter; AUG, adenine-uracil-guanine (translation initiation codon).

to be adapted to fatty acid oxidation by 'zonation' (Evans *et al*. 1990). However, the liver is exceptional in that, depending on nutritional state, it may be either synthesizing fatty acids or oxidizing them. The high level of malonyl-CoA present when synthesis is high ensures that oxidation is suppressed, thereby avoiding a potential futile cycle. Thus, in the liver ACC-α may have a dual role in this respect. It is possible, however, that ACC-α and ACC-β subunits could target selectively among different compartments within individual cells. However, although some authors (Allred *et al*. 1989) have reported enrichment of ACC-β within mitochondria, others have not (Winz *et al*. 1994). Second, although inhibition of ACC-α expression in INS-1 pancreatic β-cells results in a decreased potential for glucose-induced insulin secretion and an inherent increase in fatty acyl β-oxidation (Zhang & Kim, 1998), similar experiments to inhibit ACC-β expression in H9c2 cardiomyocytes did not result in increased β-oxidation (Kim *et al*. 1997). Instead, differentiation of the cells to form multinucleated fibres and the expression of myosin was impaired. Interestingly, transcription of the ACC-β gene is stimulated by the muscle-specific transcription factor MyoD, which thus suggests a developmental role for ACC-β in the muscle cell phenotype (Kim *et al*. 1997).

Interestingly, major differences between ACC-α, ACC-β and also the N-terminal variant of ACC-α induced in ovine mammary gland are exhibited by their distinct N termini, and thus implies that the N terminus has a crucial role in ACC function. Whether the N terminus of any of these ACC isozymes is involved in subcellular targetting remains to be

established. The junction point at which these ACC isozymes diverge at the N terminus also overlaps with some of the phosphorylation motifs that are important in the control of ACC enzyme activity. Thus, the ACC isozymes may differ in the degree to which they are good substrates for the regulatory kinases or phosphatases. Indeed, it has been demonstrated that ACC-β can be more rapidly phosphorylated by cyclic AMP-dependent protein kinase than ACC-α (Winz *et al*. 1994). Whether this process relates to the phosphorylation motifs proximal to the divergent N termini or to other distinct sites is not known. In this respect the diverse N termini may also exert important regulatory influences over the formation of active ACC polymers, and thus result in homo-polymers with distinct kinetic characteristics. The demonstration that ACC-α and ACC-β may form hetero-polymers in rat liver (Iverson *et al*. 1990) could further provide diversity in ACC kinetic indices. Interestingly, ACC-β does not appear to be expressed to any appreciable extent in adipose tissue, suggesting that the ACC-α isozyme is principally involved in *de novo* lipogenesis. However, ACC-α in this tissue is transcribed from two distinct promoters, resulting in a minimum of four mRNA species, due principally to the inclusion or exclusion of exon 4 in the 5′ untranslated region (Fig. 4). The significance of this transcript diversity is unknown, although it could relate to a distinction between fatty acids synthesized for storage and those synthesized for the replenishment of cell membrane components. Transcript diversity could result in targetted translation in distinct cellular compartments. Clearly, many critical details of the structure, expression and function of these ACC isozymes need to be defined.

Transgenesis

Transgenesis provides a powerful approach for investigating both the control of lipogenesis and also for modulating the process to improve animal performance. However, studies on lipogenesis have been limited and have been confined to laboratory rodents.

Mice have been produced which either lack endogenous lipoprotein lipase (Weinstock *et al*. 1997) or overexpress human lipoprotein lipase (Zsigmond *et al*. 1994; Shimada *et al*. 1995) in their adiopocytes. Surprisingly, neither modification appears to alter adiposity. In mice lacking lipoprotein lipase, fatty acid composition of adipose tissue lipids indicated a compensatory increase in *de novo* fatty acid synthesis (Weinstock *et al*. 1997), while, more curiously, overexpression of lipoprotein lipase was associated with an apparently compensatory increase in expression of hormone-sensitive lipase, which catalyses hydrolysis of triacylglycerol in adipocytes (Shimada *et al*. 1995).

Transgenic animals with different amounts of ACC or fatty acid synthase have not been reported, but production of an adipocyte cell-line in which the amount of ACC was reduced using a ribozyme construct resulted in a decreased rate of fatty acid synthesis (Ha & Kim, 1994). A number of studies have described mice in which the amount of insulin-responsive glucose transporter 4, the glucose translocase of adipocytes and muscle, has been enhanced. Increased expression of glucose transporter 4 in adipocytes increases glucose uptake and utilization in general, with a disproportionately large increase in fatty acid synthesis; insulin sensitivity was also enhanced (Tozzo *et al*. 1995). In addition, adipocyte lipoprotein lipase activity and its response to refeeding was muted (Gnudi *et al*. 1996).

Conclusions

Thus, although lipogenesis has been studied extensively both in animals and human subjects, there are still many questions, especially with respect to regulation, which remain to be resolved. In addition, advances in molecular biology are revealing new complexities and also offer powerful approaches both for investigating the process and for manipulating it to improve animal performance.

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