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Manipulation of adiposity by somatotropin and β-adrenergic agonists: a comparison of their mechanisms of action

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A major objective of Animal Science is to develop feasible strategies to reduce lipid accretion during growth of meat animals. Historically, this objective has been sought because of the importance of reducing the quantity of nutrients used to produce body fat and the need to produce lower-fat meat. A fact of long-standing is that an animal's feed efficiency decreases as the proportion of fat increases in body-weight gain. Thus, reducing fat deposition will improve feed efficiency (i.e. lower the feed:gain ratio) and, hence, increase productive efficiency (gain/feed). A reduction in fat content of fresh meat also will benefit consumers who wish to decrease their intake of saturated fatty acids (SFA). A reduction in total fat intake to less than 30% of energy and SFA intake to less than 10% of energy is advised in the USA because of the positive relationship that exists between the quantity of SFA consumed and an elevation in plasma low-density-lipoprotein-cholesterol (LDL-C) which is a major risk factor for coronary heart disease (for review, see Kris-Etherton *et al.* 1988).

During the past 10 years remarkable progress has been made in identifying feasible and effective strategies to reduce lipid accretion (Etherton & Kensinger, 1984; Boyd & Bauman, 1989; Etherton & Smith, 1991). The two strategies most likely to be used in production agriculture involve administering somtatotropin (ST) or β-adrenergic agonists to meat animals. In the present paper our current understanding of the mechanisms whereby ST and the β-agonists reduce adipose tissue growth will be reviewed.

THE EFFECTS OF ST ON LIPID METABOLISM

Downs (1930) and Bierring & Nielsen (1932) were the first to show that an alkaline extract of the anterior pituitary gland reduced carcass fat in rats. This was verified by Lee & Schaffer (1934) who reported that pair-fed rats injected with a crude alkaline extract of bovine pituitaries not only gained more weight but also contained proportionately more muscle and less fat. The latter paper introduced the concept that the 'growth hormone' of the anterior pituitary gland could affect the quantity of fat in animals. It was not until 1945, however, that growth hormone was isolated from the anterior pituitary (Li *et al.* 1945). This allowed Li *et al.* (1948) to conduct the first experiment to show that ST mimicked the effects the alkaline pituitary extract had on carcass fat in rats. Rats were treated 6 d/week for 437 d with a graded injection regimen increasing from 0·4 mg/d to 2·0 mg/d. Carcass fat was reduced by 47%.

Greenbaum (1953) was the first to suggest that ST stimulated fat catabolism. After 40 years it is still obvious that many scientists believe that a major metabolic effect of ST in

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adipose tissue is to stimulate lipolysis (based on the rat literature). With respect to farm animals, however, it is clear that the metabolic effects of ST on lipid metabolism are dependent on the energy balance of the animal; if animals are in positive energy balance ST decreases lipogenesis and does not appreciably affect lipolysis, whereas if the animal is in negative energy balance the predominant effects of ST are to stimulate lipolysis (see p. 422).

The early studies which showed that preparations of ST could decrease carcass fat of rats prompted a number of studies to evaluate the effects of pituitary preparations of porcine ST (pST) on growth and carcass composition of pigs (Giles, 1942; Turman & Andrews, 1955; Henricson & Ullberg, 1960). These studies were inconclusive, probably because the ST preparations were not pure. Machlin (1972) provided the first evidence that pituitary-derived pST promoted weight gain and reduced backfat thickness in pigs. However, it was not until 1988 that the extent to which pST could reduce carcass fat was established (Evock *et al.* 1988). The delay in establishing the dose–response effects of pST on carcass lipid accretion occurred, in large part, because there was no feasible means of economically purifying sufficient quantities of pituitary-derived pST for industry-wide application. By the late 1970s, however, it became apparent that advances in molecular biology would provide a means to produce large quantities of recombinantly derived proteins. This served as an impetus to initiate studies looking at the effects of highly purified preparations of pST on growth performance of pigs (Chung *et al.* 1985; Etherton *et al.* 1986, 1987*b*). By the late 1980s, additional studies had established the efficacy of recombinant pST (Evock *et al.* 1988; Boyd & Bauman, 1989; McLaren *et al.* 1990) and unequivocally established that pST markedly reduced lipid accretion rates (Table 1). Maximally effective doses of pST can reduce lipid accretion rates and adipose tissue mass by as much as 80%. In addition, maximally effective doses of pST can

Table 1. *Representative effects of porcine somatotropin (pST) on lipid accretion in pigs*

Study	pST dose (μg/kg BW per d)	Duration	Response to dose	
Evock *et al.* (1988)	0,35,70,140	77 d approx. (27–110 kg)	Dose:	Lipid in carcass (%):
			0	34
			35	24
			70	23
			140	11
Boyd & Bauman (1989)	0,30,60,120,200	45–100 kg	Dose:	Lipid deposited (g/d):
			0	300
			30	240
			60	180
			120	100
			200	35
Campbell *et al.* (1989)	0,100	60–100 kg	Dose:	Lipid deposited (g/d):
			0	462
			100	223
Campbell *et al.* (1990)	0,100	60–90 kg	Dose:	Lipid deposited (g/d):
			0	375
			100	165

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Table 2. *Biological effects associated with somatotropin (ST) in domestic animals**

Tissue	Physiological process affected
Skeletal muscle	↑ Protein accretion
	↑ Protein synthesis
	↑ DNA accretion
Adipose tissue	↓ Glucose uptake and glucose oxidation
	↓ Lipid synthesis if in positive energy balance
	↑ Basal lipolysis if in negative energy balance
	↓ Insulin sensitivity
	↓ Insulin stimulation of glucose metabolism and lipid synthesis
	↑ Catecholamine stimulated-lipolysis
	↑ Ability of insulin to inhibit lipolysis
	↓ In glucose transporter protein (GLUT4)†
	↓ Fatty acid synthase‡ and GLUT4 mRNA†
Liver	↑ Glucose output
	↓ Ability of insulin to inhibit gluconeogenesis
Systemic effects	↑ IGF-I and IGFBP-3
	↓ IGFBP-2
	↓ Amino acid oxidation and blood urea nitrogen
	↓ Glucose clearance
	↓ Glucose oxidation
	↓ Response to insulin tolerance test
	↑ FFA oxidation if in negative energy balance

* For reviews, see Peel & Bauman, 1987; Bauman *et al.* 1989; Etherton, 1989*a*,*b*.

† For details of the effects of ST on fatty acid synthase mRNA level, see Mildner & Clarke, 1991 and for details of GLUT4 mRNA and protein, see I. Louveau, M. Coleman, S. Chaudhuri and T. Etherton (unpublished results).

‡ *EC* 2.3.1.85.

IGF-I, insulin-like growth factor-I; IGFBP-3, IGFBP-2, insulin-like growth factor-binding protein-3, -2 respectively; FFA, free fatty acids.

increase average daily gain by as much as 10–20%, improve feed efficiency by 15–30% and increase protein deposition by 50% (for review, see Boyd & Bauman, 1989; Etherton, 1989*a*,*b*).

The remainder of the present discussion about ST focuses on the mechanisms by which ST exerts its biological effects. The objective is to provide an overview of the mechanisms; numerous other reviews address more specific aspects of ST action in domestic animals (Etherton & Walton, 1986; Peel & Bauman, 1987; Bauman *et al.* 1989; Boyd & Bauman, 1989; Etherton, 1989*a*,*b*; Vernon & Flint, 1989).

MECHANISMS OF ST ACTION

It is extraordinary that ST has such dramatic biological effects on lipid metabolism. The precipitous decrease in the rate of lipid accretion graphically illustrates the remarkable changes that occur in adipocyte metabolism. It is important to appreciate that ST not only alters nutrient utilization by the adipocyte but that this change is coordinated with many other diverse physiological processes in different tissues to enable more nutrients to be used for muscle accretion. Research conducted during the past 10 years has established the diversity of the biological effects of ST (see Table 2). ST affects numerous

target tissues in ways that are highly coordinated to effect marked changes in nutrient partitioning among these tissues. Many of the metabolic effects are a direct action of ST, involving a variety of tissues and the metabolism of all nutrient classes, i.e. carbohydrate, lipid, protein and minerals (see Table 2).

These metabolic changes are important because they: (1) establish the rate of lipid accretion and, therefore, the extent to which ST affects carcass composition in a growing animal, (2) play a key role in redirecting nutrients (e.g. glucose), normally destined to be deposited as lipid, to other tissues thereby supporting the nutrient needs for the increased lean tissue accretion during growth and (3) result in improvements in feed efficiency because of the reduction in the proportion of nutrients used for synthesis of body fat.

When animals are in positive energy balance, ST causes a reduction in lipogenic rate whereas the effects on lipolysis are minimal (Etherton & Walton, 1986; Walton & Etherton, 1986; Walton *et al.* 1986, 1987; Sechen *et al.* 1989; Dunshea *et al.* 1992*a*,*b*). This represents the typical situation for growing animals treated with ST, but is also observed for bovine ST (bST) treatment of lactating cows which are in substantial positive energy balance. In contrast, when animals are in negative energy balance rates of lipogenesis are already low and ST treatment affects adipose tissue by increasing rates of lipid mobilization (Machlin, 1972; Eisemenn *et al.* 1986; Bauman *et al.* 1988). This situation typically occurs in early lactation in dairy cows during the first weeks of bST treatment (before the increase in voluntary intake) but is also observed in growing animals when energy intake is restricted.

The ability of pST to reduce lipid accretion in growing pigs is the result of a decrease in insulin sensitivity of the adipocyte resulting in a marked decrease in insulin-regulated events such as glucose transport and lipid synthesis (Walton *et al.* 1987; Magri *et al.* 1990). When porcine adipose tissue is cultured for 48 h in a defined medium, pST decreases the ability of insulin to maintain lipogenic capacity in a dose-dependent manner (Walton & Etherton, 1986; Walton *et al.* 1986, Evock *et al.* 1988). Similar findings have been reported for ovine and bovine adipose tissue (Vernon, 1982; Etherton *et al.* 1987*a*). The precipitous in vitro decline in lipogenesis in response to pST occurs because of a reduction in the activity of several key lipogenic enzymes (Magri *et al.* 1990). A recent in vivo kinetic study with pigs has corroborated these findings. Dunshea *et al.* (1992*b*) determined the rate of glucose incorporation into lipid in subcutaneous adipose tissue before and during a hyperinsulinaemic euglycaemic clamp and found that insulin-stimulated rates of glucose incorporation were significantly lower (approximately 70%) in pigs treated with pST for 10 d and that the reduction in the biological effects of insulin were the result of an impairment in insulin sensitivity. The decrease in insulin sensitivity does not appear to be the result of an impairment in insulin-binding or insulin-receptor tyrosine kinase (*EC* 2.7.1.112) activity in porcine adipocytes since pST treatment does not alter either (Magri *et al.* 1990).

It is important to appreciate that the biological effects of ST are chronic rather than acute. The effects of ST are not observed in short-term (2 h) incubations with adipose tissue but only become apparent after 24 h (Walton & Etherton, 1986, 1987; Walton *et al.* 1986). This suggests that ST acts to inhibit nutrient utilization in adipose tissue by changing the mass of glucose transporter proteins or key lipogenic enzymes, or both, either by transcriptional or post-transcriptional regulation. Recent evidence provides support for this hypothesis. Mildner & Clarke (1991) have shown that pST decreases

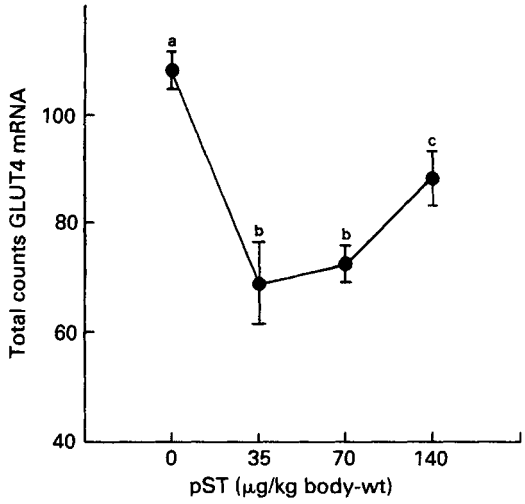

Fig. 1. Slot blot analysis of total RNA from adipose tissue from pigs treated with different doses of porcine somatotropin (pST) (n = 5 per dose). [a,b,c] Means with different superscript letters were significantly different (P<0·05). Gilts were treated daily for 7 d with 0, 35, 70 or 140 μg recombinant pST/kg body-weight. Adipose tissue was removed and RNA extracted (Louveau *et al.* 1991). Human glucose transporter protein (GLUT4) cDNA was nick-translated and used for quantification of GLUT4 mRNA.

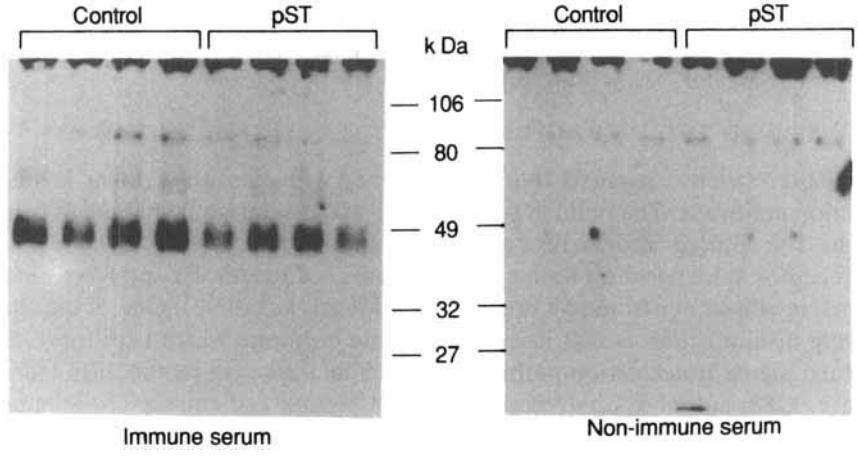

Fig. 2. Immunoblot of glucose transporter (GLUT4) protein in adipose tissue from control and porcine somatotropin (pST)-treated pigs. A total membrane protein (50 μg) from adipose tissue was submitted to SDS-PAGE, transferred to nitrocellulose and analysed by immunoblot using an immune serum against (a) GLUT4 or (b) a non-immune serum (normal rabbit serum). The membrane was then submitted to autoradiography for 10 h at −70°. Immunolabelled bands were visualized with ^{125}I-labelled protein A.

fatty acid synthase mRNA levels by 75% in pig adipose tissue. Furthermore, when pigs are treated with pST for 7 d there is a 20–40% decrease in glucose transporter (GLUT4) mRNA in adipose tissue (Fig. 1); the decrease in GLUT4 mRNA levels is associated with a 40% decrease in GLUT4 protein (Fig. 2).

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The reduction of GLUT4 by pST is important because in rat adipocytes 90% of the cell glucose transporters are GLUT4 and the acute stimulation of glucose transport by insulin is due to the translocation of GLUT4 from an intracellular pool to the plasma membrane (Birnbaum, 1989; Fukumoto *et al.* 1989; James *et al.* 1989). Although it is not known whether pST affects the ability of insulin to stimulate GLUT4 translocation in porcine adipocytes, this is likely since pST inhibits the stimulatory effects of insulin on glucose transport (Magri *et al.* 1990). The fact that fatty acid synthase and GLUT4 mRNA levels are reduced by pST provides direct evidence to promote the hypothesis that pST can regulate adipocyte metabolism by altering transcription of key metabolic genes.

The steady-state level of a particular mRNA represents the balance of synthesis, nuclear processing and degradation, transport to the cytosol and cytosolic degradation (O'Brien & Granner, 1991). Most attention has been given to studying how a hormone influences the transcription of specific genes. With respect to transcriptional regulation of the GLUT4 gene in adipocytes, we are unaware of any study that has used run-on transcription assays to establish that ST, in fact, regulates mRNA abundance at this level. Because normal levels of expression of GLUT4 appear to be insulin-dependent in rat adipose tissue (Charron & Kahn, 1990; Cusin *et al.* 1990; Sivitz *et al.* 1990), it is likely that the effect of pST in porcine adipose tissue is to antagonize the insulin-dependent expression of GLUT4. However, it is also possible that pST acts directly (e.g. via ST response elements in the GLUT4 gene). With respect to this possible explanation, an ST response element 5′ of the serine protease inhibitor (Spi) 2.1 gene has recently been identified in rat liver (Yoon *et al.* 1990). The fact that pST reduces insulin sensitivity of adipose tissue, however, is compelling evidence to support the likelihood that the intracellular ST signal pathway impedes the insulin signal pathway(s) leading to a diminution in transcription of insulin-regulated genes.

ST AND THE INTRACELLULAR INSULIN SIGNAL PATHWAYS

The available evidence suggests that ST interferes with the intracellular insulin signal transduction pathways. The point at which the ST and insulin signal pathways converge is unknown. The limited information available implies that the effects are distal to the insulin receptor since insulin binding and activation of insulin-receptor tyrosine kinase are normal in adipocytes from pST-treated pigs (Magri *et al.* 1990). One of the challenges in studying insulin action is that it is a pleiotropic hormone which uses more than one intracellular signal transduction pathway to regulate adipocyte metabolism (for review, see Farese, 1988; Saltiel & Cuatrecasas, 1988). A second and equally challenging task is that relatively little is known about the insulin signal pathway(s) of adipocytes from domestic animals *v.* rat adipocytes. To experimentally test the hypothesis that ST antagonizes the insulin signal pathway(s) it is obligatory that a working model be established for adipocytes from domestic animals that define these intracellular signal transduction pathways for insulin. This will be a challenge because of the magnitude of research required and the probability that some of the information from studies with rat adipocytes may not be applicable to porcine or bovine adipocytes.

The difference in insulin responsiveness of rat and pig adipocytes illustrates the potential problems that may exist in extrapolating results from rat studies to experiments conducted with farm animals. For example, fatty acid synthesis and glucose transport are much more responsive to insulin in rat than pig adipocytes. This point is illustrated by the

observation that 3-O-methyl D-glucose transport activity in rat adipocytes is usually stimulated twentyfold or more by insulin (Glieman & Rees, 1983). This is a much greater increase than observed for pig adipocytes (approximately 80%; Magri *et al.* 1990). Although the reasons for this difference are not clear, it appears that basal (or constitutive) rates of glucose transport are much lower in rat adipocytes. For example, when insulin-stimulated glucose transport rate in rat adipocytes (Simpson & Cushman, 1986; Lawrence *et al.* 1990) is compared with that of pig adipocytes the maximal rate is quite similar (approximately 70–80 pmol/s per 10^6 cells; Magri *et al.* 1990). In contrast, there are extraordinary differences in basal transport rate (rat approximately 1–2 pmol/s per 10^6 cells *v.* pig approximately 30–40 pmol/s per 10^6 cells). Although it is difficult to make inter-species comparisons, it is not unreasonable to speculate that fundamental differences may exist in the mechanisms by which insulin stimulates glucose uptake and metabolism in pig adipocytes given their high rate of basal glucose uptake and the fact that in vivo adipose tissue accounts for 25–40% of the glucose cleared in the pig (Dunshea *et al.* 1992*b*).

SOMATOTROPIN SIGNALLING PATHWAYS

The identity of the ST signal transduction pathway(s) and where they converge with the insulin signal pathways is obscure. An early event in the ST signal pathway appears to be phosphorylation of the ST receptor (Foster *et al.* 1988) and evidence is available to suggest that ST receptors are associated with tyrosine kinase activity in a variety of cell types (Stred *et al.* 1990). This observation is of potential significance since no tyrosine kinase domain has been identified in the ST receptor. Stubbart *et al.* (1991) have suggested that the ST receptor is part of a complex containing a distinct tyrosine kinase that is activated by ST and is capable of phosphorylating the ST receptor.

Recently, Roupas *et al.* (1991) have reported that ST interferes with the ability of a guanine nucleotide-binding protein (G protein) to mediate the activation of phosphatidylinositol phospholipase C (*EC* 3.1.4.10; PI-PLC) by insulin in mouse adipocytes. This observation suggests that the G proteins may play a role in the ST signal pathways. There is some evidence to implicate protein kinase C (PKC) in the ST signal pathway; however, this information is contradictory. It has been shown that ST stimulates the synthesis of diacylglycerol (DAG) in rat hepatocytes (Johnson *et al.* 1990) which suggests that PKC might be activated; however, PKC activity was not determined. Smal & De Meyts (1987) suggested that down-regulation of PKC reduced the insulin-like effects of hST. The significance of this observation is unclear because in vivo pST does not act in an insulin-like manner in adipose tissue and, as discussed previously, is a potent counter-regulatory hormone to insulin. Doglio *et al.* (1989) reported that human ST stimulates the expression of c-*fos* in pre-adipose Ob1771 cells by increasing DAG without any concomitant detectable effects on phosphatidylinositol turnover. A similar response has been noted by Rogers & Hammerman (1989) who found that ST increased both DAG and inositol triphosphate levels in proximal tubular basolateral kidney membranes which suggests that ST action could involve a PKC signal pathway. Recently, evidence has been published which indicates that activation of PKC is part of the ST signal pathway that leads to induction of c-*fos* (Slootweg *et al.* 1991). Moreover, Tollet *et al.* (1991) have shown that PKC is involved in the ST-dependent induction of insulin-like growth factor I mRNA in rat hepatocytes. It remains unclear, however, whether PKC is involved in the

ST signal pathway in adipocytes. For this to occur, a model of ST signal transduction has to be developed which is consistent with the observation that both the insulin and ST signal pathways appear to involve activation of PKC (for review, see Farese, 1988). This is clearly a paradox because ST cannot antagonize insulin action at this point when both hormones have been proposed to stimulate PKC activity.

The observation that the ST-binding protein (STBP; the extracellular domain of the transmembrane ST receptor) is present in nuclei of rats and rabbits (Lobie *et al.* 1991) has been interpreted to suggest that the ST–STBP complex acts directly or indirectly to regulate transcription. The fact the STBP has been identified in the nucleus is consistent with the temporal pattern of ST action; however, it is premature to draw any conclusions about whether this is an important component of the ST signal pathway.

Despite the advances made in our understanding of the mechanisms by which ST alters lipid metabolism, little is known about the intracellular ST signal pathways that alter lipid metabolism in adipose tissue. Because ST is a pleiotropic hormone it is reasonable to presume that there is a variety of ST mediators involved in the intracellular signal transduction pathway(s) and these may vary in a manner dependent on the physiological state of the animal. The nature of these signal pathways and how they converge with the insulin signal pathways in adipocytes to antagonize the biological effects of insulin remains obscure.

MECHANISMS OF β-ADRENERGIC AGONIST

In addition to ST, our understanding of how adipose tissue accretion is regulated has been facilitated by the discovery that β-adrenergic agonists (specifically, clenbuterol, cimaterol and ractopamine) can reduce carcass fat of growing meat animals (for review, see Yang & McElligott, 1989; Etherton & Smith, 1991; Moloney *et al.* 1991). It is important to appreciate, however, that the literature on the mechanisms by which β-adrenergic agonists affect adipose tissue is less than for ST. In addition, it must be emphasized that the β-adrenergic agonists represent a family of different drugs with different structures and different pharmacokinetics. Finally, the magnitude of reduction in carcass fat by the β-adrenergic agonists varies among species (Table 3). In general, the

Table 3. *Comparative responses (%) of meat animals to β-adrenergic agonists (adapted from Etherton & Smith, 1991)*

Agonist	ADG	F/G	Protein (or LEA)	Fat
Clenbuterol				
Sheep	+10	+14	+14	−20
Cimaterol				
Sheep	+22	+15	+30	−40
Pigs	+2	+5	+7	−10
Cattle	+30	+30	+41	−26
Ractopamine				
Pigs	+9	+12	+15	−14

ADG, average daily gain; F/G, feed/gain; LEA, loin eye area.

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reduction in carcass fat is less for pigs than for ruminants (Etherton & Smith, 1991). With respect to pigs, it is also clear that pST is more efficacious in reducing carcass lipid than are the β-adrenergic agonists.

β-adrenergic agonists structurally resemble catecholamines. The synthetic β-adrenergic agonists act in a manner similar to naturally-occurring catecholamines. They elicit their biological responses by binding to β-adrenergic receptors present in target cells. The subsequent stimulation of cyclic-AMP (cAMP) production may lead to induction or inhibition of a variety of cellular processes. There is evidence which indicates that β-adrenergic agonists reduce lipid deposition by stimulating lipolysis. In vivo studies have shown that free fatty acid concentration is increased in cattle and sheep which suggests that lipolysis is elevated (Beermann *et al.* 1987; Eisemann *et al.* 1988). Results from in vitro studies, however, are equivocal. Clenbuterol has been shown to stimulate lipolysis in rat (Duquette & Muir, 1985) and chicken adipose tissue (Campbell & Scanes, 1985); however, this effect has not been observed with pig (Rule *et al.* 1987) or bovine adipose tissue (Miller *et al.* 1988). In vitro studies with cimaterol and ractopamine have demonstrated a stimulatory effect on lipolysis in pig adipose tissue (Liu *et al.* 1989; Peterla & Scanes, 1990). There is also some evidence which indicates that β-adrenergic agonists decrease lipogenesis (Mersmann, 1989; Mills & Liu, 1990; Peterla & Scanes, 1990). To increase our understanding of the mechanisms by which β-adrenergic agonists affect lipid metabolism it will be necessary to conduct in vivo kinetic studies to determine the effects that the different agonists have on lipogenesis and lipolysis.

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