DEVELOPMENTAL PATTERNS OF PLASMA INSULIN-LIKE GROWTH FACTOR-I IN SHEEP

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ANCOVA	analysis of covariance
CV	coefficient of variation
°C	degrees Celsius
FEC	faecal egg counts
g	gravitational force
hr	hour
IGF	insulin-like growth factor
kDa	kilo Dalton
kg	kilogram
MANOVA	multivariate analysis of variance
ml	millilitre
NEFA	non-esterified fatty acid(s)
ng	nanogram
OMD	organic matter digestibility
RFLP	restriction fragment length polymorphism
S.E.M.	standard error about the mean
ST	somatotropin

C H A P T E R I

INTRODUCTION

Livestock Breeding Programmes and the Role of Genetic Markers

The improvement of livestock performance through selection and controlled breeding has long been of interest to farmers. Gains in performance and efficiency achieved through selection programmes are permanent and cumulative. However, selection objectives may vary over time according to changing markets and management circumstances.

While the organisation of breeding programmes varies greatly in different livestock industries and in different countries, all rely on the use of phenotypic measurements to predict genetic merit. These measurements may be derived from the animal itself, from its ancestors or siblings, or from its progeny. In New Zealand, selection for improved lean meat, wool and milk production is generally accomplished as follows:

(a) Lean meat production. In meat-producing animals such as sheep and beef cattle, a high rate of lean tissue growth is considered desirable. Selection for increased growth rate may be based on the animal's own average daily gain since the trait is expressed in both sexes prior to first mating. Information on the performance of

relatives (ancestors and siblings) can be used to more accurately estimate genetic merit but progeny testing is used infrequently (compared, for example, with selection for lactational performance in the dairy industry). Composition of growth is more difficult to assess, particularly in a non-destructive fashion. The most effective means of non-destructive composition assessment is by use of ultrasound but this is restricted to measurement of peripheral tissues (e.g. subcutaneous fat).

(b) Wool production. Fleece weight is the main trait of interest to producers of crossbred wool. As lamb fleece weight is confounded with maternal effects, exhibits low heritability, and has a low genetic correlation with later fleece weights, selection in both sexes is generally based on hogget (yearling) fleece weight. In addition, breeders may also assess fleece characteristics which affect the value of the wool per unit weight (e.g. fibre diameter, yield, colour) in the hogget fleeces.

(c) Milk production. The most common form of selection used by New Zealand dairy farmers is based on milk production and/or milk components (i.e. fat and protein). The main problems associated with selection for lactational performance are that it is not expressed in males and cannot be measured in females until after reproductive maturity. To further complicate the issue males have a large influence on genetic gain, especially where extensive use of a few superior bulls (e.g. by artificial insemination (AI)) can result in a high selection differential. As a result, New Zealand and other

countries conduct large scale progeny testing schemes to determine the genetic potential of prospective AI sires.

Limitations of Current Breeding Programmes. Associated with these methods of selection are a number of problems or limitations. In some cases, methods of estimating the genetic merit (e.g. by ultrasound backfat thickness) are poorly correlated with the actual trait of interest (e.g. lean tissue growth). Furthermore, the traits of interest often have only a low to moderate heritability due to the influence of a variety of environmental factors. Another factor which should be considered is whether phenotypic merit is confounded by maternal effects (e.g. high weaning weights may be the result of high milking potential of the dam rather than an expression of an individual's true genetic potential for growth). Selecting animals at an older age alleviates problems associated with the maternal effects and may result in increased accuracy of selection. However, it also increases the generation interval, thereby reducing the potential rate of genetic improvement. Similarly, progeny testing is a valuable tool, especially for the dairy industry, but it is expensive and also creates long generation intervals which reduce the benefit of increased accuracy of selection. All of these situations hamper the rate of selection gain.

Genetic Markers. Because of the limitations in current methods of selection, researchers have been looking for markers of genetic merit for the various production traits of economic importance in farm animals. Genetic markers can be defined as characteristics of an animal, other than its own production, which may be used to predict

genetic merit for production traits. The idea of using genetic markers to select young animals has been around for centuries (e.g. selection based on conformation or udder size in meat-producing or dairy animals respectively). However, improved understanding of metabolism of superior animals and advances in genetic engineering have resulted in research to identify new genetic markers. As well as having a high genetic correlation with the production trait of interest an effective marker must be easily and inexpensively measured (preferably in the field), have a high degree of repeatability and should not be negatively correlated to other traits of economic importance.

Use of genetic markers could provide more accurate predictions of genotype for desired traits, whether the desired traits were expressed in the individual or not. The generation interval in some programmes could be reduced provided the marker was expressed in young animals. Expression of the markers in young animals would also allow farmers to cull inferior animals at an earlier age and thereby reduce overhead costs. Furthermore, effectiveness of selection would be increased as it would be possible to screen a larger number of animals. All of these changes would potentially increase the rate of genetic gain and result in financial benefits to animal industries.

Current areas of investigation for possible genetic markers include the following:

(a) Enzyme polymorphisms and enzyme activity in tissues. One approach to developing genetic markers is to investigate differences

in the form or function of key enzymes from genetically divergent lines. For example, differences have been observed between lean and obese pig lines in the activity of lipolytic enzymes in adipose tissue (Standal et al, 1973). Divergent selection for activity of NADPH-generating enzymes has been shown to result in marked divergence in adipose tissue growth, although the rate of divergence did not exceed that achieved by selection on ultrasound backfat thickness (Rogdakis, 1982; Rothfuss et al, 1984; Kalbitz and Mueller, 1988).

Different forms of enzymes (enzyme polymorphisms) may occur within a population because the genes responsible for their production may differ slightly between animals . Harris (1980) identified polymorphic forms of some enzymes which have an important function controlling metabolism in humans and animals. Although a particular enzyme may exist in different forms, that does not necessarily mean that the forms have different biological activity, or that they will be useful genetic markers. Nevertheless, as indicated by the results of Rogdakis (1982), enzyme activities may be promising genetic markers provided that tissues can be sampled from animals without detriment to their breeding ability (i.e. non-destructive sampling).

(b) Restriction fragment length polymorphisms (RFLPs). Soller and Beckmann (1985) have reviewed the possible use of RFLPs for improvement of animal selection. RFLPs are genetic polymorphisms formed by the use of restriction enzymes to cleave DNA into fragments. These fragments can potentially be used to map chromosomes, assist in the identification of strain or parentage and, perhaps most importantly, to help search for genetic variation in economic trait

loci. This means that in the future it may be possible to examine genes related to economically important traits and determine the genetic differences between superior and inferior animals. However, the technique is currently not commercially viable because it is not known which genes influence production and because of the very considerable economic resources required to screen for useful RFLPs.

(c) Metabolic parameters. The majority of the current work related to genetic markers is involved with variation in metabolic parameters between divergently selected lines or breeds, progeny tested animals or animals with a major gene compared to those without. Parameters of interest are generally either baseline concentrations of hormones/metabolites or their responses to metabolic challenges (e.g. fasting or acute exogenous hormone/metabolite infusion). The philosophy behind this approach is that genetically superior animals are likely to express variation in the control mechanisms regulating production (e.g. hormones which regulate meat, wool and milk production) or in the consequences of this regulation (e.g. differences in circulating metabolite levels or in the partitioning of nutrients between productive tissues). This variation in control mechanisms, or in the consequences of metabolic regulation, may also be expressed in young animals and so be potentially useful as a genetic marker.

Components of the Somatotropic Axis as Genetic Markers

Attempts to identify genetic markers based on metabolic parameters have involved studies of a range of hormones and metabolites in

genetically divergent groups of animals. One of the most promising areas of research involves components of the somatotropic axis. This is a logical area for study because the somatotropic axis is known to have a key role in regulating productive processes.

With respect to growth, it has been known for many years that removal of the pituitary gland (hypophysectomy) retards or prevents growth in ruminants. This could be because of the loss of any one of a number of hormones secreted by the pituitary gland. However, replacement of somatotropin (ST), also known as growth hormone, restores growth (Olson et al, 1981). Furthermore, administration of exogenous ST to intact animals has been shown to enhance growth and improve feed conversion efficiency (i.e. increased weight gain/feed consumed). In addition, carcass quality is improved through increased nitrogen retention and protein synthesis (which results in increased lean tissue deposition) and decreased amounts of carcass fat (see reviews by Bauman and McCutcheon, 1986; McBride et al, 1988).

Hypophysectomy in sheep results in follicular atrophy and cessation of fibre production. ST replacement on its own does not restore normal fibre growth, indicating that other pituitary hormones are also involved (Ferguson et al, 1965). The relationship between plasma ST and wool is not clear. Direct intradermal injections of ST did not affect local wool growth, leading Downes and Wallace (1965) to conclude that it was unlikely that ST had any direct effect on wool production. However, the discovery of the insulin-like growth factors, which mediate many of the effects of ST (see below), now

makes this conclusion questionable. Wheatley et al (1966) observed that while nitrogen retention was increased, wool growth was actually decreased, in response to exogenous ST. There was, however, a prolonged period of compensatory growth after injections ceased. These results agree with those of Wynn et al (1988). However, others (Holcombe et al, 1988) found that short-term (30 days) ST treatment had no effect on wool growth in fine-wool ewes while Johnsson et al (1985) found that 12 weeks of ST treatment increased greasy fleece weight.

Studies involving lactating animals have shown that hypophysectomy during lactation reduces milk production. Restoration of milk yield to pre-hypophysectomised levels can be achieved by the combined administration of several hormones, including ST (Cowie, 1969). As with fibre production, ST replacement on its own will not restore milk yield to normal levels. However, once lactation has been re-established by hormone replacement therapy, milk secretion is severely hampered if ST treatment is withdrawn (Cowie, 1969). In addition, positive responses in milk production of intact dairy cows treated with exogenous ST have been recognized for several decades. During the last decade short-term studies evaluating the galactopoietic potential of ST have reported increased milk production ranging from 10 to 40% (see reviews by Johnsson and Hart, 1986; Peel and Bauman, 1987; McBride et al, 1988). Recent long-term studies have also shown positive responses in milk yield to exogenous ST (Elvinger et al, 1988; Soderholm et al, 1988; McCutcheon et al, 1989).

Given these associations between ST and productive traits, many groups have examined the possible use of ST levels as a genetic marker. This has been done initially by attempting to identify differences in ST levels between lines genetically divergent in growth or milk production.

The success of these attempts to identify differences between divergent lines has been limited. Some of the studies of divergent meat-producing lines have identified higher levels of ST in lean, fast-growing animals (Ringberg Lund-Larsen and Bakke, 1975; Althen and Gerrits, 1976; Wangsness et al, 1977; Dodson et al, 1983; Carter et al, 1989; Morgan, 1989). However, others have found no significant difference in ST between divergently selected lines (Van Maanen et al, 1989). Similarly, some studies have identified higher plasma ST levels in cows, bulls or calves of superior genetic merit for milk production (Barnes et al, 1985; Kazmer et al, 1986; Mackenzie et al, 1988; Sartin et al, 1988; Xing et al, 1988) but others have not (Land et al, 1983; Tucker et al, 1974).

The differences in these results may possibly be explained by two characteristics of ST. First, somatotropin is secreted from the pituitary gland in a pulsatile fashion and appears in plasma as a series of episodic pulses or spikes (Klindt et al, 1985). Furthermore, the effects which ST has on metabolism are functions of the pulse frequency and amplitude as well as the average baseline concentration. The combination of all these factors means that an intensive blood sampling scheme is required to accurately determine the ST concentration. Such blood sampling schemes require the use of

indwelling catheters and could not readily be accomplished in the field. Second, ST levels in the blood increase in underfed animals. Therefore, if genetically divergent lines are compared at unequal energy balances, differences in circulating levels of ST (and other hormones) between the lines will be confounded with differences in energy status. For example, Hart et al (1978) found that when lactating Friesian and Hereford-cross cows were fed equal amounts, the cows of superior genetic merit (Friesians) had higher circulating levels of ST. However, when the experiment was repeated with cows fed to equal energy balance, no difference was apparent between the groups in circulating ST (Hart, 1983).

The pulsatile secretion of ST and its dependence on nutritional status therefore limit its usefulness as a genetic marker. However, research has indicated that some, if not all, of the actions of ST may be mediated by the insulin-like growth factors (IGFs). As will be discussed later the IGFs may be more promising genetic markers than is ST.

Discovery and Structure of Insulin-Like Growth Factors (IGFs)

The insulin-like growth factors (IGFs) are a family of peptides, structurally resembling insulin, with insulin-like and growth-promoting activities. They were originally discovered through two independent lines of research. First, Salmon and Daughaday (1957) reported that the somatic growth-promoting actions of ST were mediated by a serum factor. As a result of their ability to incorporate ^{35}S into incubated cartilage, this family of peptides was originally

termed "sulfation factors". After further anabolic activities of these factors became apparent the family was renamed "somatomedins" (Sm) (Daughaday et al, 1972), with Sm-A and Sm-C being the two main factors.

In what was then thought to be unrelated work, Froesch et al (1963) observed a non-suppressible insulin-like activity (NSILA) in serum. Upon purification of two NSILA polypeptides, Rinderknecht and Humbel (1976a,b) renamed the peptides insulin-like growth factor-I and -II (IGF-I and IGF-II) because of their apparent structural and functional relationship to insulin.

Studies comparing the Sms and IGFs revealed that Sm-C was identical to IGF-I (Klapper et al, 1983) and that Sm-A was a deaminated form of IGF-I (Enberg et al, 1984). In an effort to unify the Sm/IGF concepts the terms IGF and Sm became synonymous in 1986 and, to prevent further confusion, Daughaday and his colleagues recommended that the growth factors be referred to as IGF-I and IGF-II (see Daughaday et al, 1987).

IGF-I and IGF-II are both single chain polypeptides which display 60% homology with each other. They are also highly homologous with human proinsulin. IGFs have A and B domains which correspond with the A and B insulin chains and are joined by two disulphide bridges. The IGFs have a connecting peptide region corresponding to the C-peptide in proinsulin but differing in amino acid sequence. Unlike proinsulin, the IGFs have a short carboxy-terminal extension on the A-domain, termed the D-peptide. IGF-I is a basic peptide with 70 amino

acids and a molecular mass of 7.65 kDa. IGF-II is a neutral peptide with 67 amino acids and a molecular mass of 7.47 kDa (for further details see Rinderknecht and Humbel, 1978a,b).

Although current evidence suggests that both IGF-I and IGF-II may be important growth components in the somatotropic axis little is known about the role of IGF-II, especially in post-natal growth, or the factors which may affect its level in circulation. In addition, very few laboratories have the capability for extensive radioimmuno-assay of IGF-II. Conversely, and as will be discussed in detail later, there is evidence which supports the potential use of IGF-I as a genetic marker for growth. For these reasons, IGF-I will be the focus of this study.

Production of IGF-I

IGF-I was initially thought to act only in an endocrine manner, being synthesized and released (primarily by the liver) in response to ST (Daughaday et al, 1976). However, it is now apparent that IGF-I is synthesized in many, if not all, tissues (D'Ercole et al, 1984) and may therefore act locally at or near the site of synthesis (i.e. in a paracrine or autocrine fashion). The relative importance of these mechanisms is as yet unknown. To date most studies have centred on circulating concentrations of IGF-I and the results of these studies indicate a positive, but not necessarily causative, relationship between IGF-I and growth (see later).

The liver is the primary source of plasma IGF-I with at least 55% of the hormone found in circulation being synthesized in this organ (D'Ercole et al, 1984). Production and secretion of IGF-I into circulation is dependent upon ST secretion, generally being elevated in states of ST hypersecretion and often almost undetectable in total ST deficiency. The administration of ST to dairy cows (Davis et al, 1987), steers (Breier et al, 1988b) and sheep (Pell et al, 1987) result in large increases in plasma IGF-I concentrations. As with many other endocrine systems, the dose-response relationship between ST and IGF-I is curvilinear (Clemmons et al, 1981a). The regulatory effects of ST on circulating IGF-I concentrations appear to be mediated by the hepatic somatogenic receptors. ST binds to the hepatic receptors which in turn stimulate the target cells causing de novo IGF synthesis to occur from amino acids. Breier et al (1988a) found two independent classes (high- and low-affinity) of ST binding sites on hepatic membranes in young steers. The presence of a high-affinity site correlated with greater weight gain, with elevated plasma IGF-I levels and with IGF-I response to exogenous ST (Breier et al, 1988a). Because of these and other findings they proposed an active role for high-affinity somatogenic receptors in regulating the state of the somatotropic axis and postnatal growth in ruminants (Breier et al, 1988a). The significance of the low affinity site is not clear. As will be discussed later, some forms of abnormal growth may be the result of a deficiency in, or some problem with, the hepatic receptors. In such cases, individuals have normal levels of ST but the ST is unable to bind to the hepatic receptors to stimulate IGF-I synthesis, thereby resulting in low plasma IGF-I concentrations.

In some cases, the relationship between IGF-I and ST is negative. For example, Ronge et al (1988) found that concentrations of ST increased, while levels of IGF-I decreased, during peak lactation in dairy cows. The alterations which occur in the endocrine system during this period probably create an increased partitioning of nutrients to the mammary gland, thereby increasing milk yield and resulting in the characteristic negative protein and energy balances associated with peak lactation. As stated earlier, ST increases during negative energy balance but it would appear that under some circumstances the ST is unable to stimulate IGF-I production, probably because of decreased numbers of hepatic receptors. This theory is supported by the results of Breier et al (1988a) who found that high-affinity binding sites were present only in well-fed animals.

IGF-I secretion is a slow, steady process (Schwander et al, 1983). In addition, IGF-I is bound to larger molecular weight binding proteins which increase the half-life of the IGF-I. The result is a relatively constant circulating level (Hall and Sara, 1984) as opposed to the pulsatile secretion of ST. This may be conducive to field sampling of animals for use of circulating IGF-I as a genetic marker.

IGF Binding Proteins

Circulating IGFs are non-covalently bound to larger molecular weight binding proteins (Hintz and Lui, 1977; Furlanetto, 1980; Hintz, 1984) with little or no free (i.e. unbound) IGF being detectable in plasma. There appear to be two major binding proteins, a small molecular weight complex of approximately 50 kDa (kilo Dalton) and a

large molecular weight complex of approximately 150 kDa (Hintz and Liu, 1977; Furlanetto, 1980). Although both the large and small binding proteins are synthesized in the liver, other characteristics of the two binding proteins differ.

The larger molecular weight binding protein is ST-dependent (Moses et al, 1976) and has one high-affinity binding site for IGF-I or IGF-II (Martin and Baxter, 1986). Furthermore, this complex dissociates under acid conditions (pH 2) to an acid-stable subunit which is also approximately 50 kDa (Furlanetto, 1980; Hintz, 1984). The smaller molecular weight binding protein is not a component of the larger binding protein complex, does not appear to be ST-dependent (D'Ercole et al, 1980) and is acid-stable.

The physiological role of binding proteins is not yet clear. Binding proteins may play an important role in regulating the biological effects of IGFs. It has been suggested, although never proven, that binding proteins restrict or prevent excess insulin-like activity but that they do not inhibit the growth-promoting activity of IGF-I (see Ooi and Herington, 1988). Binding proteins may also function as a promoter of IGF action, by themselves binding to cell surfaces and delivering IGFs to adjacent IGF receptors. In addition to these possible roles, as mentioned above, the binding proteins prolong the half-life of circulating IGFs. Furthermore, as IGFs do not appear to be stored in any tissues, this increased half-life may be a means of storing IGFs in the serum (see review by Ooi and Herington, 1988).

The physiological roles of binding proteins may differ with stage of development since variation occurs in the proportion of different complexes present in circulation as animals age. In the fetus the majority of the binding protein complex found is in the 50 kDa form whereas the greatest proportion in adult circulation is the larger 150 kDa complex (Butler and Gluckman, 1986). The transition from the smaller to the larger binding protein complexes occurs during the first week after birth in sheep and may be a reflection of the concurrent development of somatogenic receptors in the hepatic membranes (Gluckman et al, 1983a).

IGF Receptors

Like other peptide hormones and growth factors, the IGFs interact with their target cells by binding to specific cell-surface receptors (Rechler and Nissley, 1985). Even though IGF-I and IGF-II are structurally similar, their receptors are different both in structure and in relative specificities for the IGFs and insulin. There are two major IGF receptors. Type-1 receptors are structurally and functionally similar to the insulin receptor. They have a greater affinity for IGF-I than for IGF-II and low potency cross-reaction with insulin. Type-2 receptors have a distinctive structure which does not resemble that of the type-1 or insulin receptors. Type-2 receptors have a greater affinity for IGF-II than IGF-I and do not cross react with insulin (see Nissley and Rechler, 1984; Rechler and Nissley, 1985).

The role of IGF receptors is not clear. Originally it was believed that the acute metabolic actions of insulin and IGF were mediated by the insulin receptors while the IGF receptors mediated growth-promoting actions. However, this is now viewed as an oversimplification as there are many examples of receptor homogeneity, i.e. metabolic actions mediated by IGF receptors and growth-promoting actions mediated by insulin receptors (Nissley and Rechler, 1984).

Actions of IGF-I

As stated earlier, IGF-I has both anabolic and metabolic actions. There are several reviews which cover the earlier work (primarily *in vitro* studies) supporting IGF-I involvment in insulin-like and growth-promoting activities (see reviews by Froesch et al, 1985; Baxter, 1986; Gluckman et al, 1987).

In vitro studies have shown that IGF-I stimulates glucose metabolism in adipose tissue, striated muscle, and cardiac muscle, as does insulin. IGF-I stimulates glucose transport and oxidation, as well as synthesis of lipids, glycogen and protein. It inhibits lipolysis in adipose tissue in the same way as insulin. The potency with which IGF-I exerts these effects on adipose tissue was originally thought to be 50 to 100 times less than that of insulin (Zapf et al, 1978). However Bolinder et al (1987) suggest that IGF-I is 600 to 1000 times less potent than insulin. IGF-I mimics metabolic effects of insulin in striated muscle including the stimulation of glucose transport, glycolysis, and glycogen and protein synthesis with about one-tenth to one-fifth the potency of insulin (Poggi et al, 1979). In

perfused rat heart the ability of a partially purified preparation containing both IGF-I and -II to stimulate glucose transport, glucose uptake and lactate production was one-fifth to one-half that of insulin (see review by Zapf et al, 1981a).

The mitogenic activity of IGF-I has been demonstrated in many different cell culture lines. These actions result in cell proliferation and differentiation which enhances the potential for muscle and skeletal growth. IGF-I has been shown to stimulate RNA and DNA synthesis, cell proliferation, and protein synthesis in various organ cultures (Zapf et al, 1978; Canalis, 1980). IGF-I has also been shown to inhibit protein degradation in a variety of cells and to promote phosphorylation of a number of cell proteins (see review by Baxter, 1986). The ability to stimulate incorporation of sulfate into cartilage (Zapf et al, 1978) is also an important characteristic of IGF-I.

Many conclusions regarding the biological role of IGF-I *in vivo* are based on extrapolation from *in vitro* data and associative studies between IGF-I levels and physiological state. Few *in vivo* studies have been conducted.

Demonstration of *in vivo* effects was hampered by a shortage of pure peptides until recent advances in biotechnology led to the development of recombinant-human-IGF-I (rec-h-IGF-I). The development of rec-h-IGF-I has enabled verification of earlier studies in which administration of impure IGF-I preparations resulted in increased growth (see Van Wyk, 1984). Administration of rec-h-IGF-I increased

body weight and length in Snell dwarf mice (van Buul-Offers et al, 1986), hypophysectomized rats (Guler et al, 1988), neonatal rats (Philipps et al, 1988) and in a new line of ST-deficient dwarf rats (Skottner et al, 1989). Increases were recorded in various organ weights although, with the exception of the spleen, the organs displaying these increases were not consistent between the different studies. Guler et al (1988) observed increases in the width of the tibial epiphyses, in longitudinal bone growth, and in trabecular bone formation, and a decrease in the weight of the epididymal fat pads. Philipps et al (1988) found that IGF-I treatment increased the erythropoietec cell precursors for bone marrow and resulted in precocious eye opening (a sign of epithelial cell differentiation) in neonatal rats. These effects were not influenced by nutritional levels whereas IGF-I treatment did not alter somatic or organ growth in rats deprived of nutrients (Philipps et al, 1988). O'Sullivan et al (1989) found that administration of IGF-I to starved mice reduced weight loss, while ST treatment had no effect. These findings support the hypothesis that IGF-I is an important component of the somatotropic axis which stimulates lean tissue and bone growth. However, they also demonstrate that the effects of IGF-I can be affected by other factors (e.g. nutrition).

Factors Affecting IGF-I Levels in Circulation

Ontogenetic Development. Plasma IGF-I concentrations vary with developmental stage. Work in rats (Maes et al, 1983), mice (D'Ercole and Underwood, 1980) and humans (Gluckman et al, 1983b; Hall and Sara, 1984) indicates that the general developmental pattern of plasma IGF-I

is as follows: concentrations are low in the fetus, rise gradually through adolescence, exhibit a marked rise at puberty and decrease postpubertally to prepubertal levels.

Little is known about the ontogeny of IGF-I in cattle. Breier et al (1988c) found that plasma IGF-I levels fell from birth to five weeks of age and then rose until weaning, after which levels declined. Within ten days of weaning levels began to rise again. They suggested that the decrease in IGF-I levels from birth to five weeks, and the subsequent rise, may be related to a change in the mechanisms controlling IGF-I secretion as somatogenic receptors were low in the neonatal calf and displayed an increase at six weeks.

In sheep, concentrations of plasma IGF-I are significantly lower in the fetus than in the adult. Three to seven days after parturition plasma levels increase and remain high until four weeks after birth. By 50 days of age plasma concentrations drop to levels comparable to those found in mature sheep (Gluckman and Butler, 1983). Blanchard et al (1988) found that at 5 months of age levels are significantly higher than in newborn lambs. To date there have been no studies examining the ontogeny of plasma IGF-I from prepuberty through to adulthood in sheep.

The rise of plasma IGF-I shortly after birth in sheep coincides with the detection of somatogenic receptors in the liver and may therefore represent the onset of ST-dependent IGF-I release (Gluckman, et al, 1983a; Maes et al, 1983). The decline in levels between four and seven weeks of age is probably related to a transition in diet

resulting in the development of ruminant digestion (i.e. a change in nutritional status).

As seen above, circulating IGF-I levels vary according to stage of development. Furthermore, concentrations can be affected by stage of production. During pregnancy plasma IGF-I concentrations increase by 50 to 130% in the last trimester compared to nonpregnant females (see Baxter, 1986). In humans, levels appear to be 80% higher at term than in nonpregnant women (Bala et al, 1981) with levels declining significantly postpartum to nonpregnant levels (Wilson et al, 1982). In some species ST appears not to be the sole regulator of IGF-I during pregnancy as hypophysectomy did not influence the rise in IGF-I in pregnant rats (Daughaday and Kapadia, 1978). There is, however, a significant correlation between placental lactogen (PL) and maternal IGF-I concentrations (Furlanetto et al, 1978). Therefore, it may be that PL is a major regulator of maternal IGF-I.

Sex of Animal and Gonadal Steroids. As previously discussed, circulating IGF-I levels increase at or around puberty. It has been suggested that plasma concentrations of IGF-I are more strongly related to pubertal stage than to chronological age (Luna et al, 1983; Rosenfield et al, 1983, Harris et al, 1985). Plasma concentrations of IGF-I are elevated in parallel with circulating gonadal sex steroids during normal puberty (Rosenfield et al, 1983). This suggests that the marked rise at puberty may be related to an effect of gonadal steroids.

The relationship between IGF-I and steroids has been examined by treating animals with exogenous steroids. Administration of physiological doses of oestradiol (Copeland et al 1984; Cuttler et al 1985; Breier et al, 1988b) or testosterone (Parker et al, 1984; Jasper, 1985) suggest a positive relationship with circulating IGF-I levels. Similarly, plasma IGF-I concentrations are reduced in humans with precocious puberty when their gonadal steroids are suppressed (Harris et al, 1985; Mansfield et al, 1988). It is not clear whether the effect of sex steroids on IGF-I is direct or if they act indirectly via ST. Furthermore, evidence of a relationship between gonadal steroids and plasma IGF-I is not conclusive. Siddiqui et al (1989b) examined the effects of testosterone treatment in castrated male mice from lines divergently selected on the basis of plasma IGF-I concentrations. They found that the greater pubertal growth in high-line males compared to those in the low line was not due to a greater androgenic stimulation of circulating IGF-I. Their results also suggest that testosterone does not appear to influence pubertal growth by acting on plasma IGF-I concentrations because castrates, in whom testosterone therapy was delayed, exhibited retarded growth but normal circulating IGF-I levels.

Evidence that plasma IGF-I displays sexual dimorphism (i.e. concentrations greater in one sex than the other) has been found in several different species. However, the dimorphism is not consistent between species and appears to be dependent on stage of development. Kaplowitz et al (1982) found that IGF concentrations are equal in the male and female human fetus but that females have higher concentrations than males throughout postnatal life. Copeland et al

(1985) found that female chimpanzees had higher plasma concentrations of IGF-I than did males until after puberty. Furthermore, the females reached maximum levels by six to eight years of age whereas males did not reach maximum levels until eight to ten years. A significant sex effect in prepubertal lambs has also been observed, with males having higher plasma IGF-I concentrations than females (Blanchard et al, 1988). Unlike Blair et al (1987), who found that male mice had significantly higher plasma IGF-I levels than did females, Siddiqui et al (1989a) found no significant sex effect. However, in the latter study the sampling was conducted at 21 and 42 days of age with the sex effect on plasma IGF-I levels approaching significance at 42 days. During the period from 21 to 49 days of age they found that growth velocities were influenced by sex. As a consequence they suggest that sex may have a significant effect during this period and that a more intensive sampling regimen would be required to determine the influence of sex on plasma IGF-I levels during this pubertal period.

Diurnal Variation. The diurnal variation in plasma IGF-I has not been extensively studied but Breier et al (1986) found that no diurnal variation occurred over 25 hours of serial sampling in steers. Similarly, Hall and Sara (1984) found no apparent diurnal rythym in humans. In contrast, Minuto et al (1981) found that, in humans, concentrations were stable during waking hours but declined by approximately 25% following the onset of sleep.

Nutrition. In contrast to time of day, nutritional status does affect plasma IGF-I concentrations. Fasting has been shown to result in a decline in circulating levels of IGF-I which can be restored to normal

after realimentation. Such observations have been made in dogs (Eigenmann et al, 1985), rats (Maes et al, 1983), man (Clemmons et al, 1981b) and sheep (Hodgkinson et al 1987). Similar results were observed in steers restricted to 1% dry matter of live weight per day (Breier et al, 1986, 1988b). Isley et al (1983) found that while both protein and energy were needed to restore IGF-I concentrations, an adequate supply of energy was essential.

The changes in serum IGF-I levels during fasting and refeeding are highly correlated with nitrogen balance (Clemmons et al, 1981b; Isley et al, 1983). This provides further evidence that changes in IGF-I concentrations reflect changes in protein metabolism.

The cause of the observed decline in plasma IGF-I during nutritional deprivation may be related to hepatic somatogenic receptors. Breier et al (1988a) found that while animals on a high plane of nutrition had high- and low-affinity binding sites, those on a low plane had only low-affinity binding sites. Consequently, a relative resistance to ST occurs in poorly fed animals and this in turn causes changes in the plasma ST and IGF-I levels.

Parasites. A change in plasma IGF-I levels similar to that caused by nutrition might be expected in animals infected with parasites since parasitic infections generally result in poor appetite, listless behaviour and malabsorption of ingested nutrients (i.e. infected animals experience reduced nutrient availability). In a study to investigate the effects of parasite burden on plasma IGF-I, Elsasser et al (1988) found that levels were lower in infected than uninfected

calves. Although levels were reduced to a greater extent in the infected than in uninfected calves when the two groups were fed equivalent amounts of feed, it is not possible to rule out differences in nutrient availability as the major source of variation observed. Other possible contributing factors to the decreased circulating IGF-I concentrations in infected animals may be infection-induced hepatic pathology which could affect the hepatic receptors or liver function and hence IGF-I production.

Evidence for a Genetic Association between IGF-I and Production Traits

Most of the information on plasma IGF-I has been obtained from *in vivo* or *in vitro* studies examining the phenotypic association between IGF-1 and production traits. Studies conducted in normal and abnormal individuals or subjects with genetic disorders also indicate a genetic association between plasma IGF-I concentrations and production.

Growth. Circulating IGF-I concentrations have been examined in several populations with abnormal growth as a consequence of genetic disorders. Although variation in circulating IGF-I observed in some of these populations can be directly associated with variation in plasma ST levels (e.g. acromegaly and hypopituitary or GH-deficient dwarfism), others cannot. It is this latter category (i.e. conditions in which variation in plasma IGF-I is not a consequence of variation in plasma ST) which is of most interest and will be discussed here.

Short stature, significantly reduced plasma IGF-I concentrations and normal or elevated serum ST levels are characteristic of Laron

dwarfs (Zapf et al, 1981b), adult African pygmies and individuals with the pygmy trait (Merimee et al 1981, 1982). Laron dwarfism is an inherited autosomal recessive condition in humans characterized by retarded growth. In spite of clinical symptoms of ST deficiency, afflicted individuals have elevated serum immunoreactive ST concentrations but low plasma IGF-I levels. In addition to confirming ST resistance in patients with Laron dwarfism, Geffner et al (1987) found from in vitro work that the patients were sensitive to exogenous IGF-I. Eshet et al (1984) found that individuals with Laron dwarfism are deficient in tissue somatogenic receptors. This may explain the lack of ST sensitivity and the inability to achieve normal secretion of IGF-I.

The small stature of African pygmies cannot be explained by nutrition or environmental factors. Pygmies are characterized by low circulating IGF-I levels despite normal ST secretion (Merimee et al, 1981). These characteristics have also been found in individuals of other ethnic origins who appear to have the pygmy trait (Merimee et al, 1982). Merimee and his co-workers evaluated stature and plasma IGF-I levels in pygmies. They found no significant difference in stature or serum IGF-I concentrations between control and pygmy pre-pubertal children (Merimee et al, 1987). Pygmy children, however, failed to exhibit the increase in circulating IGF-I or the growth spurt normally associated with puberty. Merimee's group suggest that the short stature of adult pygmies is due primarily to a failure to exhibit accelerated growth during puberty. They further suggest that IGF-I is the principal factor responsible for normal pubertal growth (Merimee et al, 1987).

"Constitutionally short" humans, even when given a normal diet, exhibit retarded growth during the first five years of life. Thereafter growth rate is either normal or only slightly reduced. This condition often runs in families, thereby implying a genetic basis. Even though ST response to provocative stimuli is normal in these children their plasma IGF-I concentrations are significantly lower than in children with normal growth. Using a competitive binding assay which preferentially measured IGF-I, Binoux et al (1986) found that IGF concentrations were significantly lower in these short individuals compared to normal children and that the levels of IGF binding protein were lower in approximately the same proportions. These results led them to attribute constitutional short stature to reduced expression of the gene for IGF-I (Binoux et al, 1986).

In contrast to constitutionally short subjects, "constitutionally tall" children have high serum IGF concentrations (again measured using a competitive binding assay which preferentially detects IGF-I) but low IGF binding protein levels (Gourmelen et al, 1984). These children are unusually tall (i.e. height is generally greater than that of the mean for age by three standard deviations) but do not suffer from other known causes of advanced growth (e.g. precocious puberty). The inheritance of this trait has not, however, been clearly established.

Another population with a genetic disorder affecting growth is sex-linked dwarf chickens. They have been found to have normal or increased ST levels but substantially reduced circulating IGF-I levels (Huybrechts et al, 1985).

Variations in IGF-I concentrations have also been observed in 'normal' populations of various species (i.e. populations in which no known genetic disorders or pathological conditions exist). Within the canine species breed differences have been found, with plasma IGF-I concentrations paralleling mature body size (Eigenmann et al, 1984a). The relationship between circulating IGF-I levels and body size was further demonstrated within the poodle breed, which consists of three different strains; the standard, miniature and toy (Eigenmann et al, 1984b). A large degree of variation in body size and plasma IGF-I concentrations occurs between the three strains with the order of ranking being standard>miniature>toy in both body size and IGF-I levels. Although variation occurs in IGF-I concentrations, a similar variation in ST secretion (Eigenmann et al, 1984b) or circulating somatropin-binding proteins (Lauteric et al, 1988) does not occur. Therefore, it appears that a post-somatogenic receptor defect is responsible for the variation in growth.

Lauteric et al (1988) suggested that the correlation between body size and circulating IGF-I levels found in dogs does not hold true for different breeds of pigs. However, their data indicate that a similar relationship does exist within the Yucatan breed. Ranking of the strains for both IGF-I and body size was mini>micro. Like poodles, there was no significant difference in ST levels between the strains of Yucatan pigs. However, ST-binding protein activity was significantly greater in the mini than in the micro strain. Therefore, a somatogenic receptor defect could be responsible for the lack of growth within this breed.

Blair et al (1987, 1989a) described the development of lines of mice divergently selected on the basis of plasma IGF-I concentrations. By the seventh generation of continued selection pressure, litter mean plasma IGF-I levels were significantly different between the High and Low lines. This, combined with direct estimates of heritability, confirmed that plasma IGF-I has a genetic basis (Blair et al, 1987, 1989a). In addition to a divergence in plasma IGF-I concentrations between the lines, body weight also diverged with the High line mice being significantly heavier than the Low line (Blair et al, 1988, 1989a; Siddiqui et al, 1989a). Siddiqui et al (1989a) evaluated the body growth of these mice. They found that the High line mice had significantly greater nose-anus length and growth velocity. Even though High line mice had a faster rate of accretion of protein, water and fat, the proportion of body components was not altered, indicating that plasma IGF-I increases body growth without altering composition (Siddiqui et al, 1989a). Work in transgenic mice expressing human IGF-I (Mathews et al, 1988a,b) provides further support for the genetic involvment of IGF-I in growth.

Reproduction. The reproductive performance and fetal growth in females from the divergently selected lines of mice (Blair et al, 1989a) have also been examined (Kroonsberg et al, 1989). Their results show that High line females had an increased number of fetuses as well as heavier individual fetuses and placental units compared to Low line females (Kroonsberg et al, 1989).

Lactation. Little information is available on the genetic relationship between of plasma IGF-I and lactation. The results of

Kroonsberg et al (1989) showed that High line females had greater mammary gland weights than the Low line females. In addition, mammary gland weight increased with the number of fetuses in the High line but not in the Low line. However, these effects were apparently related to differences in maternal body weight (Kroonsberg et al, 1989). As actual lactational performance was not measured it is not possible to determine whether High line females had greater milk production per unit mammary gland weight than did females of the Low line. Work with dairy bulls indicates that plasma IGF-I concentration of bulls is correlated with the milk production potential of their daughters as determined by progeny testing (Ahlborn-Breier et al, 1987).

Purpose and Scope of the Investigation

Physiological traits could potentially be used as a selection criteria for production in domestic animals provided that they were genetically correlated with the production traits of interest and displayed other characteristics required of a genetic marker. These include:

1) They should be easily and inexpensively measured at a young age and in both sexes. For example, hormones should exhibit a stable secretion pattern/concentration which does not display a series of spikes and troughs or marked diurnal variation. Assay costs should ideally be low.

2) They should display a high degree of repeatability. They should preferably be little affected by non-genetic factors and be measurable

without resorting to highly controlled conditions.

3) They should not be adversely correlated with other desirable traits.

The preceding discussion provides evidence that the plasma concentration of IGF-I is genetically related to growth and milk production. Plasma IGF-I concentrations may therefore be useful as a genetic marker in domestic animals, particularly as our understanding of the genetic relationship between plasma concentrations of IGF-I and production traits improves. However, further information about the effect of non-genetic factors and the ontogeny of plasma IGF-I concentrations in the different species must first be obtained.

Little is known about the ontogeny of plasma IGF-I and the non-genetic factors which influence circulating concentrations of this hormone in sheep. Therefore, the objectives of this study were to establish the developmental pattern of plasma IGF-I in young sheep and to determine the effects of sex, rearing rank, age and stage of development on plasma concentrations of IGF-I. This was done with the objective of providing information which could be used to develop optimum sampling strategies for the evaluation of plasma IGF-I as a potential genetic marker.

C H A P T E R II

EXPERIMENTAL

DEVELOPMENTAL PATTERNS OF PLASMA INSULIN-LIKE GROWTH FACTOR-I IN SHEEP

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Abstract

A study was undertaken to examine the ontogeny of circulating insulin-like growth factor-I (IGF-I) concentrations in Romney sheep. The trial was a balanced 2x2 factorial design incorporating the effects of sex and rearing rank with a total of 48 animals. Blood sampling was initiated four weeks post-weaning (about 3 months of age) and continued every 2 weeks for 6 months and then every 4 weeks for 7 months. Faecal egg counts and plasma concentrations of non-esterified fatty acids (NEFA) were also determined.

IGF-I concentration was positively related to live weight throughout the trial, even when adjusted to a common sex and rearing rank. Sex of lamb had a significant effect on plasma IGF-I concentrations with concentrations in males being greater than those

in females. Puberty in females, as determined by date of first oestrus, was associated with an increase in plasma IGF-I concentrations. Although puberty in males was not measured, a surge in plasma IGF-I concentrations around the approximate time of puberty in males resulted in a marked divergence between the sexes which remained throughout the study. Rearing rank did not influence plasma IGF-I concentrations beyond 7 months of age.

Plasma IGF-I concentrations tended to be negatively associated with plasma NEFA concentrations and faecal egg counts but relationships were only occasionally significant.

IGF-I levels were highly repeatable, concentrations at the first sample being significantly correlated with those at all subsequent samples. Implications of these results in relation to potential use of plasma IGF-I concentration as a genetic marker for productivity in sheep are discussed.

Introduction

Variation in plasma concentration of IGF-I is commonly associated with variation in growth rate or mature body size (Merimee et al, 1981, 1982; Eigenmann et al, 1984a,b; Binoux et al, 1986; Blair et al, 1987; Merimee et al, 1987; Eigenmann et al, 1988). The recent development of lines of mice divergently selected for litter mean plasma concentrations of IGF-I (Blair et al, 1989a) has provided further evidence of a role for IGF-I in the regulation of somatic growth and as a mediator of a genetic component of growth. After 7

generations, the divergence between the Low and High lines in plasma IGF-I concentration and live weight at 42 days of age was approximately 20% and 25%, respectively (Blair et al, 1989a). Significant differences between the lines have been reported in fetal and postnatal growth, body composition, reproductive performance and organ growth (Siddiqui et al, 1989a; Kroonsberg et al, 1989; R.A. Siddiqui - pers. comm.). Work in dairy bulls has shown that plasma concentrations of IGF-I in the bulls are related to the milk production of their daughters (Ahlborn-Breier et al, 1987). This observation, and the fact that selection for high IGF-I levels in mice increases mammary gland weight (Kroonsberg et al, 1989), suggests that there may also be a genetic association between plasma IGF-I concentrations and milk production.

As the degree of genetic relationship between IGF-I and production traits becomes more clear, it may be possible to use plasma concentrations of IGF-I as a selection criterion in domestic animals. However, it will be necessary to first determine the developmental pattern of IGF-I and the degree to which animals retain their ranking for this trait as they age. This is particularly important given that marked variation in plasma concentrations of IGF-I with age or stage of development has been reported in humans (Merimee et al, 1987), mice (Siddiqui et al, 1989a) and cattle (Breier et al, 1988c).

Plasma concentrations of IGF-I are influenced by a number of genetic and non-genetic factors other than growth potential. These include sex (Ringberg Lund-Larsen and Bakke, 1975; Luna et al, 1983; Copeland et al, 1985; Blair et al, 1987; Merimee et al, 1987; Siddiqui

et al, 1989a), litter size or birth rank (Blair et al, 1987), nutrition (Breier et al, 1986, 1988b), weaning (Breier et al, 1988c) and internal parasite load (Elsasser et al, 1988). If IGF-I concentrations were to be used as a selection criterion, it would be necessary to identify and adjust for any non-genetic factors which contributed to the variability in plasma IGF-I levels so as to improve the accuracy of ranking animals prior to selection. The objective of this study was therefore to establish the developmental pattern of plasma concentrations of IGF-I in young sheep and to determine the magnitude of non-genetic factors which must be adjusted for when selecting on the basis of plasma IGF-I concentrations.

Materials and Methods

Animals and Experimental Design. The experiment was designed as a 2x2 factorial incorporating the effects of sex (male vs. female) and rearing rank (single vs. twin), with 12 animals per cell. The sheep (New Zealand Romney lambs) were derived from a commercial flock and birth dates were therefore not available. Rearing ranks were identified by observing ewes with their lambs at weaning. The lambs were weaned at approximately 12 weeks of age and transported to Massey University's Sheep and Beef Cattle Research Unit for an adjustment period of four weeks prior to commencement of the study.

The animals were run at a low stocking rate (equivalent to 14 mature ewes per hectare) on mixed ryegrass-white clover pasture. At approximately 210 days of age the lambs were separated into two mobs

according to sex. All animals were drenched every 28 days with anthelminthic ("Ivermec", MSD Agvet NZ Ltd, Auckland, New Zealand).

Live Weights and Blood Sampling. Live weights were measured, and blood samples collected, between 08.30hr and 13.00hr every two weeks for six months then every four weeks for seven months. On each occasion, single blood samples (7 ml/sheep) were collected by jugular venipuncture into vacutainers ('Neotube', Nipro Medical Industries Ltd, Tokyo) containing sodium heparin as the anticoagulant. Blood was stored on ice, and within two hours of collection was centrifuged at 2500 g for 20 minutes at 4°C. Plasma was harvested and stored at -20°C until assayed.

Chemical Analyses. Plasma IGF-I concentrations were determined by radioimmunoassay after acid-ethanol extraction (Gluckman et al, 1983b). The intra- and inter-assay coefficients of variation (CV) were 5.0% and 9.8%, respectively. Plasma concentrations are expressed with respect to recombinant human-met-IGF-I (rh-met-IGF-I, batch #742-44; Dr. B. D. Burleigh, International Minerals and Chemicals, Pitman-Moore, Northbrook, IL, USA). Non-esterified fatty acid (NEFA) concentrations were determined using the colourimetric method ("NEFAC", WAKO Pure Chemical Industries Ltd, Japan) modified as described previously (McCutcheon and Bauman, 1986). Intra- and inter-assay CV were 2.6% and 3.8%, respectively.

Faecal Egg Counts. Faecal samples were collected per rectum every four weeks at the time of blood sampling. Samples were placed on ice and within two hours of collection were stored at 4°C until analysed.

Within seven days of collection, counts of Strongylate nematode eggs per gram of faecal material (FEC) were determined by the modified McMaster method as described by Soulsby (1982).

Puberty in Females. Harnessed vasectomized mature rams were run with the females from approximately 210 to 350 days of age early April to late August) to determine the date of first oestrus. The normal breeding season for Romney hoggets in the Manawatu is April to July. Females were checked for mating marks every two weeks during this period. A subjective scoring system (1 = very light mark to 5 = solid mark) was used to discriminate between females truly in oestrus and those which had been "rape served".

Testis Diameter. At each blood sampling period the maximum diameter of each testis was measured in the anterior-posterior plane using a pair of vernier calipers with the rams in a sitting position.

Fleece Weight. The animals were shorn prior to the commencement of the trial and then again at nominal 378 and 508 days of age. Greasy fleece weights were recorded at each of the last two shearings.

Pasture Analyses. Samples of the pasture grazed by the lambs were collected every four weeks by hand-plucking. They were stored at -20°C until processed. Samples were freezed dried, ground to 1mm mesh size and *in vitro* digestibilites determined by the enzymic hydrolysis method of Roughan and Holland (1977) calibrated against six samples of known *in vivo* digestibility.

Statistical Analyses. Effects of sex, rearing rank (both treated as fixed effects), plasma NEFA concentrations and faecal egg counts (FEC) on live weight and plasma IGF-I concentrations were determined by analysis of covariance (ANCOVA) at each sampling time. Multivariate analysis of variance (MANOVA) was used to analyse the overall effects of sex and rank, and their interaction with age of the animals. Repeatability of plasma IGF-I was assessed by generating the matrix of correlations between IGF-I levels at each sampling time. The intra-class correlations (based on IGF-I levels adjusted for the effects of sex and rearing rank) were examined. The relationship between puberty (in female sheep) and circulating IGF-I levels was assessed by classifying females at each sampling date according to whether or not they had shown first oestrus. Presence or absence of oestrus was then treated as a main effect and fitted in a model which also included rearing rank. All analyses were undertaken using the 'SAS' statistical package (SAS, 1985).

Results

Live Weight. Figure 1 illustrates the effects of sex and rearing rank on growth of the lambs. MANOVA analysis showed that the effects of sex and rearing rank were highly significant ($P<0.001$) with males being heavier than females and singles heavier than twins. The sex by age and rank by age interactions were also significant ($P<0.001$), reflecting a divergence between the sexes and between the ranks as the lambs aged. Analysis of covariance at each sampling time (Table 1) showed that plasma concentrations of IGF-I were strongly related to live weight within treatment groups. Furthermore, the correlation

between live weight and IGF-I was positive. The relationship between FEC or NEFA concentrations and live weight was only occasionally significant (see Table 1).

Plasma IGF-I Concentrations. Developmental patterns of plasma IGF-I concentrations in relation to sex and rank are illustrated in Figure 2. A marked divergence between the sexes (males > females) began at approximately 214 days of age and continued until sampling ceased at 490 days. The effects of age and sex on plasma IGF-I and the interaction between age and sex were all highly significant ($P<0.001$) as determined by MANOVA. Rearing rank, when analyzed at individual sample times (see Table 2), showed significant effects (single > twin) up to 196 days of age but not thereafter. Over the whole trial, the effect of rank was non-significant. FEC had a significant negative effect only at days 238 ($P<0.05$). Similarly, the effect of NEFA concentrations was non-significant except on days 265 ($P<0.10$) and 434 ($P<0.01$) when the relationship with plasma IGF-I was negative.

Repeatability of plasma IGF-I concentrations was high (see Table 3) with the intraclass correlation between successive pairs of samples ranging from 0.47 ($P<0.01$) to 0.85 ($P<0.001$). The intraclass correlation between the first sample (day 115) and subsequent samples was also high, ranging from 0.41 ($P<0.01$) to 0.85 ($P<0.001$).

Puberty in Females. The females began to display signs of oestrus at nominal 238 days of age and, within a relatively short period of time (i.e. 42 days), 92% of the females had been marked by the rams (see Figure 3).

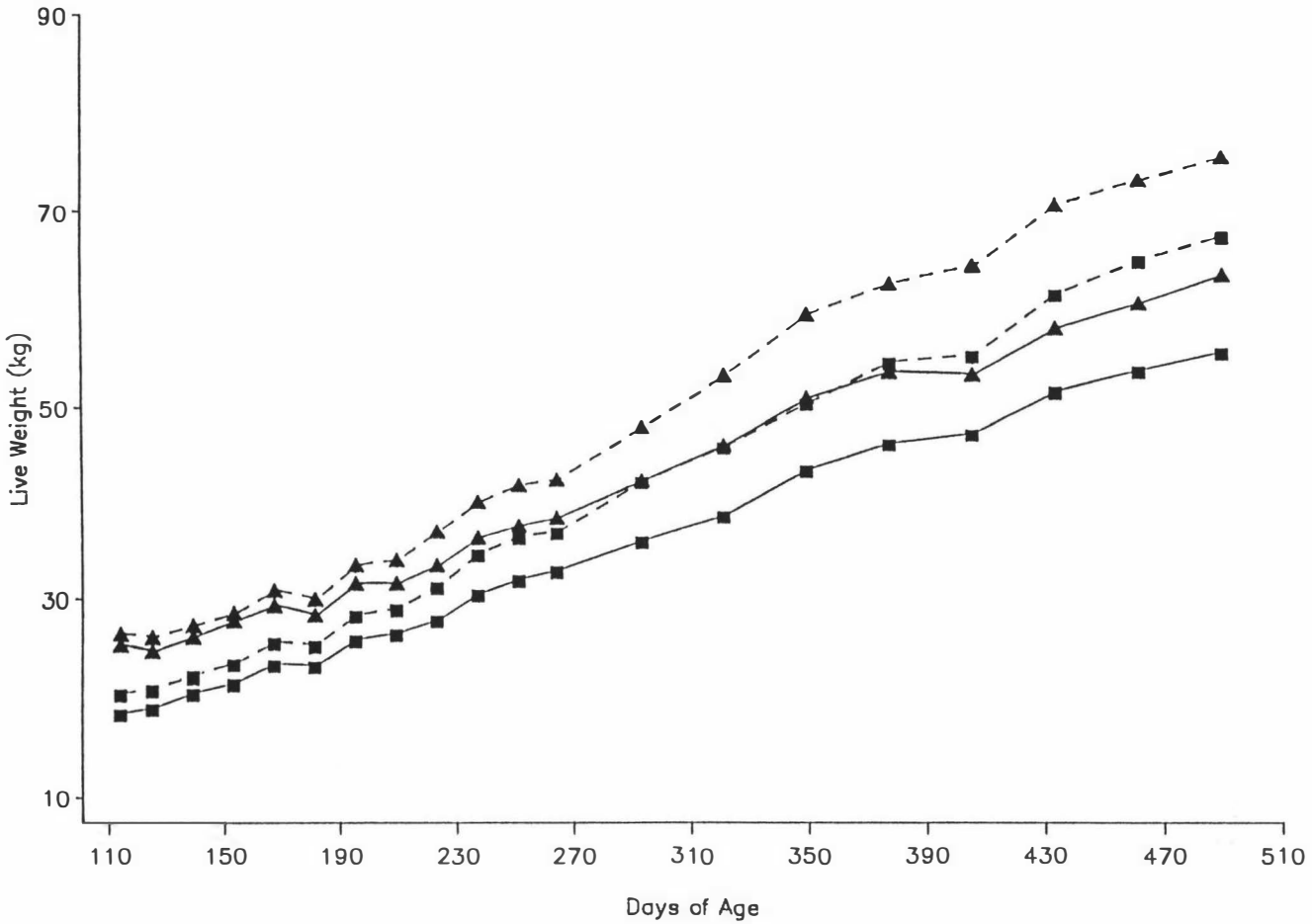

Figure 1. Live weight of female (——) and male (- -) single (▲) and twin (■) sheep over time (days of age based on an estimated date of birth as described in the text). Pooled standard errors ranged from 0.7 to 2.1 kg.

Table 1. Effects of sex, rearing rank, faecal egg counts (FEC), plasma non-esterified fatty acid (NEFA) concentrations and plasma IGF-I concentrations on live weight (by ANCOVA at each sampling time).

Source[a]	df[b]	Mean square and significance at nominal age (days)[c]									
		115	126	140	154	168	182	196	210	224	238
Sex	1	30*	33*	24	23	43*	47*	56*	54*	140**	184***
Rank	1	481***	347***	331***	380***	371***	273***	341***	279***	363***	369***
Sex*Rank	1	3	1	1	4	2	0	1	0	0	0
FEC	0/1	11	49**	-	10	-	104**	-	86**	-	156***
NEFA	1	0	12	0	0	53	3	6	25	0	20
IGF-I	1	145***	65**	89**	92**	155***	61*	163***	55*	128**	116**
Error	42/41	7	7	9	10	8	9	10	12	14	11
		252	**265**	**294**	**322**	**350**	**378**	**406**	**434**	**462**	**490**
Sex	1	223***	152**	334***	667***	689***	668***	1117***	1561***	1626***	1722***
Rank	1	355***	319***	359***	653***	912***	692***	702***	685***	655**	764***
Sex*Rank	1	0	1	6	0	6	10	28	24	5	0
FEC	0/1	-	17	9	119+	53	141+	20	1	1	3
NEFA	1	15	0	2	47	102+	1	1	240*	2	34
IGF-I	1	80	118**	406***	241**	561***	384**	185+	475**	240*	441**
Error	42/41	16	16	16	29	32	40	46	45	54	48

a. All main effects treated as fixed.
b. df for FEC and error vary according to whether FEC was in model for a particular sampling time.
c. Mean square = 0 indicates values less than 0.5.
+ P<0.10 * P<0.05 ** P<0.01 *** P<0.001

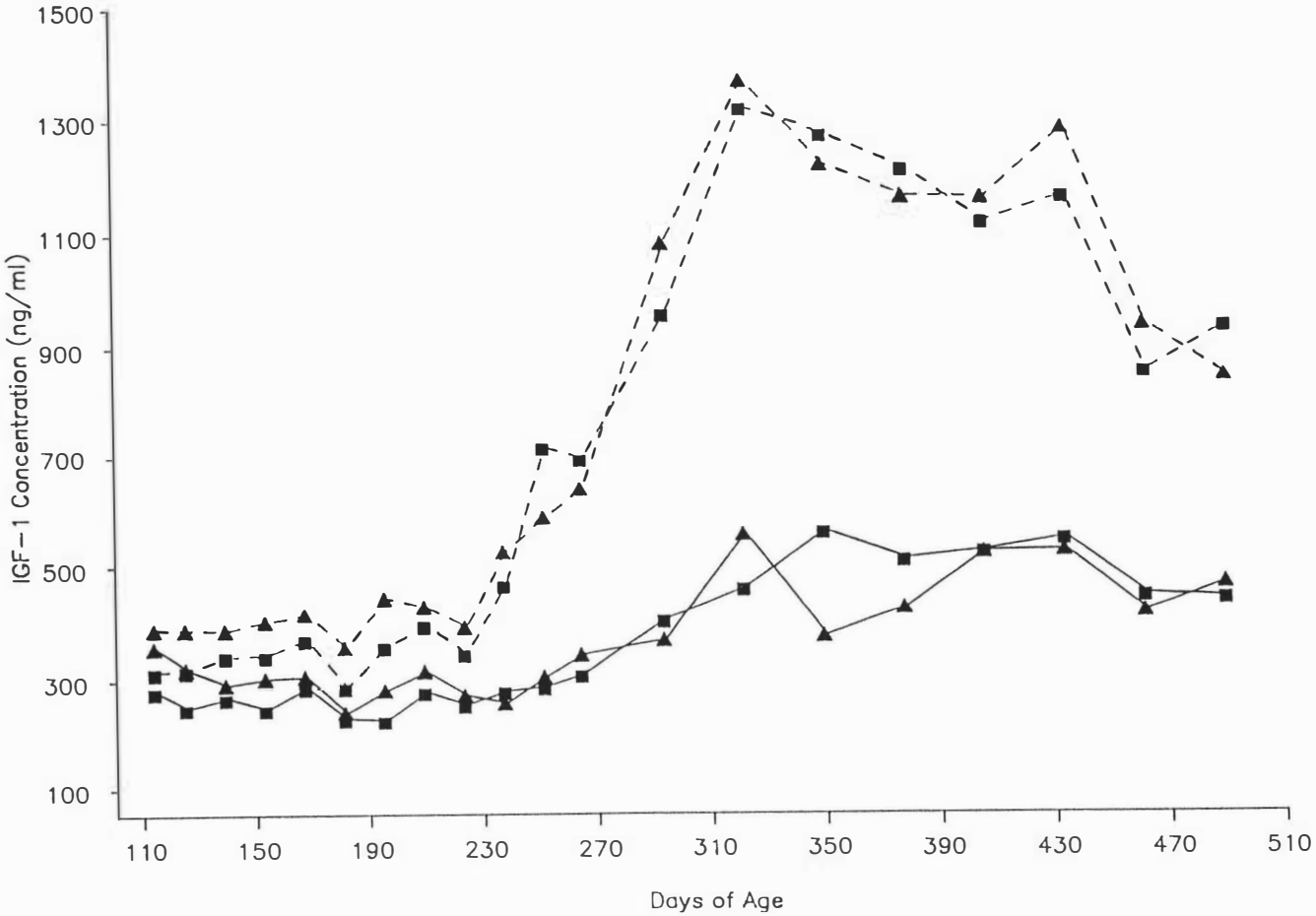

Figure 2. Plasma IGF-1 concentrations in female (——) and male (- -) single (▲) and twin (■) sheep over time (days of age based on an estimated date of birth as described in the text). Pooled standard errors were less than 30 ng/ml up to 262 days and ranged from 40 to 65 ng/ml thereafter.

Table 2. Effects of sex, rearing rank, faecal egg counts (FEC) and plasma non-esterified fatty acid (NEFA) concentrations on plasma IGF-I (by ANCOVA at each sampling time).

Source[a]	df[b]	Mean square and significance at nominal age (days):							
		115	126	140	154	168	182	196	210
Sex	1	14293	55699*	84992**	114046***	113092***	87429***	261252***	160504***
Rank	1	67208**	58415*	14833	40096*	12465	21625*	58297*	18201
Sex*Rank	1	1	2	1480	72	1829	8944	2658	279
FEC	0/1	1815	20206	-	246	-	12072	-	12831
NEFA	1	14236	10278	820	221	19	933	2931	3204
Error	43/42	8122	8388	7452	6107	8046	4446	8730	7402
		224	**238**	**252**	**265**	**294**	**322**	**350**	**378**
Sex	1	135490***	623854***	1516634***	1362767***	4489189***	8666893***	6699141***	558144***
Rank	1	12584	3795	36598	1712	19839	35206	112818	19526
Sex*Rank	1	2543	19473	65033	22375	74238	21038	86223	30947
FEC	0/1	-	50822*	-	13127	61433	17909	17516	114634
NEFA	1	8126	1916	125056	86327+	77038	7598	140440	113068
Error	43/42	8310	11240	51723	23204	52345	49716	73199	44481
		406	**434**	**462**	**490**				
Sex	1	4619064***	5539305***	2577268***	2312652***				
Rank	1	4387	33280	9240	12805				
Sex*Rank	1	5894	51636	37955	41301				
FEC	0/1	62949	1331	43478	7638				
NEFA	1	42960	405612**	67	15913				
Error	43/42	28376	48048	29711	27987				

a. All main effects treated as fixed.
b. df for FEC and error vary according to whether FEC was in model for a particular sampling time.
+ P<0.10 * P<0.05 ** P<0.01 *** P<0.001

Table 3. Pearson intraclass correlations for plasma IGF-I concentrations at nominal days of age:

	115	126	140	154	168	182	196	210	224	238	252	265	294	322	350	378	406	434	462	490
115	1.0																			
126	.85	1.0																		
140	.77	.71	1.0																	
154	.66	.58	.76	1.0																
168	.68	.64	.81	.76	1.0															
182	.62	.51	.63	.67	.74	1.0														
196	.61	.57	.73	.76	.84	.77	1.0													
210	.54	.55	.58	.64	.65	.57	.63	1.0												
224	.69	.71	.66	.59	.69	.61	.71	.77	1.0											
238	.61	.64	.67	.51	.73	.55	.63	.64	.71	1.0										
252	.42	.41	.48	.37	.44	.26	.42	.56	.49	.66	1.0									
265	.41	.40	.43	.42	.38	.31	.34	.55	.35	.56	.73	1.0								
294	.61	.54	.46	.45	.36	.37	.35	.38	.39	.51	.39	.53	1.0							
322	.43	.44	.33	.33	.45	.25	.32	.46	.37	.57	.50	.56	.67	1.0						
350	.66	.56	.57	.58	.51	.62	.54	.49	.55	.54	.28	.37	.60	.51	1.0					
378	.53	.46	.56	.41	.58	.43	.45	.43	.43	.58	.22	.17	.47	.51	.71	1.0				
406	.56	.55	.57	.61	.66	.46	.52	.52	.56	.56	.29	.36	.40	.57	.56	.51	1.0			
434	.73	.66	.61	.51	.62	.54	.49	.64	.63	.61	.31	.39	.62	.53	.71	.59	.65	1.0		
462	.53	.56	.68	.54	.75	.55	.60	.55	.61	.75	.42	.40	.41	.44	.57	.64	.71	.58	1.0	
490	.62	.61	.53	.54	.59	.55	.60	.50	.56	.53	.31	.28	.47	.40	.55	.38	.54	.66	.47	1.0

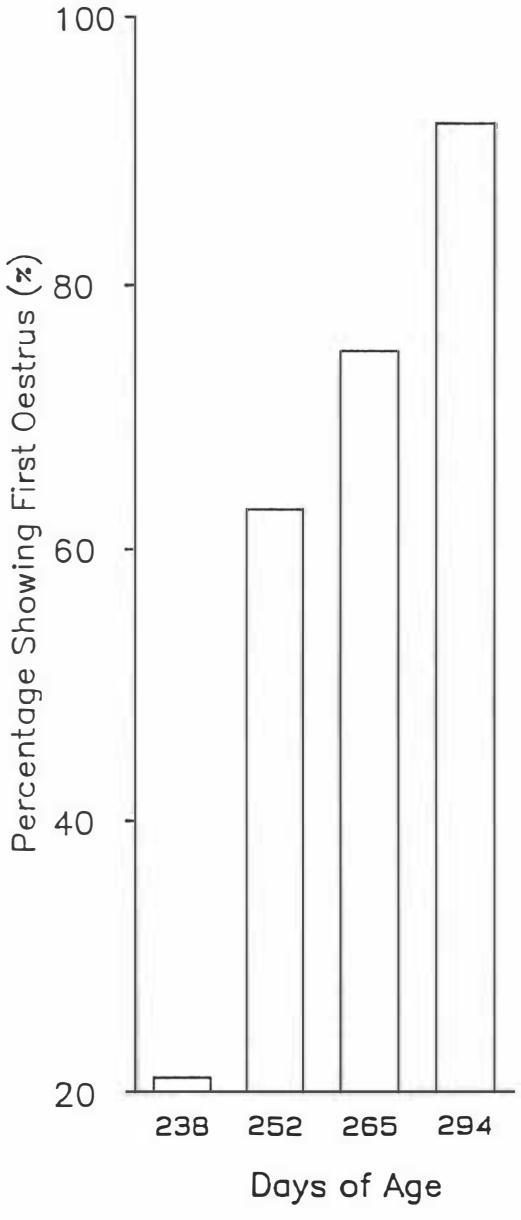

Figure 3. Proportion of females which had shown first oestrus by nominal 238, 252, 265 and 294 days of age.

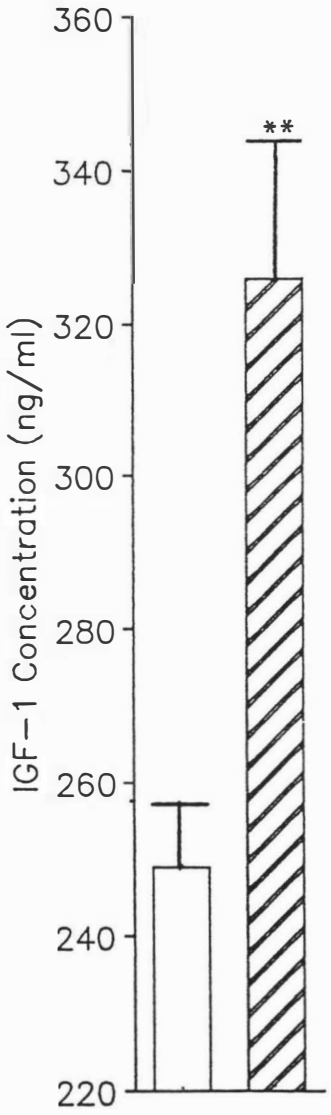

Figure 4. Plasma IGF-I concentrations in females which had attained first oestrus (striped bar) compared to those which had not (open bar) at nominal 252 days of age. Vertical bars represent the standard errors about the mean. (** $P<0.01$)

Analysis of the effect of puberty on plasma IGF-I was conducted using data from day 252, the time at which approximately equal proportions of females had and had not shown oestrus. As shown in Figure 4, IGF-I levels were significantly ($P<0.01$) greater at this time in females which had attained puberty (326 ± 16 ng/ml; mean ± S.E.M.) than in those which had not (249 ± 8 ng/ml). This effect was significant even when adjustments to common live weights and rearing ranks were made (see Table 4).

Table 4. Effects of rearing rank, live weight and puberty on plasma IGF-I concentrations in female hoggets at nominal 252 days of age.

Source[a]	df	Mean square and significance
Rank	1	2030
Live Weight	1	1489
Puberty[b]	1	30812**
Error	20	3169

a. All main effects treated as fixed.
b. Classification attained puberty vs. not attained puberty based on mating marks to day 252.
**P<0.01

Testis Diameter. Figure 5 depicts the average testis diameter of rams throughout the trial while Figure 6 shows the average testis diameter to live weight ratio. Although the average testis diameter to live weight ratio peaked at about 190 days, it was not possible to determine a definite relationship between testis diameter or diameter/weight ratio and the onset of puberty in the individual rams.

Fleece Weight. The greasy fleece weights obtained at the two shearings were summed to provide an estimate of the total greasy

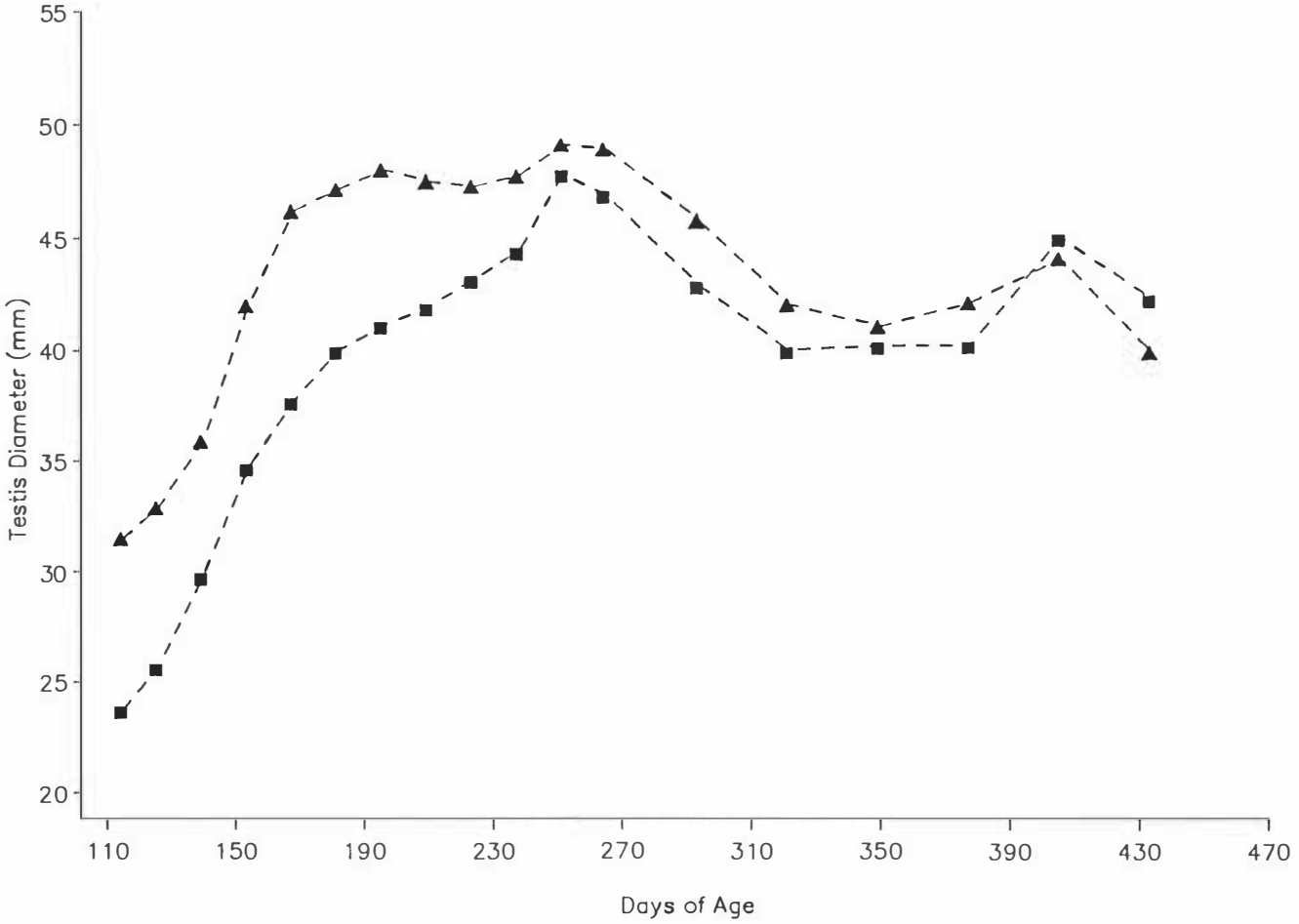

Figure 5. Average testis diameter measurements in single (▲) and twin (■) males over time (nominal days of age).

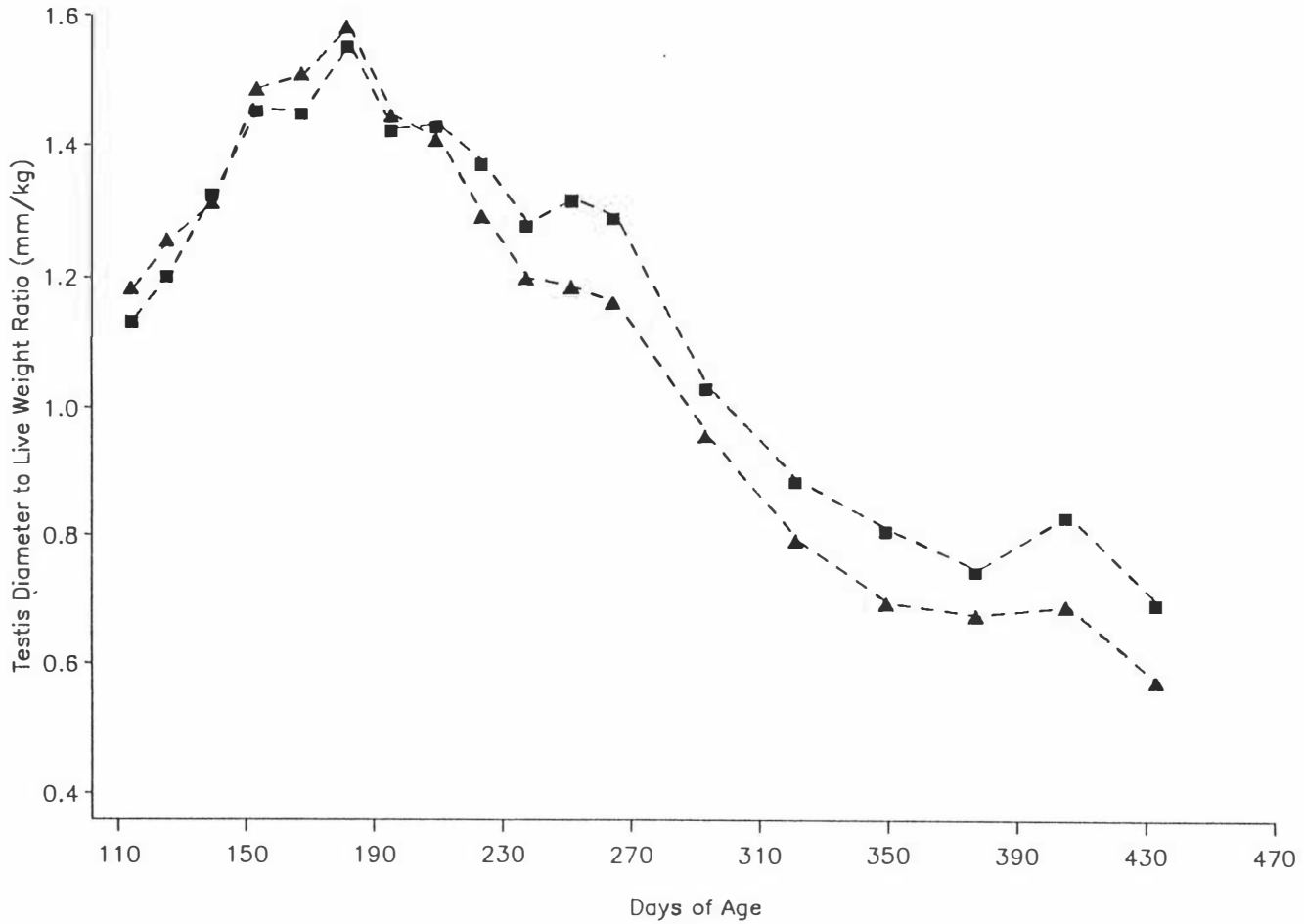

Figure 6. Testis diameter to live weight ratio in single (▲) and twin (■) males over time (nominal days of age).

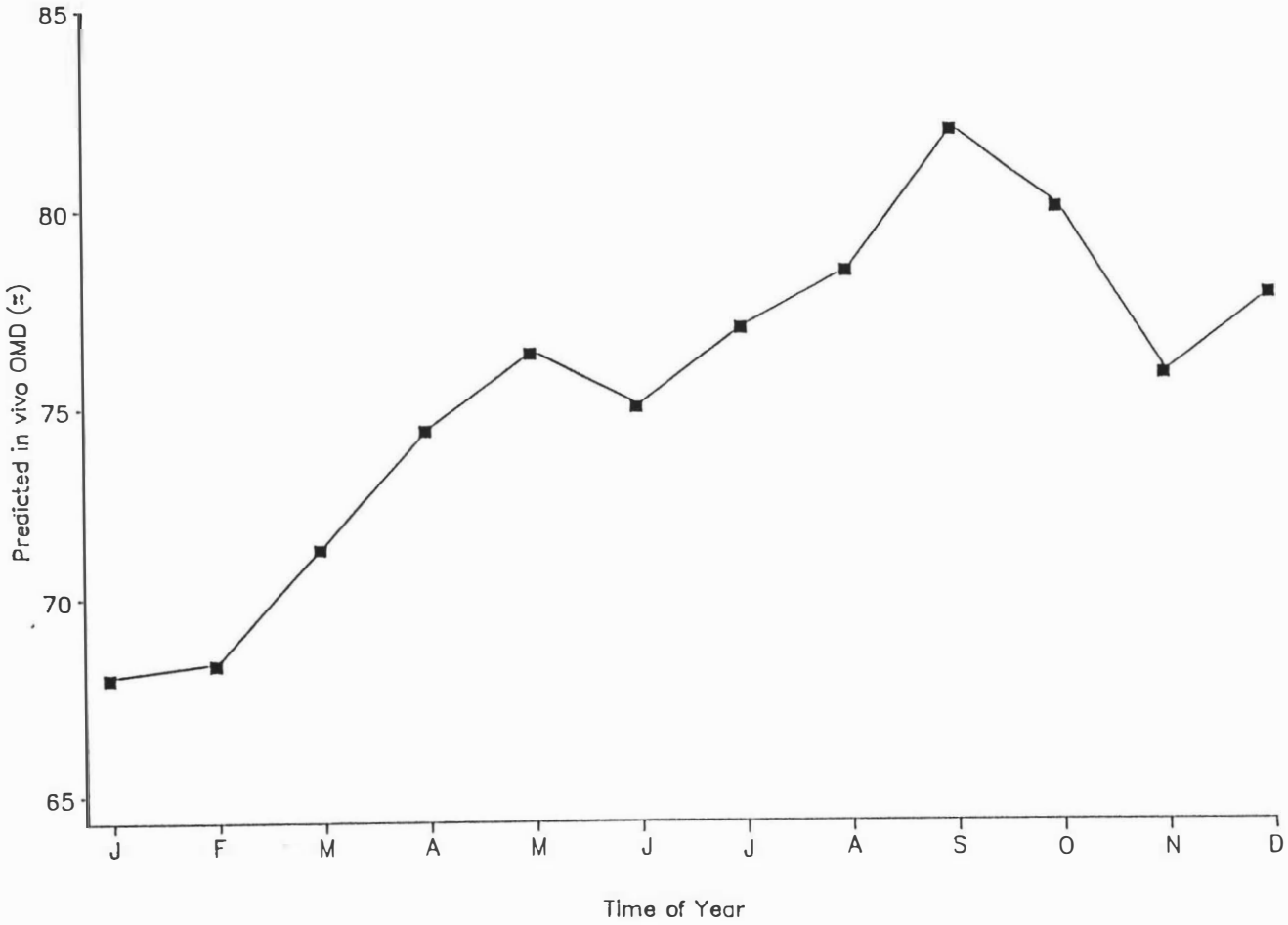

Figure 7. Predicted in vivo organic matter digestibility (OMD) of samples of pasture grazed by experimental animals throughout the experiment.

fleece weight (GFW) produced during the 420 day period (i.e. from the time shorn prior to the commencement of the study through to the last shearing. GFW (adjusted for sex and rank) was not significantly related to plasma IGF-I concentrations (see Table 5). Live weight accounted for most of the variation in GFW with heavier animals having higher fleece weights.

Table 5. Effects of sex, rearing rank, mean plasma IGF-I concentrations and mean live weight on total greasy fleece weight.

Source[a]	df	Mean square and significance[b]
Sex	1	2*
Rank	1	5**
IGF-I	1	1
Live Weight	1	10***
Error	40	0 (actual value = 0.39)

a. All main effects treated as fixed.
b. Mean square = 0 indicates values less than 0.5.
*P<0.05 **P<0.01 ***P<0.001

Pasture Analyses. The predicted *in vivo* digestibilities obtained from the pasture samples indicated that pasture quality was good throughout the year (Figure 7). The poorest quality pasture was during the dry summer months (January, February). Quality increased during the autumn/winter period, peaked in early spring and then decreased in late spring/early summer.

Discussion

The results of this study show that a number of non-genetic factors contribute to variation in plasma IGF-I concentrations. Plasma levels

increased with age in both sexes, and initiation of a two- to three-fold increase in the males around 238 days of age resulted in a marked divergence from the females. This divergence persisted throughout the remainder of the study. The sexual dimorphism in plasma IGF-I concentrations paralleled the effects of sex on live weight. Evidence of sexual dimorphism in circulating IGF-I has been found in other species (Copeland et al, 1985; Blair et al, 1987; Merimee et al, 1987). However, the ranking (i.e. males > females or vice versa) may vary with stage of development and may not be constant between species.

In an effort to determine the timing of puberty in the males, testis diameters were recorded and the ratio of diameter to live weight examined. Although the graph of average diameter/weight ratio for all the males showed the expected relationship (i.e. acceleration then deceleration of testis diameter relative to live weight), considerable variation in the individual animals made it impossible to accurately determine the time of puberty in each ram. The onset of the observed increase in plasma IGF-I levels in the males (230 days) was, however, close to the time of peak testis diameter to live weight ratio (190 days). Puberty in the females, as indicated by the onset of first oestrus, was associated with significantly increased plasma IGF-I concentrations. This agrees with work in other species including humans (Luna et al, 1983; Hall and Sara, 1984; Merimee et al, 1987), chimpanzees (Copeland et al, 1985) and mice (Siddiqui et al, 1989a). There is evidence that the rise in plasma IGF-I concentrations at puberty may be related to a rise in circulating sex steroids, as plasma IGF-I levels are increased by treatment with

physiological doses of oestrogens (Cuttler et al, 1985; Breier et al, 1988b) and androgens (Parker et al, 1984; Jasper, 1985). However, these results are not conclusive as Siddiqui et al (1989b) found that testosterone did not appear to influence pubertal growth of male mice via plasma IGF-I.

NEFA concentrations in this study occasionally had a significant relationship with plasma IGF-I concentrations. However, the animals were fed at or near ad libitum levels on good quality pasture so that nutritional status generally was not limiting. Where it occurred, the association between plasma IGF-I and NEFA was negative, i.e. animals with high NEFA concentrations (indicative of undernutrition) had reduced plasma IGF-I concentrations. This is in agreement with other work (Hawker et al, 1985; Breier et al, 1986; Ahlborn-Breier et al, 1987) which showed that plasma IGF-I concentrations were reduced during times of poor nutritional status in humans, steers and dairy bulls, respectively.

A regular drenching programme was used during this trial to suppress parasite load and resulted in low faecal egg counts (average less than 600 eggs per gram). Using faecal egg counts (FEC) as an indicator of parasite load resulted in only sporadic significant relationships with plasma IGF-I. Animals with high FEC (indicative of high parasite load) tended to have reduced plasma IGF-I levels. This agrees with a previous study which showed that parasite loads reduced plasma IGF-I concentration to a degree greater than that which could be explained by the associated decrease in nutrition (Elsasser et al, 1988).

In conclusion, the results of this study suggest that plasma IGF-I levels meet many of the criteria required of a genetic marker. Plasma concentrations of IGF-I appear to be genetically related to growth and production (Merimee et al, 1981, 1982; Eigenmann et al, 1984a,b; Binoux et al, 1986; Ahlborn-Breier et al, 1987; Merimee et al, 1987; Blair et al, 1989a), although the basis of genetic variation in plasma IGF-I and its covariation with productive traits requires further study. The high repeatability of circulating IGF-I in this trial implies that there would be little benefit in using more than one IGF-I determination as the basis for selection (i.e. the additional accuracy provided by further samples would not outweigh the costs of collection and analysis.) The marked effect of puberty on concentrations of IGF-I in plasma suggests that time of sampling in relation to pubertal stage will be an important consideration. In addition, if sampling were conducted after seven months of age it is unlikely that adjustments for rearing rank would be required. Finally, the conditions under which the selection is carried out may be important if variability in plasma concentrations of IGF-I is not to be limited by undernutrition or high parasite loads.

C H A P T E R III

GENERAL DISCUSSION

The results of this study lend further support to the view that plasma IGF-1 concentration is likely to be useful as a marker for live weight gain in sheep, and perhaps in other species. Earlier the attributes required of genetic markers were described. The following discussion summarizes the degree to which circulating IGF-I fulfills these criteria and identifies priority areas for future research in this regard.

Plasma IGF-I concentrations were shown to be highly repeatable in this study, indicating that a single sample would be adequate to measure circulating levels. In addition, it appears that plasma IGF-I does not display marked diurnal variation. This further supports the use of a single sample to evaluate an animal's status for plasma IGF-I. However, it must be acknowledged that diurnal variation in plasma IGF-I has not been extensively studied and that the repeatability estimates obtained in this study were based on samples taken at a standard time each day. Even though sampling can easily be done in the field, caution is required to ensure that poor nutrition or parasite burdens do not hinder the expression of genetic variation in plasma IGF-I. Conversely, one might argue that sampling should be conducted during a 'challenge' (i.e. while the animals are burdened with parasites or on a low level of nutrition). In as much as it is not known whether such a challenge would result in the selection of animals more resistant to external factors and therefore better able

to perform in an uncontrolled environment, further work is required to investigate the consequences of different sampling regimes.

The fewer samples required to measure an animal's status for plasma levels of IGF-I compared to other metabolic parameters currently being investigated as potential genetic markers (e.g. growth hormone, glucose, urea) would likely result in less expensive assessment for selection purposes. However, there are relatively few laboratories in the world capable of effectively conducting radioimmunoassay assessment of IGF-I which limits the immediate possibilities for using this hormone as a genetic marker.

The results from this study showed a marked decline in plasma IGF-I concentrations from November (430 nominal days of age). This was most apparent in the males but it was also observed in the females to a lesser extent. From this study it was not possible to determine the cause of this decline. As discussed previously, work in some species (e.g. humans, rats and mice) would suggest that the decline was part of an inherent ontogenic pattern. However, results from other species would suggest that it is a seasonal response. Suttie et al (1988) found that circulating IGF-I levels had a seasonal pattern in red deer stags, peaking in October to a level significantly greater than those observed at other times of the year. Although the cause of this pattern is not known, it did follow a peak in mean ST levels in the stags (Suttie et al, 1988). If seasonal variation does occur in plasma IGF-I concentrations it is likely that responsiveness to seasonal pattern may vary between animals and species. For example, red deer are more seasonal than many breeds of sheep. Hence their

plasma IGF-I levels may be more responsive to seasonal changes. If this is true it would be necessary to determine the magnitude of seasonal variation between genetically different animals (i.e. do genetically superior individuals show more or less response to seasonal changes compared to inferior individuals) in order to use plasma IGF-I as a genetic marker. It might also be interesting to determine what had the greatest influence on plasma IGF-I levels in deer, puberty or seasonal variation.

In order to assess seasonality of plasma IGF-I in sheep it will be necessary to separate the effects of age and season, for example by comparing spring-born and autumn-born lambs at equal ages but in different seasons (or vice versa). Other aspects which should be considered in relation to seasonal variation are indoor comparison studies of animals to separate the effects of photoperiod and feed quality/allowance, and the role of melatonin in regulating seasonal effects on plasma IGF-I.

In order to be useful as a genetic marker, a trait must be heritable and be genetically correlated with the production trait of interest. Estimates of the heritability of IGF-I have been derived in mice (Blair et al, 1987, 1989a) and cattle (Davis and Bishop, 1989). In mice, Blair et al (1987) found estimated heritabilities of 0.40 ± 0.27, 0.19 ± 0.21 and 0.17 ± 0.28 at 35, 80 and 110 days of age, respectively. It should be noted that although a fostering experiment was specifically designed to estimate the heritability at 35 days of age in the absence of confounding maternal effects, the estimate (i.e. 0.40 ± 0.27) was calculated from limited data and should therefore be

taken only as an indication of possible genetic variation (Blair et al, 1987). This is further supported by a realised heritability of 0.15 ± 0.12 calculated in mice after seven generations of divergent selection for litter mean plasma IGF-I (Blair et al, 1989a). The value for realised heritability was reduced relative to earlier estimates by a very limited response to divergent selection during the last two generations of selection. However, the cause of the decline in response is unknown. Davis and Bishop (1989) found that circulating IGF-I levels in Simmental crossbred identical twin heifers (produced by embryo splitting and transfer) had a range of estimated heritabilities from 0.08 ± 0.38 to 0.68 ± 0.21 over five ages ranging from 291 to 684 days of age. It should be noted the number of animals in their trial was low (8 sets of twins) and the estimates which they gave should only be used as a guideline. Nevertheless, these results suggest that there is genetic variability in circulating levels of IGF-I although the heritability may vary according to age/stage of development at time of sampling. Furthermore, the variation in heritability with age/stage of development may not be specific between species. Clearly extensive studies to accurately determine the heritability of circulating IGF-I are now required.

There are two main ways of determining the genetic correlation between plasma IGF-I and live weight or other production traits. These are analyses of covariation between relatives (e.g. full- or half- sibling relationships or parent-offspring relationships) and comparisons of divergent selection lines. The development of divergent selection lines also provides a unique model for studying the physiological basis and consequences of genetic diversity between

sheep of differing plasma IGF-I concentrations as well as the direct and correlated responses to divergent selection.

Following the encouraging results obtained in mice (Blair et al, 1987, 1988, 1989a) and this experiment, Massey University has initiated a selection experiment to develop lines of Romney sheep divergent for circulating IGF-I levels. Briefly, 111 twin Romney ram lambs from three different sires were weaned, weighed on four occasions and blood sampled twice during the first six weeks post-weaning. A fifth weight and a fleece weight from five months of growth (from weaning) were recorded at nine months of age. Twelve of these rams were selected to become the foundation sires for the divergent selection lines, based on their plasma IGF-1 concentrations. Four rams from each extreme (high and low) were selected, with four other rams being randomly chosen for the control line. Rams were randomly mated to 25 ewes each (100 ewes/line) and records were collected from 245 offspring in the first generation.

The preliminary results from the selection study (H.T. Blair - pers. comm.) indicate that there was a significant sire effect on plasma IGF-1 concentrations. This agrees with the results from the initial group of 111 ram lambs, in which significant effects of sire on plasma IGF-I were apparent (Blair et al, 1989b). Although sire effects were significant, line effects were not. Thus phenotypic levels of IGF-I in the ram lambs which were subsequently used as sires apparently did not accurately reflect their genetic ranking for this trait. This could have been due to poor nutritional levels at the time of blood sampling the ram lambs. The ram lambs which were to

become first generation sires in the selection lines were blood sampled during a dry hot summer. This may have limited their ability to fully express genetic variation for IGF-I. It could be possible to determine if the ranking of the rams for plasma IGF-I still agrees with the original ranking by re-sampling the rams since the results of the present study showed that plasma IGF-I was highly repeatable over extended periods of time.

In the first generation of the selection lines, sex of the animals did not have a significant effect on circulating IGF-1 at the time of sampling (H.T. Blair - pers. comm.). This is in agreement with the results from the present study when compared at equivalent ages (i.e. at the first sample of the present study, the sex effect was non-significant but thereafter the effect became highly significant). In contrast to the present study, the effect of rearing rank was also non-significant in the selection lines. The cause of this difference is not clear but the average weight of singles four weeks post-weaning was 3.7 kg heavier than that of twins in the selection study compared to 6.4 kg in the present study. This may suggest that twin lambs in the selection trial were not influenced or handicapped by maternal effects to the same extent as twins in the present study. Another possible factor is nutrition, as sampling of the first generation progeny was again conducted during a dry, hot summer and feed supply could have been limited.

Based on one cycle of selection the realised heritability for plasma IGF-I in sheep was estimated to be 0.2 (H.T. Blair - pers. comm.). This again indicates that plasma IGF-I has a heritable

component in sheep. Furthermore, the difference in plasma IGF-I levels between lines was 26 ng/ml which reflects the apparently low heritability of the trait. Such a small difference would make it difficult to observe a significant line effect during a single year of selection but a cumulative effect might be apparent over a period of years. This may explain the lack of significant line effect mentioned above.

This study showed that plasma IGF-I levels are highly correlated with live weight in sheep although they appear to have no significant relationship with greasy fleece weight (other than through the relationship with live weight). In addition, results from mice after seven generations of divergent selection based on plasma IGF-I have shown that high line mice are heavier than those from the low line but that there is no apparent compositional difference at similar live weights (Siddiqui et al, 1989a). Kroonsberg et al (1989) found that high line females were 14% heavier than those of the low line at mating. This increase in mature live weight was also accompanied by increases in the number of fetuses per dam, the weight of individual fetuses and the weight of the mammary gland (Kroonsberg et al, 1989). If similar results occurred in sheep selected for plasma IGF-I, the increase in mature live weight would result in increased ewe flock maintenance requirements, a potential disadvantage in commercial sheep operations. However, when considered on a metabolic live weight basis, the increase would not be particularly great (i.e. a 14% increase in live weight would reflect a 10% increase in metabolic live weight for a 55 kg ewe). Furthermore, the positive effects on reproductive performance as well as increases in weaning weights of lambs would

counteract the negative influence of increased maintenance requirements. Thus, present results suggest that selection on the basis of plasma IGF-I would not have any adverse effects on productive parameters other than the possible disadvantage of increased ewe live weights and maintenance requirements.

In conclusion, there is increasing evidence to support the role of plasma IGF-I as a genetic marker for live weight. It is unlikely, however, that selection will ever be based on plasma IGF-I alone. Rather, a number of metabolic traits are likely to be combined into a selection index. The exact nature of such an index and other possible components of the index are yet to be determined and can only be assessed by extensive progeny testing. However, as discussed here, it is likely that plasma IGF-I will be one important component of the index in situations where selection for high live weight gain is desirable.

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