This is to certify that the

dissertation entitled

Expression of Alpha Actin in Tissues of Livestock Species, Ractopamine and Neonatal Testosterone Effects on Skeletal Muscle and Protein Metabolism in Pigs

presented by

David Møberg Skjaerlund

has been accepted towards fulfillment
of the requirements for

Doctor of Philosophy degree in Animal Science

Robert A. Merkel

Robert A. Merkel
Major professor

Date July 15, 1993

EXPRESSION OF ALPHA ACTIN IN TISSUES OF LIVESTOCK SPECIES, RACTOPAMINE AND NEONATAL TESTOSTERONE EFFECTS ON SKELETAL MUSCLE AND PROTEIN METABOLISM IN PIGS

By

David Møberg Skjaerlund

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1993

ABSTRACT

EXPRESSION OF ALPHA ACTIN IN TISSUES OF LIVESTOCK SPECIES, RACTOPAMINE AND NEONATAL TESTOSTERONE EFFECTS ON SKELETAL MUSCLE AND PROTEIN METABOLISM IN PIGS

By

David Møberg Skjaerlund

Four boars and four barrows were allotted to one of six groups to assess skeletal muscle growth and protein metabolism. Castration was performed within 24 h of birth,and all pigs remained with their dams until slaughtered at either 1,2 or 4 wk of age. Castration at birth did not affect muscle weights, nucleic acid concentrations or content, nor in vivo or in vitro protein synthesis rates. However, developmental changes in all measures of skeletal muscle protein metabolism were seen during this 4 wk neonatal period.

In the second experiment, a human skeletal alpha actin cDNA probe was characterized for use with livestock species. Three market weight animals were slaughtered in order to obtain tissue samples from each of the meat producing livestock species: porcine, bovine, ovine and avian. The four tissues of interest were skeletal muscle, heart, smooth muscle (stomach or gizzard) and liver. No hybridization was observed with RNA from liver or smooth muscle from any of the species

suggesting little or no hybridization to nonmuscle and smooth muscle beta and gammma actin isoforms. The probe hybridized to RNA from skeletal muscle of pigs, cattle, sheep and chickens although relative hybridization was 75% less with chicken RNA.

In the third experiment, sixty crossbred barrows were used to study the effect of ractopamine (a phenethanolamine-beta-adrenergic agonist) treatment (2, 4, 6 wk) and its withdrawal (1, 3, 7 d) on muscle growth and on the relative abundance of skeletal muscle alpha actin mRNA. Ractopamine increased longissimus muscle weight, total DNA, RNA and protein content at 4 wk and this increase was maintained when ractopamine was withdrawn for 7 d. The relative abundance of skeletal muscle alpha actin mRNA was increased 41 and 62% only at 2 and 4 wk, respectively. These results indicate that the ractopamine-enhanced muscle growth may result from increased myofibrillar gene expression at the pretranslational level.

ACKNOWLEDGEMENTS

I would like to thank the Department of Animal Science for their financial support and the opportunity to pursue my PhD. I am also very grateful for the teaching and research experience. The friendships and memories resulting from the privilege of coaching the meat judging team will be long lasting. My appreciation and gratitude is extended to Dr. Hogberg for these great opportunities and for his input, starting with my first "hog consultation" as a youngster.

My deepest gratitude is extended to Dr. Merkel for his exemplary devotion to teaching, research and his graduate students. Words cannot express all my appreciation for his continual encouragement, his open door and availability to talk, his understanding and his never-ceasing concern, not only for my academic career but also for my family life. He has been a genuine role model and an excellent mentor, whose advice I have cherished greatly over the many years. Thank you for all your guidance and for the honor to work as your student.

My appreciation is extended to Dr. Bergen for the opportunity to work in his lab and for all his input into my research over the years. I am very grateful for his patience and support while I attempted to learn the ropes of molecular biology. I am also indebted to Dr. Helferich for the use of his lab in completing this research and for his expertise and input. My appreciation is also extended to Dr. Grant, fellow graduate student, whose unselfish help, assistance and friendship will not be forgotten.

I wish also to thank the rest of the faculty, Drs. Ames, McIntosh, Miller and Romsos, who have provided assistance and guidance with the completion of my graduate program. I have appreciated working with and have enjoyed the friendship over the years of Tom, Dora, Bob, Bea, Debbie, Aubrey, Sally, Alan, Buddy, Don, Jan and the many others who have passed through the halls of the Meat Lab.

This dissertation would not have been completed without the constant encouragement and prayers of my entire family. Special thanks to my wife, Marcia, for patience, understanding and encouragement to keep pressing on. Most of all, I have appreciated her never-wavering belief in me. Thanks Mom for all your prayers which have carried us all through difficult times and for those meals which were like "manna" in graduate school. Thanks Dad for your attitude of perseverance and optimism and for molding into me the character which has enabled me to pursue and complete this degree. Your wisdom has been greatly valued and cherished.

I wish I could thank again my brother, John, M.D., Ph.D., D.V.M., M.S., M.S., (1954-1992) for his example of diligence, honesty, integrity, discipline, selfless and flawless moral character. I will never forget all the exciting hours spent, even on mountain tops, discussing science, new discoveries in research and the world around us. His friendship and love has been and will continue to be greatly missed. Special thanks goes to my sister, Anne Fege, Ph.D., and to all the other family members and friends for the frequent and persistent question, "How is the thesis going?" Yes, it is finished!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Understanding the mechanism and regulation of muscle growth is of vital importance to meat animal agriculture. Total muscle mass represents 55 to 60% of total carcass weight and 30 to 40% of total live weight (Mulvaney, 1981). The goal of meat animal agriculture is to increase efficiency of lean meat production.

Muscle growth can occur by hypertrophy, an increase in cell size, or by hyperplasia, an increase in cell number. Prenatal muscle growth is primarily a result of hyperplasia. During embryonic development there is a tremendous increase in cell number but muscle cell number does not increase substantially after birth (Allen et al., 1979). There are some reports of modest increases in fiber number, depending upon the extent of embryonic development at the time of birth (Goldspink, 1972; Swatland, 1976). Most of the muscle growth postnatally can be attributed to hypertrophy as myofibrillar proteins accumulate.

Muscle hypertrophy is the difference between protein synthesis and protein degradation. Both processes are important and each is regulated independently. Protein synthesis can be regulated at the level of translation or by a pretranslational event such as transcription. Millward (1980) suggested that fractional synthesis rate varies inversely as a function of the protein:DNA ratio, or DNA unit.

Lewis et al. (1984) suggested that fractional synthesis rates are dependent upon the concentration of RNA and ribosomes. Caravatti et al. (1982) demonstrated that abundance of mRNA is closely correlated to protein synthesis rates and transcription of RNA may be an important indicator of protein synthesis. By gaining an understanding of the changes in the protein synthetic machinery (DNA, mRNA, rRNA and tRNA) that occur during the growth of an animal, more specific questions can then be addressed as to how the specific control points of protein synthesis are regulated throughout postnatal development.

Advancements in molecular biology have provided new tools of research that enable us to readily and more specifically answer questions regarding the genomic and pretranslational regulation of muscle growth. Animal agriculture needs to utilize such tools for research with livestock species. In order to understand more about the genomic regulation of a major muscle protein, e.g. actin, in livestock species, the specific aim of this project was to validate the use of a human skeletal muscle alpha actin cDNA to monitor the abundance of porcine skeletal muscle alpha actin mRNA during postnatal development. With the ultimate goal of understanding gene regulation of myofibrillar proteins, a major portion of the work was directed toward characterizing the use of this skeletal muscle alpha actin cDNA probe in livestock species in order to gain information regarding

pretranslational regulation of growth (Skjaerlund et al., 1984; Helferich et al., 1988; Helferich et al., 1989; Skjaerlund et al., 1989).

Intact males are more efficient in conversion of feed to gain and produce carcasses at market weight with 20 to 30% less fat than barrows (Field, 1971; Mulvaney, 1984; Knudson et al., 1985a, 1985b). Gonadally intact males also have 8 to 15% greater muscle mass than castrated males (Prescott and Lamming, 1967; Mulvaney, 1984; Knudson et al., 1985a). Postpubertal circulating testosterone appears to enhance skeletal muscle growth and improve carcass traits. Circulating testosterone concentrations are also elevated in boars during the first few weeks after birth (Colenbrander et al., 1978; Ford, 1983). The objective of this study was to determine the effect of elevated perinatal testosterone concentrations on skeletal muscle growth and protein metabolism.

Similar to the effect of testosterone on skeletal muscle growth, administration of the phenethanolamine, ractopamine, to finishing pigs increases muscle mass, total muscle protein and RNA content, and fractional synthesis rates (Bergen et al., 1989). A time-course study in which muscle growth and abundance of skeletal alpha actin is monitored during administration and withdrawal of ractopamine to pigs is necessary to identify mechanisms mediating ractopamine-induced muscle hypertrophy. The last objective of this study was to

monitor changes in skeletal muscle protein metabolism and skeletal alpha actin mRNA abundance in pigs fed ractopamine during a 6 wk feeding period and a subsequent 7 d withdrawal period.

LITERATURE REVIEW

Protein Turnover

Muscle is in a dynamic state of turnover as protein is continually being synthesized and broken down. Schimke (1970) estimated that only a small pool of free amino acids is present within a cell, representing approximately .5 % of the total amino acids (Figure 1). The continual breakdown of protein provides most of the amino acids needed for synthesis. Millward et al. (1975) have suggested that some 80% of the amino acids derived from the degradation of protein are reutilized for new protein synthesis. Protein turnover is a major process and in the rat, growing pig and man, protein synthesis appears to account for between 15 and 20% of overall heat production (Waterlow et al., 1978; Reeds and Lobley,

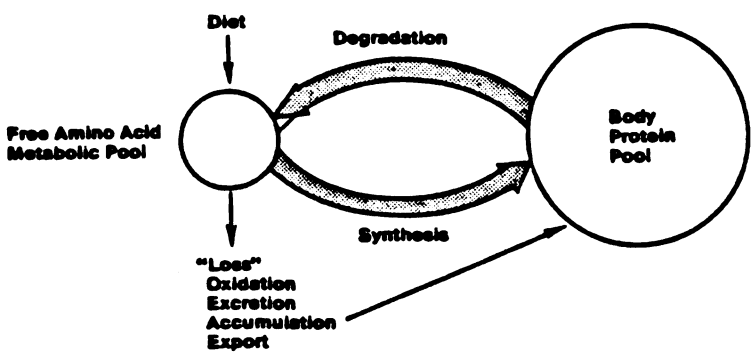


FIGURE 1. Relationship of amino acid pools in protein turnover. Reprinted from Bergen et al. (1987).

1980). Garlick et al. (1976) estimated that 17% of the total metabolic rate could be accounted for by protein turnover.

The extent of protein accretion is dependent on both protein degradation and protein synthesis. Muscle growth occurs when the rate of protein synthesis exceeds the rate of protein breakdown. Millward et al. (1976) indicated that during a steady state situation, the two rates are equal. During growth, the amount of protein synthesized each day exceeds the net amount of protein accumulated each day by a factor of two to three (Swick, 1982). Fractional protein synthesis rate (FSR), fractional breakdown rate (FBR) and fractional accretion rate (FAR) are the respective rates relative to total protein and are interdependent such that FAR = FSR - FBR. Fractional rates are normally expressed as the percent of total protein pool that is synthesized, degraded or accumulated per day.

A high rate of protein turnover appears to be a wasteful process. Young and Pluskal (1977) demonstrated that only a small percentage of the total muscle protein synthesized is for actual accretion. Mulvaney (1981) estimated only 20% of the skeletal muscle protein synthesized per day is actually deposited in young growing boars. Laurent and Millward (1980) have estimated that total protein synthesis can be partitioned into 68% for normal replacement, 9% for growth and 23% for wastage during stretch induced hypertrophy of adult fowl. Goldberg and Chang (1978) suggested that protein

breakdown releases amino acids that can be utilized for gluconeogenesis.

Schimke (1977) stated that protein breakdown is essential in removing abnormal proteins and that cells have a general mechanism for protein degradation rather than a specific process for the removal of only abnormal proteins or proteins that are no longer needed. As Swick (1982) elaborated, degradation allows tissues and organs to remodel and restructure during growth. The high rate of protein turnover seems to have a biological significance, perhaps allowing for greater sensitivity in regulating the amount of specific proteins. With a rapid turnover, enzyme quantity can be more quickly altered than with proteins that have a low rate of turnover. In addition, small changes in protein synthesis and protein degradation allows for large, compounded changes in the final protein quantity. The energetic efficiency of muscle accretion could be greatly increased by altering the rates of protein synthesis and breakdown.

The rates of protein synthesis per kilogram of body weight are greatest in the smallest animals. Garlick et al. (1976) demonstrated that the rat has two to three times greater fractional synthesis rates than the pig. The differences are less when rates are compared on the basis of body weight$^{.75}$, with the growing pig having a faster rate than the rat, sheep or man (Waterlow et al., 1978). Turnover rates also vary between tissues. Garlick et al. (1976) reported

that rates of protein synthesis for 75 kg pigs in liver and kidney (24%) were three times those of the brain (8%), while the rates for heart (6.8%) and skeletal muscle (4%) were lower. Protein synthesis in skeletal muscle is a major contributor to total body protein synthesis. Garlick et al. (1976) state that muscle contributes 42% of whole body protein synthesis in the pig and only 19% in the rat. Even though 70% of liver protein is replaced every 4 to 5 days (Schimke, 1977), liver represents only approximately 10% of whole body protein synthesis in both animals (Garlick et al., 1976).

Protein synthesis rates vary between muscle fiber types. In young growing animals, protein synthesis rates for muscle with predominantly white fibers were greater than those for muscle with predominantly red fibers, but the opposite is true in adults (Arnal et al., 1976; Maruyama et al., 1978). In adult rats, protein synthesis rates of 10 to 12%, 8 to 10% and 4 to 5% were reported for heart, soleus (red muscle) and quadriceps (white muscle), respectively (Arnal et al., 1976). Protein turnover rates in chick anterior latissimus dorsi (slow tonic, red muscle) were estimated by Laurent et al. (1978) to be three times greater than in the posterior latissimus dorsi (fast twitch, white muscle) and five times greater than white breast muscle. Mulvaney (1983) showed that the red portion of porcine semitendinosus muscle had greater in vitro protein synthesis and protein degradation rates than the white portion. This observation is consistent with data

reported by Millward (1980). Richmond and Berg (1982) suggested that the relative growth rate of muscles appears to be related to muscle function. They found that those muscles associated with mobility and propulsion showed much earlier development than those concerned with posture. Postural muscles (red fiber types) appeared to grow at the same relative rate as total muscle, with a proportionally greater increase later in life.

Turnover rate of sarcoplasmic proteins is greater than that of the myofibrillar proteins. Bates and Millward (1983) demonstrated that sarcoplasmic proteins in adult rats were synthesized and degraded at twice the rate of myofibrillar proteins. Individual myofibrillar proteins also differ in their turnover rates and half-lives. Using SDS gel filtration, Schreurs et al. (1985a) demonstrated that the turnover rate of myosin heavy chains and myosin light chains relative to actin was 1.5 and 3.3 times greater, respectively. This suggests that turnover of the functional unit, i.e. the myofibril, does not turnover as a whole but that the individual subunits are replaced at individual and independent rates. Contractile proteins are more sensitive to nutritional and physiological states, increasing to a greater extent than the soluble proteins during rapid growth, and decreasing more extensively during starvation (Millward and Waterlow, 1978; Bates and Millward, 1983). For example, Schreurs et al. (1985b) demonstrated that the rate of [^{14}C]tyrosine

incorporation into muscle and liver decreased 50% and 20%, respectively, when 3 month old rats were fed a protein-free diet.

Muscle protein turnover is unique in that it shows a marked developmental decrease as the animal matures. In general, protein synthesis and protein degradation are positively correlated with both being elevated during rapid growth and both decreased during slow stages of growth (Waterlow et al., 1978). Protein degradation rates in rats were elevated during rapid growth and breakdown was reduced during slower growth (Millward et al., 1981) On the other hand, Ogata et al. (1978) reported that the increased growth in young rats is the result of high protein synthesis and low protein degradation.

The decline in total protein turnover during development is due to a combined decrease in both the fractional synthesis rate and the fractional degradation rate (Millward, 1980). Quadricep muscles of 3 week old rats had fractional synthesis rates greater than 22% and by 1 year values were less than 5% (Millward et al., 1975). Waterlow and Stephen (1967) also observed an overall decline in protein degradation rates with increasing body weight of the rat. Shrivastava and Chaudhary (1969) showed a decline in in vivo and in vitro incorporation of [^{14}C]-leucine into proteins of skeletal muscle during the development of the mouse from birth to 1 year of age. In lambs, the fractional protein synthesis rates decreased from

24% at 1 week to 2% at 16 weeks (Arnal et al., 1976). A decline in fractional synthesis rates from 25 to 8% was observed by Maruyama et al. (1978) in muscles from chickens between 1 to 2 weeks of age. In young rats, myofibrillar protein synthesis rates declined more rapidly than the sarcoplasmic protein synthesis rates from 35 to 100 g body weight (Bates and Millward, 1983). After 100 g body weight, the decline in myofibrillar protein synthesis rates paralleled that of the sarcoplasmic proteins. No comprehensive study documenting the birth to puberty changes in protein turnover has been reported for pigs.

Declining turnover with age has also been demonstrated for humans. Using 3-methylhistidine excretion as an indicator of muscle protein degradation, Munro (1976) and Tomas et al. (1979) showed a decline in excretion from neonatal infants to mature adults. Waterlow (1967) stated that the grams of protein synthesized per day were lower for adults than for young men or children. The decline in protein turnover and subsequently lower metabolic rates are the reason that man and animals need to decrease caloric intake as they mature to avoid excessive deposition of fat.

Measurement of Protein Turnover

Many procedures have been developed to measure protein synthesis and protein degradation. These have included invasive in vivo procedures and to a lesser degree, noninvasive in vivo methods or in vitro approaches and either

measure whole body protein turnover or the protein turnover of a specific tissue or protein. In vivo methods are time consuming and expensive due to enormous isotope costs for large animals as well as the cost of the animals and their disposal. Protein accretion or FSR can easily be measured by determining the net gain in protein or muscle over a given period of time (e.g., 1 week). Protein synthesis rates can be determined directly with the administration of a radiolabeled tracer, such as [^{14}C]-tyrosine, and the rate of its appearance in protein is measured. Having determined FAR, and FSR with continuous infusion of a radiolabeled amino acid, protein degradation is normally calculated using the formula: FBR = FSR - FAR.

Some of the specific approaches have included polysomes profiles (Noll, 1969), initiation and translation assays (Alexis et al., 1972; Bergen, 1974), in vitro and in situ perfusions (Goldberg et al., 1975, sections 1-4), in vitro tissue incubations (Fulks et al., 1975; Skjaerlund et al., 1988), in vitro tracer methodology and protein synthesis rate measurement based on single or constant tracer administration and whole body protein synthesis based on amino acid flux (Waterlow et al., 1978; Zak et al., 1979; Wolfe, 1984). An excellent review of some of these procedures has been described by Bergen et al. (1987) which includes a detailed and specific discussion of the kinetics and factors involved.

Methodology for measuring protein synthesis is based on

several assumptions. First, it is assumed that the precursor pool or free amino acid pool is homogenous and in a steady state. Secondly, it is assumed that the isotope enters the precursor pool only by exogenous administration and a complete and random mixing of the precursor pool occurs. Finally, it is assumed that there is a constant amount of isotope incorporated per unit of time (Waterlow et al., 1978; Reeds et al., 1980).

There has been a long standing debate as to the origin of the precursor pool. Observations by Hider et al. (1969, 1971) appeared consistent with a model based on the premise that amino acids were incorporated directly from the extracellular pool without first equilibrating with the intracellular pool. Li et al. (1973) and Alemany (1976) proposed that the free intracellular pool resembled a precursor for protein synthesis while the extracellular pool did not. Airhart et al. (1974) and Ward et al. (1984) suggested that amino acids for protein synthesis are derived from a combination of both intracellular and extracellular pools and that perhaps the majority comes from the extracellular pool. Recently, Irvine et al. (1990) suggested that sarcoplasmic proteins may exist in at least two sub-populations which have different turnover rates and which also may obtain their amino acids for synthesis from different precursor pools. Because of the variation in precursor pool location and the possible dilution of labeled tracer with recycled amino acids within the tissue, all measurements of

precursor pool specific activity are subject to some degree of error.

Common methods of administering an isotope tracer for FSR determination of a specific tissue is through a single tracer dose injection, a single massive flooding dose tracer infusion or a continuous tracer infusion (Bergen et al., 1987). The single dose injection of a tracer amino acid is the easiest method to administer and the period of label incorporation is kept relatively short. With this method, a single dose of labeled tracer is incorporated and the specific activity of the free amino acid is intially high and then declines rapidly. Several data points must be collected to determine the specific activity of the tissue free and bound protein pools. Because many data points must be obtained to accurately estimate the rapidly declining precursor pool specific activity (Zak, 1979), a large number of animals is necessary which becomes cost prohibitive with livestock species. In short term studies, the difference between extracellular and intracellular specific activity also becomes a problem. Henshaw et al. (1971) found that following injection of a large flooding dose of unlabeled amino acid along with the labeled tracer, the specific activity of the free pool in plasma, muscle and liver rapidly rises to near that of the specific activity of the injected label and remains relatively constant for a short period of time. Thus, a large number of animals are no longer needed to accurately

determine the specific activity of the precursor pool.

Constant specific activity of the precursor pool is obtained with the continuous infusion method developed by Waterlow and Stephen (1967, 1968) and modified by Garlick et al. (1973). With the continuous infusion of a labeled amino acid at a constant rate for several hours, the specific activity of the precursor pool reaches a plateau which is maintained during the course of infusion. Protein synthesis rates, based upon specific activities of the free and bound tissue pools, are then determined at the end of infusion during which labeled tracer incorporation into protein is linear. The rise in tissue free pool specific activity parallels that of the plasma specific activity, although the specific activity of the tissue pool is much lower (Waterlow and Stephen, 1968; Garlick et al., 1973). The tissue free pool specific activity is lower due to the dilution by unlabeled amino acids resulting from degradation of protein (Waterlow et al., 1978). Contribution of recycled amino acids to the tissue free pool can range from 20 to 30% (Gan and Jeffay, 1967; Aub and Waterlow, 1970). The continuous infusion method has the advantage of determining FSR in vivo using only one animal per determination. The disadvantages of applying this method with livestock species is the cost of large quantities of isotope required for these animals as well as their high cost of disposal.

A more cost efficient method would be to measure protein

synthesis and degradation rates in vitro on biopsy samples which requires smaller quantities of isotope and the remaining portions of the animal can be salvaged. In order to alleviate some of these problems, a procedure for calculating in vitro protein turnover rates (Fulks et al., 1975) has been modified for use in livestock species (Mulvaney et al., 1983; Skjaerlund et al., 1984; Bergen et al., 1987; Skjaerlund et al, 1988). Small intact muscles or muscle strips are used to determine protein synthesis by measuring the rate of incorporation of a radiolabeled amino acid into protein and protein degradation is measured by tyrosine released from the muscle into the medium. This method requires small amounts of isotope for meat animal species and the analytical procedures are less involved than in vivo methods. The limitation of this procedure is that it is characterized as having a net negative nitrogen balance and degradation rates are often higher than synthesis rates. The procedure cannot determine absolute rates but does reflect relative rates observed in vivo (Skjaerlund et al., 1984).

Protein synthesis is a complex process and can be considered to consist of two stages (Figure 2). The first stage involves the nucleus, in which DNA is transcribed into various ribonucleic acids (mRNA, tRNA, and rRNA) for transfer to the cytoplasm where they are utilized for polypeptide synthesis. This stage includes transcriptional (synthesis of RNA from DNA) and pretranslational (processing and transport

of RNA) events. The second major stage is limited to the cytoplasm where the polypeptide chain is synthesized, assembled and modified (translational and posttranslational events). Regulation of protein turnover can occur at several points along the entire protein synthesis pathway, which affects the final protein. Most traditional methods of determining protein synthesis rates measure the final end product and cannot determine whether changes in synthesis rates have resulted from changes in transcriptional, pretranslational, translational or posttranslational events. In order to more specifically understand the regulation of protein synthesis, a focus on transcriptional or

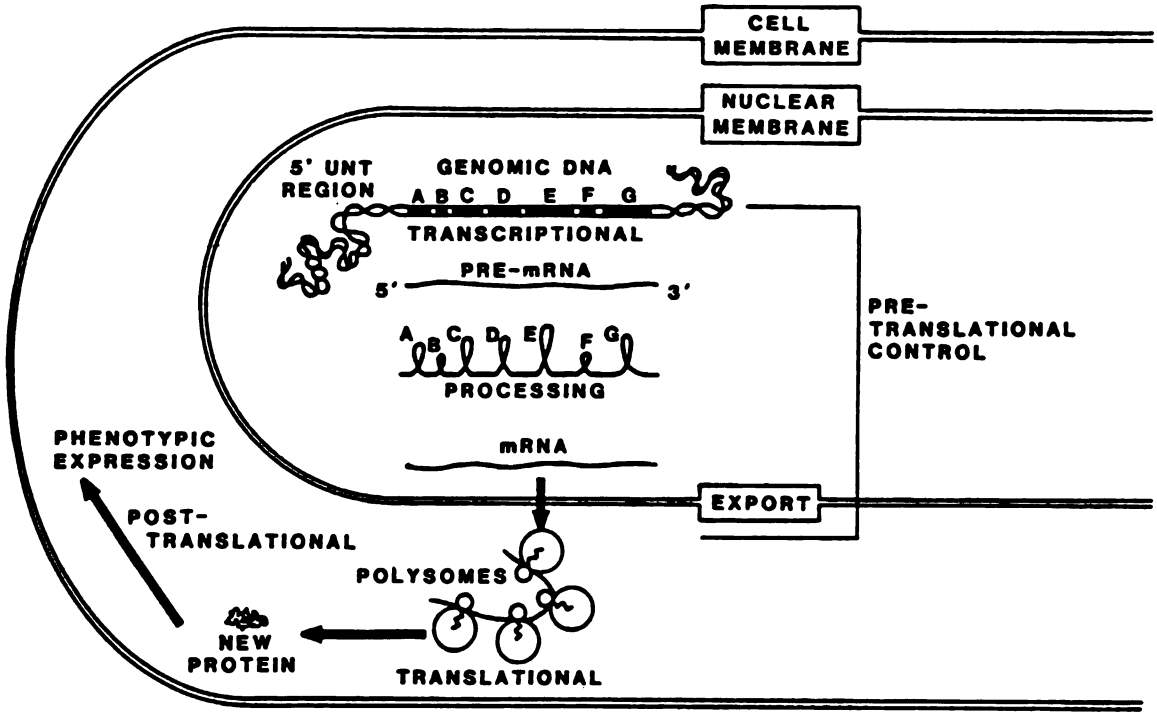


FIGURE 2. Key regulatory points in protein synthesis.

pretranslational events, namely those dependent on DNA or RNA content is included.

Nucleic Acids and their Relation to Growth

Skeletal muscle growth can occur by an increase in number of myofibers (hyperplasia), or by an increase in cell size (hypertrophy) which is associated with the addition of myofibrils and nuclei. Even though total muscle fiber number appears to be essentially determined at birth, 80% or more of the DNA in muscle accumulates after birth (Winick and Nobel, 1966; Allen et al., 1979). Enesco and Puddy (1964) and Leblond (1972) state that the increase in DNA content of the multinucleated cells of skeletal muscle represents an increase in nuclei number, not an increase in cell or fiber number.

Since muscle nuclei are not capable of DNA synthesis or division, the source of the new DNA material was investigated by Mauro (1961) and Moss and Leblond (1970, 1971). Using electron microscopy and labeled thymidine incorporation studies, they discovered the source of new nuclei was a population of small, heterochromatic, mononucleated, spindle shaped cells they called satellite cells. These cells lie between the plasma membrane and the basement membrane of myofibers. The differences in myonuclei number of the rat extensor digitorum longus muscle and the soleus muscle were directly related to the satellite cell number of the respective muscles (Kelly, 1978). Reznik (1969) demonstrated an increase in [^{3}H]-thymidine uptake and satellite cell

activity of regenerating skeletal muscle following injury. Mulvaney et al. (1988) also showed increased porcine satellite cell proliferation and DNA accumulation during periods of rapid postnatal growth and following androgen treatment as shown by autoradiographic assessment of [^{3}H]thymidine incorporation.

DNA content can be used to estimate the number of nuclei in muscle cells since the DNA content of the diploid nucleus is constant (Mirsky and Ris, 1949; Leblond, 1972). Enesco and Leblond (1962) estimated the amount of DNA per diploid nucleus to be approximately 6.2 picograms. However, it is important to realize that not all the DNA within muscle tissues can be attributed only to myonuclei. Grove et al. (1969) indicated that over half of the nuclei in cardiac muscle are from fibroblasts. In skeletal muscle, Enesco and Puddy (1964) estimated that about 20 to 30% of all nuclei are located outside the skeletal muscle fiber, with the majority being satellite cells as well as other nonmuscle cells such as those of the connective tissues.

Cheek et al. (1971) have defined the term DNA-unit to be an imaginary volume of cytoplasm managed by a single nucleus. The size of these DNA-units would be defined by the protein:DNA ratio and the number of DNA-units would depend on the total amount of DNA within a given muscle. The ultimate muscle size is determined by the number of DNA-units (Millward and Waterlow, 1978). For example, the smaller soleus and

plantaris muscles have a small amount of DNA compared to the larger gastrocenemius and quadriceps muscles which contain greater amounts of DNA. Size of the muscle is not determined by or related to the size of the DNA-unit. Cheek et al. (1971) have suggested that hyperplasia may be used to describe the increase in number of DNA-units, whereas hypertrophy would describe the increase in the size of DNA-units.

The majority of DNA accumulates postnatally and the total amount of DNA dramatically increases during development (Winick and Noble, 1966). Skeletal muscle, kidney and liver total DNA increased three- to fivefold from 1 month old weanling rats to 1 year old mature rats (Waterlow et al., 1978). Heart DNA showed a smaller increase and there was no change detected within the brain during this period. In rats, DNA accumulation occurs at a rapid, linear rate very early in development and then begins to taper off around 84 days of age (Winick and Noble, 1966). DNA accretion appears to parallel the accumulation of muscle protein early in development and then lags in later development (Allen et al., 1979). Data by Moss (1968b) suggest that the rapid accumulation of DNA may be a prerequisite for periods of rapid growth. Robinson (1969) also has reported the increase in total DNA content during growth. Moss (1968a) and Swatland (1977) showed a direct correlation between muscle fiber diameter and total number of muscle fiber nuclei.

The protein:DNA ratio increases dramatically during

development. Waterlow et al., (1978) reported that the mg protein:mg DNA ratio increased from 126 to 452 for 1 month old vs 1 year old rats. Millward (1980) also demonstrated a three- to fourfold increase in the DNA-unit size and that muscles with oxidative metabolism had the lowest DNA-unit size or protein:DNA ratio. Concentration of DNA is inversely related to the protein:DNA ratio and declines during growth, perhaps due to a dilution effect of the additional protein mass of muscle.

Total RNA also increases during growth and seems to closely parallel total protein accumulation, whereas RNA concentration decreases during development. This has been shown in rats (Devi et al., 1963; Srivastava and Chaudhary, 1969; Enesco and Puddy, 1964; Winick and Noble, 1966), in pigs (Robinson, 1969; Gilbreath and Trout, 1973; Hakkarainen, 1975) and in chickens (Moss, 1968a, 1968b). Gilbreath and Trout (1973) found the DNA and RNA concentrations in porcine longissimus muscle were greatest at 1 day of age but decreased dramatically by 2 weeks and continued the decline as the animal aged. Hakkarainen (1975) stated that the decline in RNA concentrations is the result of a dilution effect due to increased protein accumulation.

Waterlow et al. (1978) suggested that the capacity for protein synthesis is indicated by the RNA:protein ratio, and the extent to which the capacity is utilized is indicated by the rate of protein synthesis to RNA ratio or RNA activity.

The RNA:protein ratio provides an approximation of ribosome concentration, since most of RNA is rRNA. Srivastava and Chaudhary (1969) showed that the amount of cellular protein supported per unit of total muscle RNA increases during development. The RNA activity is held within fairly narrow limits, with perhaps a slight decline with age. Waterlow et al. (1978) reported that parallel changes in FSR occur with changes in RNA concentration; the changes in FSR reflect changes in RNA content. They suggested that the main factor in determining rate of protein synthesis in different muscles is RNA concentration. Millward et al. (1975) and Lewis et al. (1984) showed that the quantity of protein synthesized per unit of RNA is remarkably constant during growth.

Winick and Noble (1966), Powell and Aberle (1975) and Millward et al. (1975) suggest that the ratio of RNA to DNA is indicative of the capacity to synthesize protein. Devi et al. (1963) and Winick and Noble (1966) reported an increase in the RNA:DNA ratio during the first few weeks of postnatal development in the rat. After this early period no change was observed in the RNA:DNA ratio. Only in the case of rapid compensatory muscle growth of rats after nutritional deprivation (Millward et al., 1975) and in stretch-induced hypertrophy (Laurent et al., 1978) did the RNA:DNA ratio increase greatly. Thus small changes in growth due to nutritional or hormonal factors could result from increases in RNA, or transcription rate, or even from increased efficiency

of translation. During development it appears that the synthetic machinery, i.e. RNA, is relatively constant per nucleus or unit of DNA.

If, during normal growth and development of an animal, neither RNA activity nor RNA:DNA ratio change, then synthesis rate per unit of DNA, or DNA activity would also remain fairly constant. In adult animals, DNA activity appears to be the same in different muscles as was reported for 300 day old rats (Waterlow and Millward, 1978). In fast growing rats, Waterlow and Millward (1978) also showed that DNA activity did not change markedly during development, whereas in slow growing rats DNA activity was slightly elevated. Waterlow et al. (1978) found slightly higher DNA activities in young rats but that the decrease during growth, which is in the order of 30%, is much smaller than the decline in FSR. The developmental change also may be the result of additional nonmyonuclei of fibroblasts which change in concentration during growth and development.

Millward (1980) concluded that FSR varies inversely as a function of the DNA unit size; the smaller the DNA-unit, the more rapid the protein turnover rate. This appears to be an inevitable consequence during development because as the DNA-unit size increases, concentration of RNA declines. This naturally leads to a decrease in ribosome concentration and overall concentration of mRNA per milligram tissue, which are needed for synthesis of protein. The synthetic machinery,

indicated by RNA content, is relatively constant per nucleus during development. Thus, the FSR, i.e., the percent of total protein synthesized, is greater when the protein:DNA ratio is smaller.

Increases in total RNA appear to be a precondition for increasing the deposition of protein (Hakkarainen, 1975) and RNA is a measure of the machinery needed for protein synthesis (Waterlow et al., 1978) as well as FSR (Garlick et al., 1976). Approximately 80% of total RNA is rRNA and only about 2% is actually poly-adenylated mRNA, the template used for protein synthesis (Young, 1970). The relative abundance of 28S rRNA to total RNA is maintained throughout development as observed in the spleen, liver, and brain in 6, 24 and 36 month old rats (Slagboom et al., 1990). Measurements of total RNA content only provide an indication of the machinery for the translational steps in protein synthesis. Protein synthesis can be regulated at several steps, including transcription of mRNA, processing, transport and stability of mRNA, ribosome availability, translation factors, tRNA charging or even posttranslational modifications (Young, 1974). The abundance of mRNA is affected by the rate of transcription from DNA, or gene expression, and also is to a certain extent dependent on the stability and extent of processing of the message. Either an increase in message or an increase in stabilty, allows the message to be translated a greater number of times and both could lead to an increase in protein synthesis rate.

The rate of protein synthesis can be controlled by changes in the amount of mRNAs or by the activation of preexisting, stable mRNAs. As reviewed by Paul (1974) and Weinberg (1977), activation of stored mRNA may play an important role in protein synthesis during early embryogenesis, resulting in an increase in the efficiency of total protein synthesis machinery. However, in other experiments it has been suggested that selective species of mRNA were activated for translation (Rosenthal et al., 1980). Androgenic steroids can increase the concentration of specific mRNAs in the kidney (Berger et al., 1986). By directly measuring gene transcription rates in vitro, Berger et al. (1986) reported that induction of these mRNAs in the kidney was not accounted for by stimulation of gene transcription but must have resulted from events at the level of mRNA processing altering stability or turnover. Their results suggest that relative mRNA levels within cells can be altered not only by gene transcription but also through posttranscriptional-pretranslational events selectively acting on specific mRNAs. Many studies have shown a close correlation between the abundance of a specific mRNA and synthesis of the corresponding protein (McKnight and Palmiter, 1979; Swaneck et al., 1979). Shani et al. (1981, 1987) suggested that during myogenesis in cell lines, activation of stored mRNA is not a major mechanism for controlling the time at which differentiation and synthesis of the myofibrillar proteins

occur. Instead, they demonstrated that the transcription of new myofibrillar protein mRNA is directly responsible for the onset of protein synthesis.

In studies utilizing a mouse cell line, Caravatti et al. (1982) determined that the corresponding mRNAs coding for myosin heavy chain and for alpha actin are detectable immediately before the initiation of myofibrillar protein synthesis. Their results demonstrated a close temporal correlation between muscle mRNA accumulation and protein synthesis during myogenesis. Similar results were obtained in a primary chick muscle culture (Schwartz and Rothblum, 1981) and in a rat muscle primary culture and the L8 cell line (Shani et al., 1981). During stages of early recovery from atrophied muscle caused by immobilization of gastrocnemius-plantaris muscle of the rat, alpha actin mRNA abundance increased parallel to the increase in actin synthesis rate, suggesting pretranslational regulation (Morrison et al., 1987). After day 4, there appears to be an increase in translational efficiency since mRNA no longer was correlated with the continued rise in protein synthesis rates. Devlin and Emerson (1979) concluded that during myogenesis, contractile protein synthesis is regulated by changes in mRNA. Monitoring the abundance of mRNA can result in specific information regarding protein synthesis regulation and whether or not it occurs at the pretranslational level. Since transcription of mRNA precedes the synthesis of protein,

modulating the abundance of mRNA could allow for enhanced protein synthesis.

The first step in gene activation may actually be the unfolding of the tightly packed chromatin structure to allow for transcription. Most actively transcribed genes, approximately 10 to 20% of total DNA, are in an unfolded chromatin conformation in which they are susceptible to DNase I activity (Mathis et al., 1980; Weisbrod, 1982). The sensitivity to DNase I may however only reflect the potential for a gene to be transcribed rather than transcription itself. Expression of a gene is a highly complex process regulated by several factors that are responsible for transcription and its control, even at the level of promotor interaction.

The advancement of molecular biology has enabled the monitoring of the abundance of specific mRNA species. With nucleic acid probes, complementary to the mRNA coding for a specific protein, it is possible to investigate the regulation of protein synthesis at the pretranslational level. It is important for these probes to be specific to the protein of interest. With the emphasis on regulation of muscle growth, it is essential to characterize probes that can be used in livestock species for investigating the regulation and control of myofibrillar gene expression. With the characterization of such probes, one can then easily monitor the response to hormonal, nutritional or physiological changes and understand more specifically how they may effect protein synthesis at the

pretranslational level.

Myofibrillar Proteins

Emphasis is placed on the myofibrillar proteins since they represent slightly more than 50% of the total protein content of skeletal muscle (Young and Allen, 1979). Myofibrillar proteins refer to structural proteins of the thick and thin filaments that compose the sarcomere as well as proteins that regulate contraction of skeletal muscle. During postnatal growth, fiber diameter is increased due to the addition of myofibrils and muscle fibers increases in length due to the addition of sarcomeres (Griffin et al., 1971; Stromer et al., 1974; Goldspink, 1980). Myosin, representing the thick filament, is the predominant myofibrillar protein accounting for 43 to 45%, and actin, representing the thin filament, accounts for 22 to 23% of total myofibrillar protein (Yates et al., 1983). Since actin represents over 10% of all protein within muscle, it is important to determine the regulation and expression of this major gene family in order to more fully understand muscle growth.

Synthesis of myofibrillar proteins is a highly synchronous process and accumulation of the contractile proteins during myogenesis is closely coordinated (Devlin and Emerson, 1978, 1979; Young and Allen, 1979; Affara et al., 1980). Devlin and Emerson (1978) have shown that the accumulation of several myofibrillar proteins begins at the same time, they have similar synthetic rates, and they reach

their steady state levels at the same time. Using a cell free translation assay, Devlin and Emerson (1979) demonstrated that coordination of protein synthesis in cell cultures was regulated at the level of mRNA. Shani et al. (1981), utilizing cDNA probes for skeletal muscle actin, myosin heavy chain and myosin light chain, found that the coordinated expression of mRNA is correlated to the coordinated synthesis of the respective proteins. In addition to the apparent coordinated regulation of the myofibrillar gene sets, Gunning et al. (1987) suggested that genes corresponding to each transcript may also be regulated on an individual basis. Studies using 4 kg rabbits (Schreurs et al., 1985a) suggest that there are different relative turnover rates for myosin and actin and that the myofibril does not turnover as a whole unit but each subunit or protein is replaced independently. Because of the generally coordinated expression, monitoring the synthesis of one myofibrillar marker protein and its respective gene transcript will help determine the regulation and assembly of myofibrils in muscle. It is essential that regulation of the expression of specific muscle proteins be studied on an individual basis if we are to understand fully the regulation of muscle protein accretion during development.

Actin is selected here as the representative myofibrillar protein due to its abundance, limited number of isoforms, including only one which is skeletal muscle specific, and it is known that actin is an excellent marker of striated muscle

tissue during differentiation, embryonic development and during postnatal growth of the animal (Jockusch et al., 1984; Sassoon et al., 1988). Actin is composed of a single polypeptide chain of 374 amino acids, including one 3-methyl histidine residue, with a molecular weight of 41,785 (Elizinga et al., 1973). Actin is a globular protein that can polymerize in the presence of ATP and physiological ionic conditions (50-100 mM potasssium chloride or .1-10 mM magnesium chloride) to form a long two-stranded helix of F-actin, which together with tropomyosin and the troponin complex, comprises the thin filament. During contraction, actin forms crossbridges with myosin thick filaments. Actin has the unique property of activating the ATPase of myosin, which is a vital step in muscle contraction.

Besides being found in skeletal, cardiac and smooth muscle, actin is also found in other cell types, functioning as microfilaments which aid in cytoplasmic movement and provide cytoskeletal structural support for cells. Actin has been identified in a large number of cells and tissues including amoeba (Pollard and Weihing, 1974) and even in higher order plants (Condeelis, 1974; Jackson and Doyle, 1977). It now seems apparent that actin is ubiquitous to all eukaryotes, perhaps because of its vital function in cytoskeletal support and cytoplasmic movement.

Even though the molecular weight of actin from diverse groups of organisms is similar, their amino acid sequences

differ. Electrophoretic studies using isoelectric focusing have resolved six distinct actin isoforms in mammals which have been isolated from cardiac muscle (cardiac alpha), skeletal muscle (skeletal alpha), smooth muscle (beta, gamma) and nonmuscle cells (beta, gamma) (Whalen et al., 1976; Vandekerckhove and Weber, 1978a). No fiber type specific isoforms of actin (i.e., fast and slow or red and white muscle) have been found. Actin is an excellent marker for studying the regulation of myofibrillar protein synthesis since there is only one actin isoform predominantly present within skeletal muscle. Even though myosin is more abundant, there have been over 12 myosin isoforms identified in skeletal muscle which presents greater complications than just studying the one skeletal muscle alpha actin isoform (Buckingham et al., 1984; Buckingham, 1985; Buckingham et al., 1987).

Comparison of Actin Isoforms

As mentioned, six different genes coding for actin have been identified in mammals (Barton et al., 1987). This however is not the case in all organisms. Only one isoform and one gene have been identified in yeast (Gallwitz and Sures, 1980). Yeast actin also has one intervening sequence between the codons encoding for amino acids 3 and 4. On the other hand, slime mold has 17 actin genes that have been identified, not all of which are functional and none of the genes has any intervening sequences (McKeon and Firtel, 1981). In contrast to all of the above, sea urchins have 11 genes

that code for actin and show great variability in their number of intervening sequences (Scheller et al., 1981). A great deal of variability exists among the diverse groups or types of organisms.

The beta and gamma actin isoforms are slightly more alkaline due to a highly charged region of 4-5 amino acids near the N terminus allowing the separation of alpha, beta and gamma actin on 2- dimensional gels (Obinata et al., 1981). Cardiac and skeletal muscle alpha actin have similar isoelectric points due to conservation of their amino acid compositions. Skeletal muscle alpha actin differs in only 4 noncharged amino acids (<2% of the total) from cardiac alpha actin (positions 2, 3, 298 and 357) while 24 to 25 amino acid substitutions (<7% of the total) are present in nonmuscle beta and gamma actin (Vandekerckhove and Weber, 1978b, 1979). These 25 replacements are not randomly distributed as residues 18-75 and 299-356 are constant, whereas residues 2-18 and 259-298 show many substitutions (Vandekerckhove and Weber, 1978c). Skeletal muscle actin also differs in 6 to 8 amino acids from the smooth muscle beta and gamma actins (Vanderkerckhove and Weber, 1979).

These results indicate a very close relationship between the four muscle actins in comparison to the nonmuscle actins but the amino acid substituion patterns indicate that smooth muscle actins appear to be more closely related to the nonmuscle beta and gamma actins rather than to cardiac and

skeletal alpha actins. The latter two actins are more closely related. In spite of the large degree of sequence identity, Cavadore et al. (1985) demonstrated that structural differences exist between skeletal muscle and aortic actins around the C- terminal region and at regions near residues 227 and 167. These structural differences may be responsible for the variability in the extent of Mg-ATPase activation of the two isoforms.

Use of immunological procedures to study the development and expression of actin isoforms has been limited due to the shared common antigenic determinants, as expected from the high degree of amino acid conservation. Antiserum against chicken embryo brain actin bound to bovine cardiac muscle, rabbit skeletal muscle, bovine brain and chick embryo brain (Morgan et al., 1980). However, cardiac actin antiserum bound only cardiac and skeletal actin and not bovine brain actin which expresses nonmuscle beta and gamma isoforms (Morgan et al., 1980). Use of antibodies for the detection of isoforms, very specific antigenic sequences must be used. However, utilizing immunological methods or determining amino acid sequences provide only limited information regarding the expression of the various isoforms during growth and development.

In order to more easily distinguish between similar actin isoforms, molecular biological techniques have been applied to differentiate and characterize actin isoforms. cDNA clones

specific to skeletal muscle alpha actin and cardiac alpha actin have been isolated from the rat (Shani et al., 1981; Garfinkel et al., 1982), mouse (Minty et al., 1981; Sassoon et al., 1988), chicken (Paterson et al., 1984; Gordon et al., 1988) and human (Ponte et al., 1983; Gunning et al., 1983; Hanauer et al., 1983). No cDNA probes for alpha actin have yet been developed from meat producing animals, i.e., pigs, cattle or sheep. Both cardiac and skeletal muscle actin mRNA are approximately 1650 nucleotides in size with a coding length of 1122 bases and a 3′ nontranslated region of 300 nucleotides with the cardiac 3′ untranslated region being 70 nucleotides shorter (Garfinkel et al., 1982; Mayer et al., 1984). Nonmuscle actin mRNAs are generally 2100 nucleotides in length (Minty et al., 1981).

There is a greater sequence diversity in the nucleotide sequence of mRNA as compared to the amino acid sequences due to the degeneracy of the genetic code. As a result of different codon usage, approximately a 15% difference in the nucleic acid sequence between skeletal and cardiac alpha actins results (Buckingham et al., 1984; Buckingham, 1985). There is an ever greater diversity between alpha actin and the nonmuscle, beta and gamma actins (Ponte et al., 1984). For example, analysis of the DNA sequences of the 5′ end demonstrated that although beta and gamma actin genes start with a methionine codon (MET-Asp-Asp-Asp and MET-Glu- Glu-Glu, respectively), the human alpha actin gene starts with a

methionine codon followed by a unique cysteine codon (MET-CYS-Asp-Glu-Asp-Glu) (Zakut et al., 1982; Gunning et al., 1983).

Shani et al. (1981) used a full length rat skeletal alpha actin cDNA probe and found that it hybridized well with RNA extracted from rat, rabbit, dog and chicken skeletal muscle and, to a much lesser extent with rat heart muscle. Hybridization to RNA from rat stomach and brain was detected at low stringency conditions (50°C, .1 x SSC) but not at higher conditions (60°C, .1 x SSC). They also utilized a cDNA probe containing sequences specific to the 3′ untranslated region of rat skeletal muscle alpha actin and found specific binding only to rat skeletal muscle RNA and to a much lesser extent to rat cardiac RNA. The 3′ probe also hybrized to RNA extracted from rabbits and dogs but not from chickens. No hybridization was detected with other tissues. These results indicate that the coding regions of actin genes are highly conserved, whereas the 3′ nontranslated regions show great divergence and less conservation of sequence than the coding region. Hanauer et al. (1983) determined the nucleic acid sequence for a human cDNA clone for human skeletal muscle alpha actin and found that it confirmed the complete conservation of amino acid sequence within human, rabbit and rat alpha actins. The 5′ untranslated region of human skeletal alpha actin showed good sequence identity with the corresponding rat gene but a lesser degree with the 3′ untranslated region.

It now appears that certain segments within the 3′ untranslated region are similar across species and yet other sequences within the 3′ untranslated region can be not only isoform specific but also species specific and unique to the species of interest (Gunning et al., 1984). There appears to be greater homology and conservation of sequence across species for the same isoform than there is between actin isoforms of the same species. The coding region is highly conserved across species, even among the various actin isoforms. Furthermore, there appears to be conservation of the 5′ untranslated region. The 3′ untranslated region shows the greatest diversity between isoforms even though select segments are conserved across species (Gunning et al., 1984). The comparisons among species and isoforms suggest that actin divergence among higher order vertebrates involves limited tissue divergence rather than species specificty. Alpha actin mRNA tissue expression and sequence identity and conservation have not been determined in pigs, sheep or cattle. However, based upon these observations, it would appear possible to use a full length cDNA probe from another mammalian species to study alpha actin mRNA expression in livestock species.

Developmental Expression of Alpha Actin

Isoforms also can arise and dominate during specific stages of muscle development. Development can be divided into three distinct stages: 1) embryonic stage (differentiation at which time individual functioning muscle units and

innervation are established), 2) neonatal stage (limited movement becomes possible), 3) postnatal and adult stage (full locomotive capability and load-bearing ability). Actin itself does not have developmental isoforms but the expression of the various isoforms varies greatly throughout development of the various tissues.

During early embryonic development, myogenic cells, called presumptive myoblasts, are mononucleated with a high mitotic activity that synthesize cytoplasmic proteins similar to other cell types. During differentiation, the presumptive myoblasts begin synthesizing muscle specific proteins and cease dividing (Holtzer, 1970). After transition to the myoblast stage, the cells begin to fuse and form multinucleated myotubes which synthesize myofibrillar proteins and assemble myofibrils. In addition DNA replication and nuclear division is halted (Okazaki and Holtzer, 1966).

Much research has been performed for the purpose of understanding the process of myogenesis and differentiation. Using isoelectric focusing, it was demonstrated that mononucleated myoblasts in culture contain large amounts of beta and gamma actins and that after cellular fusion, alpha actin becomes the major actin isoform in the multinucleated myotubes (Garrels and Gibson, 1976; Whalen et al., 1976; Rubenstein and Spudich, 1977; Paterson et al., 1984). Schwartz and Rothblum (1981) observed low amounts of alpha actin mRNA in replicating prefusion presumptive myoblasts and

the majority of actin mRNA was accounted for by beta and gamma actin. Beginning at myoblast fusion, they discovered that alpha actin mRNA accumulated and reached peak levels within 95 hours when myotube formation was complete. Conversely, beta and gamma actin began to decline at the onset of fusion and was not detectable at the end of myotube formation. In an interesting case involving the study of carcinogenesis, accumulation of alpha actin mRNA and alpha actin synthesis was inhibited by the transformation to tumorigenicity (Leavitt et al., 1985). Shutdown of alpha actin expression appears to be a reproducible transformation-sensitive marker in rodent fibroblasts.

Utilizing a mouse skeletal muscle cell line in culture and isotype-specific cDNA probes, Bains et al. (1984) showed that the skeletal muscle alpha actin mRNA pool took several days to reach its peak and then had reached only 15% of the level in adult skeletal muscle. However, they demonstrated that cardiac alpha actin reaches a peak six times greater than the skeletal alpha actin peak within 24 hours of the initiation of differentiation. In cloned human satellite cells, cardiac actin was shown to be the major alpha actin mRNA in fusing cells with skeletal alpha actin induced to a lesser extent (Gunning et al., 1987). Bains et al. (1984) also showed that the decreases in beta and gamma actin after the onset of fusion were not coordinately regulated as gamma actin decreased most rapidly.

Using isoform specific probes, it has been shown that genes coding for muscle-specific proteins are not preferentially sensitive to DNase I in proliferating mononucleated cells of the myogenic cell line L8 (Carmon et al., 1982; Melloul et al., 1984). Actively transcribed genes are sensitive to DNase I digestion and reflect a potential for gene transcription, whereas nontranscribed genes are not as sensitive to DNase I digestion (Weisbrod, 1982). The changes which render myofibrillar protein genes and alpha actin preferentially sensitive to DNase I take place during the transition to terminal differentiation and the onset of myotube formation (Carmon et al., 1982).

In chick embryonic skeletal muscle, alpha actin is present in very low amounts at early myogenic stages but abundance is high in terminally differentiated cells coupled with decreased expression of the beta and gamma isoforms (Ordahl et al., 1980; Shimizu and Obinata, 1980). Cardiac alpha actin is the predominant isoform present in chick embryonic muscle immediately after differentiation and then decreases as development proceeds (Paterson and Eldridge, 1984; Paterson et al., 1984). The abundance of the various isoforms depends on whether one measures the cytosolic or myofibrillar fraction. In embryonic skeletal muscle, proportions of the three actin isoforms are in the order beta > gamma > alpha in the soluble fraction while alpha > beta > gamma in the myofibrillar fraction (Shimizu and Obinata,

1980). Cardiac alpha actin also has been discovered to be the major isoform in early developing skeletal muscle of the rodent. In late fetal limb muscle of the mouse, cardiac actin represents about 40% of striated muscle actin and declines to 20% immediately after birth (Minty et al., 1982; Vandekerckhove et al., 1986). The maximum accumulation of cardiac actin mRNA occurs in 17 day old fetal muscle of mice which corresponds to the time when maximum increase in muscle mass is taking place (Buckingham et al., 1984). Garner et al. (1989) and Alonso et al. (1990) showed that amount of alpha actin mRNA (skeletal and cardiac combined) is much higher in heart than in skeletal muscle. On the basis of nanograms of alpha actin, their results indicate that the amount of alpha actin mRNA (skeletal and cardiac combined) in cardiac muscle is almost three times that in skeletal muscle. This may be due to the higher turnover rate of the cardiac muscle.

The case is similar for development of the heart as both cardiac and skeletal muscle alpha actin isoforms are coexpressed. In late fetal and newborn rats, skeletal muscle alpha actin accumulates, although at this stage of development cardiac alpha actin is the predominant isoform in the heart (Mayer et al., 1984; Schwartz et al., 1986). Sassoon et al. (1988) showed that cardiac alpha actin can first be detected around 7 days in the developing heart of the mouse embryo. They also observed that skeletal muscle alpha actin mRNA accumulated in lower amounts but coexpression was observed

throughout embryonic development of the mouse heart. For the chick heart, Ordahl (1986) reported similar amounts of cardiac and skeletal muscle alpha actin mRNA as early as 2.5 days in ovo.

Skeletal muscle alpha actin can also be expressed in the adult heart at low levels (Mayer et al., 1984; Buckingham et al., 1987). Shani et al. (1981) first reported that probes derived from the 3′ untranslated region of a rat skeletal muscle alpha actin gene hybridized to adult rat heart RNA at about 2% of that of rat skeletal muscle RNA. Minty et al. (1982) also concluded that skeletal muscle alpha actin mRNA is expressed at less than or equal to 2% of cardiac alpha actin mRNA abundance in the adult mouse heart. Garner et al. (1989) found that adult mice have 95.8% cardiac alpha mRNA and 4.2% skeletal muscle alpha actin mRNA in the heart. In the case of BALB/c mice, which have a mutation in the cardiac alpha actin gene locus but not in the actin coding sequence, skeletal muscle alpha actin mRNA abundance is increased to 47% of total actin in the adult heart and cardiac alpha actin only represents 53% (Garner et al., 1986; Garner et al., 1989). Under conditions of aortic stenosis which leads to cardiac overload and consequent cardiac hypertrophy, high amounts of skeletal muscle alpha actin accumulated in adult rodent hearts in addition to the cardiac alpha actin normally present (Schwartz et al., 1986). Izuma et al. (1988) and Schiaffino et al. (1989) both reported the accumulation of skeletal

muscle alpha actin mRNA in the heart shortly after the onset of pressure overload. Gunning et al. (1983) reported that skeletal muscle alpha actin mRNA accounts for about 50% of the total actin mRNA in a diseased adult heart from transplant surgery. It appears that skeletal muscle alpha actin can account for about 2 to 10% of the actin mRNA transcripts in the normal adult heart from mammals (Gunning et al., 1983; Vandekerckhove et al., 1986; Kedes, personal communication).

During postnatal growth, cardiac and skeletal muscle alpha actin become the major isoforms in their respective tissues. In skeletal muscle of newborn rats, cardiac alpha actin mRNA is approximately 13% of the cardiac actin present in heart muscle (Mayer et al., 1984). During 80 days of postnatal development, cardiac alpha actin decreases by a factor of 130 in skeletal muscle and increases in heart muscle by a factor of 3.4. Mayer et al. (1984) also suggested that skeletal muscle alpha actin in newborn hearts is about 10% of that found in leg muscle. During development, skeletal alpha actin decreases twelvefold in heart muscle and increases by a factor of 2.3 in skeletal muscle. Garner et al. (1989) determined that cardiac alpha actin mRNA represents 19.6% of total alpha actin mRNA in newborn mice and 3.3% in adult mice. In adult rat skeletal muscle, skeletal muscle alpha actin is the predominant isoform accounting for over 95% of all actin, with cardiac alpha actin representing 5% or less (Caravatti et al., 1982; Gunning et al., 1983; Vandekerckhove et al.,

1986; Barton et al. 1987). A cDNA probe for skeletal muscle alpha actin could be used to specifically study alpha actin mRNA abundance and expression in adult skeletal muscle. The expression of skeletal muscle alpha actin mRNA has not been studied in skeletal muscle of pigs, cattle or sheep.

Actin Gene Structure and Regulation

Six different actin genes have been isolated in mammals; each corresponding to one of six isoforms (Nudel et al., 1983; Buckingham, 1985). In humans, the cytoplasmic actins, beta and gamma, are encoded by a multigene family, whereas skeletal and cardiac alpha actin genes are a single copy (Engel et al., 1982; Ponte et al., 1983). There may be as many as 30 copies of the actin genes within the human genome and they represent different gene loci (Engel et al., 1981; Humphries et al., 1981). Some of these multicopy fragments may actually be cytoplasmic actin pseudogenes (Moos and Gallwitz, 1983; Scarpulla and Wu, 1983). Within the mouse genome, the striated muscle alpha actins are single copy genes with more than 20 copies of the beta and gamma actin genes in addition to 20 to 50, similar but not identical, sequences which may be pseudogenes (Minty et al., 1982). Cleveland et al. (1980) have shown that the chicken genome may encode several cytoplasmic gamma genes although only one gamma actin protein has been found in chickens.

The coding region of the mouse skeletal muscle alpha actin gene which has over 90% sequence identity with the rat

or chick, is split by five introns at codons specifying amino acids 41/42, 150, 204, 267 and 327/328 (Hu et al., 1986). These intron positions are identical to the corresponding gene in chickens (Fornwald et al., 1982), rats (Zakut et al., 1982; Nudel et al., 1983) and humans (Hamada et al., 1982; Taylor et al., 1988). In a comparison of rats and mice, the intron sequences are about 75% identical and the corresponding introns of chickens are much more divergent in length and sequence (Hu et al., 1986). The respective intron locations for cardiac alpha actin are almost identical to the skeletal muscle gene in the human and mouse genome (Hamada et al., 1982; Hu et al., 1986). However, the intron locations for the cytoplasmic actins are different than those for the human striated actin genes (Hamada et al.,1982). The rat beta actin gene has five introns and positions were assigned to 6 base pairs upstream from the initiator codon ATG and codons 41, 121, 267 and 327 (Nudel et al., 1983).

Comparison of nucleotide sequences for rat, mouse, chicken and human alpha actin genes revealed several conserved sequences outside of the protein coding region, including several inverted repeat sequences which can form hairpin loops, and these sequences are not present in the beta actin genes (Hu et al., 1986). There is high sequence identity in the promoter region of chicken and rat alpha actin genes, other than the CAAT, ATA and poly-adenylation signal, AATAAA (Ordahl and Cooper, 1983).

Considerable sequence identity exist in the 5′ untranslated region between humans, rodents and, to a lesser extent, chickens. Comparison of chicken and rat alpha actin genes reveals conserved sequences around the CAAT box and about 46 to 59 nucleotides downstream from the cap site (Ordahl and Cooper, 1983). There also is very high conservation (85%) in the 5′ flanking region between the cap site and 300 nucleotides upstream of rat, mouse and chicken skeletal muscle alpha actin genes (Hu et al., 1986). Hu et al. (1986) found no cross sequence identity between the alpha and beta 5′ untranslated regions. The regions of high sequence identity in the alpha actin gene across species may be important in the regulation of alpha actin expression.

Gunning et al. (1984) investigated whether or not the coexpression of skeletal and cardiac muscle alpha actin in human skeletal muscle and heart was the result of chromosomal linkage. They discovered that the two muscle genes do not cosegregate and are on different autosomes, with the cardiac actin gene found on chromosome 15 and the skeletal muscle actin gene on chromosome 1. They concluded that coexpression is not the result of chromosomal linkage and that neither gene can be the primary target resulting in X-linked muscular dystrophies. Minty et al. (1982) and Czosnek et al. (1983) also found that the two striated muscle genes are not closely linked in the mouse genome and that the skeletal muscle actin gene is not linked to a nonmuscle actin gene. Coexpression of

the sarcomeric actin proteins does not depend on or require the close structural proximity of these genes (Roberts et al., 1985). In chicken primary myogenic cultures, skeletal muscle alpha actin appears to be regulated independently from the cardiac alpha actin gene. Accumulation of skeletal muscle alpha actin but not cardiac alpha actin mRNA can be blocked in calcium-deficient medium which arrests myoblast fusion (Hayward et al., 1988).

Minty et al. (1986) suggest that there are two steps necessary for cardiac alpha actin gene expression: activation of the gene and subsequent modulation of its transcriptional activity. These two steps can be separated and the factors involved in modulation may be distinct from those involved in gene activation. Regulation can occur by cis-acting factors, referring to a DNA locus that affects activity of DNA sequences on its own strand of DNA, or by trans-acting factors, referring to a diffusible product able to act on all receptive sites in the nucleus (Richter et al., 1989). Sharp et al. (1987) suggested that both the 5′ and 3′ untranslated region of the actin gene contain sequences important in regulating expression during development. Other investigators have confirmed that the alpha actin gene contains sequences upstream of the transcription start site in the 5′ region which modulate the developmental expression of alpha actin (Seiler-Tuyns et al., 1984; Nudel et al., 1985; Minty and Kedes, 1986).

Grichnik et al. (1986) showed that a 411 nucleotide sequence flanking the 5′ end of the skeletal muscle alpha actin gene was responsible for a 9- to 15-fold increase in CAT enzymatic activity during myoblast fusion. Walsh and Schimmel (1987), using DNA footprint analysis, showed that a segment located 78 nucleotides upstream of the transcription start site may be essential for alpha actin expression in developing myoblasts and myotubes. Unidirectional 5′ deletion analysis demonstrated that the human skeletal alpha actin gene contains a proximal cis-acting element that is located between positions -153 and -87 relative to the transcription start site which is necessary and sufficient for muscle specific expression and regulation during myogenesis (Muscat and Kedes, 1987). Bergsma et al. (1986) defined the cis- acting transcriptional control region of the chicken skeletal muscle alpha actin gene to 200 nucleotides starting at -107 and included the CCAAT and TATA box homologies.

Organization of the upstream regulatory regions for alpha actin are completely different from the beta and gamma genes and, with the definition of key regulatory domains, it has been found that these regions for skeletal muscle alpha actin are different from those of cardiac alpha actin. The nucleotide sequences of the regulatory regions for the two striated genes have very few similarities in nucleotide sequence and the cardiac alpha actin gene does not possess the same additive, enhancer-like characteristics of the respective

skeletal muscle gene (Minty and Kedes, 1986; Miwa and Kedes, 1987; Miwa et al., 1987; Muscat et al., 1988). From in vivo transcription and in vitro binding studies, Muscat et al. (1988) found that both the skeletal muscle and cardiac alpha actin gene interact with a common trans-acting factor that can regulate the expression of both genes. On the other hand, they also discovered that the cis-acting region of skeletal muscle alpha actin interacts with a trans- acting factor that does not appear to be used by the cardiac alpha actin gene promoter. It appears there may be common trans- acting factors, yet different cis-acting sequences that allow the coexpression of both skeletal muscle and cardiac alpha actin during development.

Much progress has been made in understanding the developmental expression of the various actin isoforms in the human, rat, mouse and chicken. These can provide an excellent basis for the study of actin expression in livestock species. It appears that actin has high sequence identity across all mammals and this facilitates the study of actin expression in livestock species using probes that have been isolated and characterized, such as the human probes. It is important to realize that no work has yet been published, except that of Skjaerlund et al. (1993), regarding the expression of skeletal muscle alpha actin in cattle, sheep or pigs.

Effect of Testosterone on Skeletal Muscle Growth

Several investigators have reported that boars have 3 to

20% more muscle mass than barrows (Prescott and Lamming, 1967; Field, 1971; Hansson et al., 1975; Mulvaney, 1984; Knudson et al., 1985a, 1985b). Knudson et al. (1985a) reported that body weight of boars and barrows did not differ before the onset of puberty. At 205 kg body weight, boars had 9% more muscle than barrows (Knudson et al., 1985a). Mulvaney (1984) observed no difference in muscle mass between prepubertal boars and castrates but postpubertal boars had 14% more muscle mass than castrates. Castration of male guinea pigs resulted in a 10% reduction in muscle weight relative to noncastrates and was subsequently restored with testosterone propionate administration (Kochakian et al., 1964; Kochakian, 1976). Mulvaney (1984) found that castrates implanted with testosterone also had increased muscle mass compared to sham-implanted castrates. Castrated rabbits treated with exogenous testosterone had increased semitendinosus muscle RNA, DNA and protein content compared to castrates (Grigsby et al., 1976).

Not all muscles respond to the same degree to androgen treatment. Kochakian (1976) suggested that shoulder muscles may be more sensitive to androgen stimulation than muscles from the hindquarter. The rat levator ani muscle is highly sensitive to androgens, whereas the superficial vastus lateralis muscle lacks androgen sensitivity (Boissonneault et al., 1990). Mulvaney (1984) reported that weights of the two shoulder muscles, triceps brachii and brachialis, were 30 and 31% greater in postpubertal boars than barrows while the

corresponding difference for muscles from the hindlimb, semitendinosus muscle, and from the back, longissimus muscle, were only 21 and 10% greater, respectively. The semitendinosus muscle appears to be intermediate in senitivity to androgen stimulation and in its allometric propensity for growth (Mulvaney et al., 1985).

Part of the increased muscle mass can be attributed to an increase in protein synthesis. Barrows implanted with testosterone or dihydrotestosterone had greater protein synthesis than control barrows (Mulvaney et al., 1983). Administration of testosterone to gonadally intact male rabbits also increased incorporation of [^{3}H]leucine into skeletal muscle proteins (Grigsby et al., 1976). Likewise, muscle protein synthesis was stimulated in rats administered testosterone propionate (Breuer and Florini, 1965) and trenbolone acetate (Vernon and Buttery, 1978). Cardiac muscle protein synthesis rates were decreased in castrated male rats and were stimulated following treatment with testosterone (Kinson et al., 1991). Serum from postpubertal boars added to media in which porcine skeletal muscle strips were incubated had 83% greater protein synthesis rates than serum from barrows (Skjaerlund et al., 1988). Rogozkin (1979) found a 16% increase in [C^{14}]leucine incorporation into myosin and a 16% increase in DNA dependent RNA polymerase activity in gastrocnemius muscles of rats administered methandrostenolone. Testosterone proprionate administered to castrated male rats

increased RNA polymerase activity, ribosomal activity and chromatin template activity (Breuer and Florini, 1965). Abundance of actin mRNA in the rat levator ani muscle was reduced 85% by castration but was restored by injections of testosterone propionate (Boissonneault et al., 1990).

N^{τ}-methylhistidine excretion was 23% less in castrated rats than in intact males (Santidrian et al., 1982) Castration of 15 and 75 kg boars decreased semitendinosus muscle protein degradation rates (Mulvaney et al., 1983). If testosterone elevates protein degradation rates, then protein synthesis rates must be elevated to a greater extent to allow for the increased muscle accretion. This would be consistent with the observation that boars have higher metabolizable energy expenditures for maintenance than castrates (Knudson, 1986). Vernon and Buttery (1976, 1978) observed decreased N^{τ}-methylhistidine excretion in rats after animals were treated with trenbolone acetate suggesting that perhaps the mechanism of action between testosterone and trenbolone acetate may be different (Lobley et al., 1983).

Circulating testosterone concentrations are elevated in boars during the first few weeks after birth and are similar to average concentrations present following the onset of puberty (Colenbrander et al., 1978; Ford, 1983). Colenbrander et al. (1978) found that perinatal testosterone levels were highest (1.3 ng/ml) at 2 to 3 wk after birth, whereas Martin et al. (1984) found that testosterone

concentrations peaked (1.7 ng/ml) at 5 to 7 wk of age. Thereafter, testosterone concentrations decreased to approximately .5 ng/ml until they increased at 17 or 18 wk of age or the onset of puberty. Neonatal administration of testosterone propionate to barrows increased weaning weights compared to untreated barrows (Mulvaney and Marple, 1987; Dvorak, 1981). It is not known what effect the elevated perinatal testosterone concentrations has on neonatal skeletal muscle metabolism and protein turnover.

Effect of Beta-andrenergic Agonists on Skeletal Muscle Growth

Beta-adrenergic agonists increase skeletal muscle growth and reduce fattening in many species (for reviews, see Hanrahan, 1987; Williams, 1987; Thorton and Tume, 1988; Yang and McElligott, 1989; Bergen and Merkel, 1991). A 10 to 20% increase in muscle weight is observed after treating rats with the beta-agonist clenbuterol for only 1 to 2 weeks (Emery et al., 1984; Reeds et al., 1986; McElligot et al., 1987). Lambs fed cimaterol for approximately 2 months showed a 25 to 30% increase in muscle weights compared to lambs fed a control diet (Beermann et al., 1986, 1987). In clenbuterol-treated lambs, gastrocnemius muscle increased in weight by as much as 40% (Kim et al., 1987). Ractopamine feeding for 4 weeks increased semitendinosus muscle mass in finishing pigs more than 25% compared to controls (Bergen et al., 1989).

Ractopamine and other beta-adrenergic agonists appear to modulate both protein synthesis and protein degradation so as

to increase protein accretion. Reeds et al. (1986) concluded that decreased protein degradation was the reason for increased muscle hypertrophy as FSR was not altered by clenbuterol treatment in rats. Earlier work showed that isoproterenol depressed protein turnover in acute rat hind limb perfusions (Li and Jefferson, 1977) and Garber et al. (1976) found that catecholamines decreased amino acid release from rat skeletal muscle incubated in vitro. Others have reported reduced protein degradation in vivo and in cultures of muscle cells following beta-adrenergic agonist treatment (Forsberg and Merrill, 1986; Bohorov et al., 1987; Morgan et al., 1988; Young et al., 1990). Eadara et al. (1988) showed that feeding cimaterol to rats increased FSR 32% and decreased N'-methylhistidine excretion 25%, with the greatest effect at 1 week of treatment.

Emery et al. (1984) first reported that clenbuterol fed to rats increased FSR in vivo. Bergen et al. (1989) reported increased FSR in ractopamine-fed pigs which could account for the observed muscle hypertrophy and increased FAR. FSR of skeletal muscle alpha actin was 55% greater in ractopamine treated pigs than controls (Helferich et al., 1990). Clenbutrerol feeding also increased FSR in lambs (Claeys et al., 1989) and rats (MacLennan and Edwards, 1989). This is consistent with reports of increased protein synthesis in cultures of muscle cells containing ractopamine or clenbuterol (Anderson et al., 1990), ractopamine (Adeola et al., 1989), or

cimaterol (Young et al., 1990). Ractopamine and isoproterenol each enhanced the proliferative activity of chick satellite cells in culture and this was mediated via the beta-adrenergic receptor (Grant et al., 1990). Relative abundance of skeletal muscle alpha actin mRNA in pigs was increased twofold by ractopamine feeding (Helferich et al., 1990) and myosin light chain mRNA abundance was increased in steers fed ractopamine (Smith et al., 1989). The beta-adrenergic agonist $L_{644,969}$ also increased skeletal muscle alpha actin mRNA abundance in lambs (Koohmaraie et al. 1991). These results indicate that ractopamine and other beta-adrenergic agonists may increase muscle mass by enhancing protein synthesis pretranslationally.

The effect of beta-adrenergic agonists on muscle growth is most dramatic early on and after prolonged feeding the response is attenuated (Yang and McElligott, 1989). Kim et al. (1992) found increased weight gain in skeletal muscles of rats fed cimaterol for up to 2 weeks, but no further increased occurred with feeding cimaterol for an additional 2 weeks. Rats fed cimaterol had the greatest accleration in gain within the first week and gain decelerated after 1 week (Eadara et al., 1989). Likewise, FSR was elevated at one week but no change was detected thereafer. This attenuation has also been reported by others (Reeds et al., 1986; Beermann et al., 1987; Bergen et al., 1989). The mechanism mediating the time-course effect of ractopamine on protein synthesis or enhancement of mRNA abundance in pigs is not clear. The effect of

subsequent withdrawal of ractopamine on skeletal muscle protein metabolism in pigs also is not known.

CHAPTER 1

SKELETAL MUSCLE GROWTH AND PROTEIN TURNOVER IN NEONATAL BOARS AND BARROWS

Abstract

Four boars and four barrows were allotted to one of six groups to assess skeletal muscle growth and protein metabolism. Castration was performed within 24 h of birth, and all pigs remained with their dams until slaughtered at either 1, 2, or 4 wk of age. Four additional pigs were slaughtered at birth to obtain initial body composition. All other pigs were infused with [^{14}C]tyrosine for 6 h prior to slaughter to determine in vivo fractional protein synthesis rates (FSR). At slaughter, muscle bundles were removed from the semitendinosus and incubated with [^{3}H]tyrosine to determine in vitro protein synthesis rates. Nucleic acids and protein were determined on the semitendinosus muscle. Testosterone concentrations, determined at weekly intervals, peaked in boars at 3 wk of age. Castration at birth did not affect combined weights of the semitendinosus, longissimus dorsi, triceps brachii and brachialis muscles. Likewise, neither in vitro protein synthesis rates nor in vivo FSR was affected by castration. However, a developmental decline in in vivo FSR and in vitro protein synthesis rates occurred from 1 wk to 4 wk. Neither concentrations nor total protein, RNA or DNA in the semitendinosus muscle differed between neonatal boars and barrows at any age. Concentrations of DNA and RNA at 4 wk were two- and threefold lower, respectively, than at

birth. Protein/DNA and protein/RNA ratios increased three- and sixfold, respectively, from birth to 4 wk. Testosterone concentrations had little effect on skeletal muscle growth and protein turnover rates during this neonatal period.

Introduction

Boars are more efficient in conversion of feed to gain and produce carcasses at market weight with 20 to 30% less fat than barrows (Field, 1971; Mulvaney, 1984; Knudson et al., 1985a, 1985b). Gonadally intact males also have 8 to 15% greater muscle mass than castrated males (Prescott and Lamming, 1967; Mulvaney, 1984; Knudson et al., 1985a). Five weeks after castration at 15 and 40 kg, body weight and skeletal muscle protein accretion of barrows did not differ from that of boars. In contrast, postpubertal boars had greater muscle protein accretion rates than barrows that were castrated at 75 kg (Mulvaney, 1984). Protein synthesis rates of boars were greater than barrows that had been castrated at either 40 or 75 kg body weight (Mulvaney, 1984). Castration reduced proliferative activity of satellite cells in skeletal muscle of neonatal pigs (Mulvaney et al., 1988). Circulating testosterone concentrations are elevated in boars during the first few weeks after birth and are similar to average concentrations present following the onset of puberty (Colenbrander et al., 1978; Ford, 1983). The objective of this study was to determine the effect of elevated perinatal testosterone concentrations in neonatal boars (birth to 4 wk of age) on skeletal muscle growth and protein metabolism compared with that of barrows castrated at birth.

Materials and Methods

Animals, Treatments and Experimental Protocol. Twenty-eight cross-bred boars (Yorkshire x Hampshire x Duroc) from a total of eight different litters were randomly allocated at birth to seven groups of four pigs each. Four pigs were slaughtered at birth to determine initial body composition. The boars in three of the remaining six groups were castrated within 24 h of birth. Four boars and four barrows were then slaughtered at 1, 2 and 4 wk of age. All pigs remained with and nursed their dams at the MSU Swine Research Unit until they were infused and slaughtered. No supplemental feed was provided. The pigs were weighed and blood samples for determination of serum concentrations of testosterone were collected at weekly intervals. Fractional protein synthesis rates (FSR) were measured in skeletal muscle at 1, 2 and 4 wk by continuous infusion of radiolabeled tyrosine (Bergen et al., 1987). The pigs were removed from their dam during the 6 h infusion period and then immediately euthanized (5 cc, 50% w/v phenobarbitol) and exsanguinated. The left semitendinosus, longissimus dorsi, brachialis and triceps brachii muscles were removed, dissected free from fat and weighed. A subsample of the left semitendinosus muscle was frozen immediately in liquid nitrogen and stored at -80°C for determination of in vivo FSR (Mulvaney et al., 1985; Bergen

et al., 1987).

At slaughter, muscle strips were teased free and removed from the right semitendinosus muscle for determination of in vitro protein synthesis rates according to the procedure described by Bergen et al. (1987) and Skjaerlund et al. (1988). The soft tissues of the left side were dissected free from skin and bone and then ground along with the muscles listed above for determination of total soft tissue protein. Protein was determined by micro-Kjeldahl method (AOAC, 1980). Nucleic acids (DNA and RNA) from the semitendinosus muscle were assayed according to a modified procedure (Munro and Fleck, 1969) as described by Bates et al. (1985).

Testosterone Determination. Blood samples were collected weekly from each pig until slaughtered. The blood was allowed to clot overnight at 4°C, and serum was harvested by centrifugation at 2500 x g for 30 min. Serum testosterone was quantified by radioimunoassay using MSU antitestosterone #74 raised against testosterone-3-oxime-human serum albumin. The assay was validated by Kiser et al. (1978) and previously used for testosterone detection in boars (Kattesh et al., 1979).

Determination of In Vivo Fractional Protein Synthesis Rate. The FSR were determined according to procedures described by Mulvaney et al. (1985) and Bergen et al. (1987). Twenty-four hours before infusion, pigs were anesthetized with halothane, and catheters were surgically inserted into the right and left jugular veins. Catheters were kept patent with

heparinized sterile saline. The pigs were infused with L-[U^{14}C]tyrosine (Amersham, Arlington Heights, IL) dissolved in sterile .9% NaCl at a rate of .011 μCi/g body weight for 6 h. Blood samples were obtained from the contralateral vein to determine plasma specific activity of tyrosine (Bergen et al., 1987). Following infusion, the pigs were euthanized.

The semitendinosus muscle samples were powered with Dry Ice at -70°C and a .5 g subsample was treated with 3 mL cold (4°C) 2N perchloric acid for determination of the free-pool tyrosine specific activity. Following centrifugation (4000 x g, 15 min, 4°C), 1 mL of saturated potassium citrate was added to the supernatant and centrifuged at 4000 x g for 10 min. The supernatant was dried with a heating block under a nitrogen airstream and then resuspended in 3 mL .5 M sodium citrate, pH 5.5, for subsequent decarboxylation of tyrosine to yield tyramine.

The pellet was dried with the following separate and sequential steps: 5 mL 1% potassium acetate in ethanol; 5 mL ethanol-chloroform (3:1); 5 mL ethanol-ether (3:1); 5 mL ether and 5 mL hot 2 N perchloric acid. For determination of bound tyrosine specific activity, the pellet was hydrolyzed with 20 mL 6 N HCl and autoclaved for 20 h at 121°C. The hydrolysates were evaporated to dryness and resuspended in .5 M sodium citrate, pH 5.5. Enzymatic conversion of L-tyrosine to tyramine was accomplished with L-tyrosine decarboxylase (E.C. 4.1.1.25) and following selective extraction tyramine was

quantified fluorometrically (Ambrose, 1974). Specific activities of the free and bound tyrosine pools were determined from liquid scintillation counts (Bergen et al., 1987; 1989). FSR was calculated using the equation described by Garlick et al. (1974) and previously applied by Mulvaney et al. (1985) and Bergen et al. (1987). Daily protein accretion rate was calculated as the difference in total semitendinosus muscle protein between adjacent infusion groups (i.e., wk 1 - birth; wk 2 - wk 1; wk 4 - wk 2) divided by the number of days. Thus, semitendinosus protein pool size was the average over 7 d for the first two slaughter groups and 14 d for the last slaughter group. Fractional accretion rate (FAR) was then calculated as daily accretion rate divided by pool size. Fractional breakdown rate (FBR) was calculated as the difference between FAR and FSR (Millward et al., 1975).

Determination of In Vitro Protein Synthesis Rate. A modification (Bergen et al., 1987; Skjaerlund et al., 1988) of the method described by Fulks et al. (1975) was followed to determine in vitro protein synthesis rates. Immediately after exsanguination, bundles of muscle fibers (5 mm wide, approximately 10 to 12 mm long and 1.0 to 1.5 mm thick) from the right semitendinosus muscle were bluntly dissected free. Four muscle strips per pig (approximately 80 mg each) were clamped off at rest length in situ and then excised. The strips were preincubated for 30 min at 37°C in a shaking water bath in 4 mL of oxygenated (95 O_2:5 CO_2) Krebs-Ringer

bicarbonate buffer, pH 7.4 (Umbreit et al., 1964), containing insulin (.1 U/mL), glucose (10 mM) and 5 x porcine plasma concentration of amino acids (Bergen et al., 1987). The strips were removed, blotted and placed in a second vial with 4 mL of fresh, oxygenated buffer and 2.5 μCi/mL L-[2,3,4,6-^{3}H]tyrosine (Amersham, Arlington Heights, IL). After incubation for 2.5 h at 37°C, the strips were removed, homogenized (Brinkman Polytron) in 2 mL of cold .01 mM potassium phosphate buffer, pH 7.4, and protein precipitated with .5 mL 50% trichloroacetic acid. After centrifugation at 23,500 x g for 20 min, the pellet was washed with .01 mM potassium phosphate buffer, dissolved in .5 mL NCS^{R} tissue solubilizer (Amersham, Arlington Heights, IL) and assayed for ^{3}H by liquid scintillation in aqueous scintillant (Skjaerlund et al., 1988). An aliquot of the supernatant was also counted for ^{3}H and the tyrosine content determined fluorometrically (Ambrose, 1974) for calculation of specific activity of the free intracellular pool. Protein synthesis rates were calculated from ^{3}H tyrosine incorporation (dpm/mg) divided by specific activity of the intracellular tyrosine pool (dpm/pmol) and expressed as pmol tyrosine incorporated.mg^{-1}.h^{-1}.

Statistical Analysis. Data were analyzed by least square analysis of variance using the general linear models procedure of the Statistical Analysis System (SAS, 1987). The data were analyzed using a one-way analysis of variance with treatment (boars vs barrows) and age (birth, 1, 2, 4 wk) as data

classes. Fractional accretion rates cannot be statistically compared since different pigs were slaughtered at the two ages and FAR were estimated from the average semitendinosus muscle protein content. Likewise, FBR cannot be statistically analyzed because FBR is calculated by the difference between FAR and FSR.

Results and Discussion

Serum testosterone concentrations determined at weekly intervals are presented in Figure 3. Castration reduced testosterone below the detection level of the assay (.28 ng/mL) by 1 wk. Average circulating testosterone concentration in boars increased approximately threefold from birth to the peak value (2.6 ng/mL) at 3 wk. Colenbrander et al. (1978) and Ford (1983) also reported that testosterone concentrations of neonatal boars peaked between 2 and 3 wk postnatally and then declined to relatively low concentrations (.47 ng/mL) until the onset of puberty. These results are consistent with changes in the steroid-histochemical activity (Van Straaten and Wensing, 1978) and morphological differentiation of the testis (Van Straaten and Wensing, 1977). Colenbrander et al. (1977) also reported a similar, but earlier occurring, secretory pattern for serum LH concentrations. The decline of serum testosterone concentrations after 3 wk of age may be due to maturation of the hypothalamo-hypophysial-gonadal feedback system as suggested by Colenbrander et al. (1978).

Castration did not significantly affect live body weight or total soft tissue protein content at 1, 2 or 4 wk of age (Table 1), although boars tended to have greater body weights at each age. Knudson et al. (1985a) reported that body

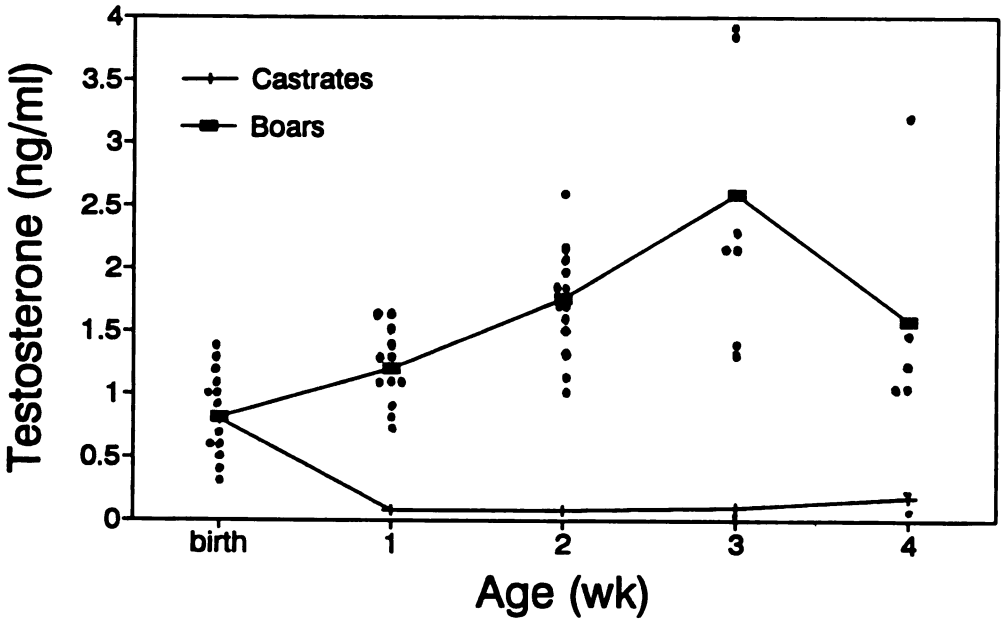


FIGURE 3. Serum testosterone concentrations of neonatal boars and barrows. Blood samples were collected at weekly intervals and serum testosterone concentrations were quantified by radioimmunoassay. Barrows were castrated within 24 h of birth. Concentration is presented for each boar and the line represents the average at the weekly interval.

TABLE 1. Live body weights, carcass soft tissue protein, and muscle weights of neonatal boars and barrows

Weight		1 wk		2 wk		4 wk		
		B[a]	C[a]	B	C	B	C	SEM[b]
Body wt, g	1300	2574	2308	4462	4036	8253	8103	522
Soft tissue protein, g	18.6	68.0	59.1	140.6	121.5	280.2	282.8	20.4
Muscle wt[c], g	24.4	52.4	41.3	101.6	85.2	195.5	189.7	13.5

[a] B = boars and C = boars castrated at birth.

[b] Standard error for treatment means. Means for boars and castrates did not differ at 1, 2 or 4 wk of age (P>.05).

[c] Combined weight of the semitendinosus, longissimus dorsi, triceps brachii and brachialis muscles from the left side.

weight of boars and barrows did not differ before the onset of puberty in boars. Variation in body weight increased with age from birth to 4 wk, apparently due to differences in nursing ability of the dams. In a similar study, boars, and castrates implanted with testosterone propionate weighed 18 and 32% more at 1 and 3 wk of age than untreated castrates, but no differences in weight were observed at 2 wk (Mulvaney et al., 1988). Neonatal administration of testosterone propionate to barrows increased weaning weights compared to untreated barrows (Mulvaney and Marple, 1987; Dvorak, 1981).

Although nonsignificant, combined weights of the semitendinosus, longissimus dorsi, triceps brachii and brachialis muscles of boars were numerically greater, especially at 1 (27%) and 2 wk (19%) of age, than barrows (Table 1). Similar results were observed by Mulvaney (1984) for these same four muscles between barrows and prepubertal boars compared at either 40 and 60 kg body weight. He also found that total carcass muscle of prepubertal boars did not differ from barrows, but boars tended to have greater total muscle mass. At 105 kg body weight, however, postpubertal boars had greater total carcass muscle and greater combined weight of the four muscles than barrows (Mulvaney, 1984).

Boars tended to have greater ($P > .05$) protein contents of the left hemicarcass soft tissues at 1 (15%) and 2 wk (16%) than barrows (Table 1). At 4 wk the protein contents were nearly identical. Similar observations were noted by Mulvaney

(1984) for barrows and prepubertal boars. However, postpubertal boars had greater protein contents of soft tissues than did barrows when compared at 105 kg body weight (Mulvaney, 1984).

Castration at birth did not alter ($P > .05$) semitendinosus muscle weight at 1, 2 or 4 wk of age, although muscle weights were numerically greater (21%) in boars than barrows at 4 wk (Table 2). These observations are nearly identical to those noted by Mulvaney (1984) for differences in semitendinosus muscle weight (19%) between barrows and prepubertal boars compared at 40 and 60 kg body weight. Additionally, Mulvaney et al. (1988) observed no differences in individual muscle weights until 3 wk of age when the triceps brachii muscle weight of boars was 18% greater than that of castrates.

Muscles appear to differ in sensitivity to androgens. Kochakian (1976) suggested that shoulder muscles may be more sensitive to androgen stimulation than muscles from the hindquarter. Boissonneault et al. (1990) reported that the levator ani muscle of the rat is sensitive to androgens, whereas the superficial vastus lateralis muscle lacks such sensitivity. Mulvaney (1984) observed no difference in weight of shoulder muscles (triceps brachii and brachialis) and those of the hindlimb (semitendinosus) or back (longissimus dorsi) between barrows and prepubertal boars. In contrast, weights of these two shoulder muscles were 30 and 31% greater in

TABLE 2. Semitendinosus muscle weight, protein and nucleic acid content of neonatal boars and barrows

Item	Birth	1 wk		2 wk		4 wk		SEM[b]
		B[a]	C[a]	B	C	B	C	
Semitendinosus wt, g	2.4	4.6	4.6	9.6	9.5	23.7	19.6	1.5
Protein, %	9.7	15.7	15.8	17.7	17.0	17.4	17.4	.5
Total protein, mg	231	722	731	1701	1614	3761	3421	273
RNA, mg/g	10.01	5.48	5.20	3.74	3.69	2.90	3.29	.46
Total RNA, mg	24.3	25.4	24.1	35.6	33.9	63.7	63.7	4.2
DNA, mg/g	2.68	2.34	2.26	1.78	1.73	1.51	1.48	.09
Total DNA, mg	6.3	10.7	10.3	17.1	15.8	31.0	28.8	1.9
RNA/DNA	3.79	2.39	2.32	2.11	2.15	1.95	2.23	.13
Protein/DNA	36.4	68.6	70.4	100.0	101.6	119.5	119.6	6.0
Protein/RNA	9.7	29.6	30.8	47.8	47.9	62.8	54.8	3.6

[a] B = boars and C = boars castrated at birth.

[b] Standard error for treatment means. Means for boars and castrates did not differ at 1, 2 or 4 wk of age (P>.05).

postpubertal boars than barrows compared at 105 kg, while corresponding differences for the hindlimb and back were 21 and 10% greater. These observations support the postulation that muscles of the shoulder are more responsive to androgens than hindlimb or back muscles, and they also indicate that muscle responsiveness is amplified postpubertally.

Protein percentage as well as total protein content of the semitendinosus muscle did not differ between boars and castrates at 1, 2 or 4 wk of age (Table 2). Protein percentage, however, increased 62% from birth to 1 wk and increased approximately another 10% from 1 to 2 wk, but no change occurred between 2 and 4 wk of age.

Neither concentration nor total content of RNA and DNA in the semitendinosus muscles of boars and barrows differed at 1, 2 or 4 wk of age (Table 2). Likewise, none of the nucleic acid ratios differed between boars and barrows at any of the slaughter periods (Table 2). Concentrations of RNA and DNA in semitendinosus muscles of boars castrated at 15 and 75 kg were similar to those of boars determined 5 wk after castration (Mulvaney, 1984). When castrated at birth, barrows at 100 kg body weight had only 81% of the semitendinosus muscle DNA content of littermate boars at this weight (Knudson et al, 1985a). At 3 wk of age, neonatal boars had greater satellite cell proliferative activity than barrows in the triceps brachii muscle suggesting that boars had more DNA (Mulvaney et al., 1988).

Treatment of cultured rat myogenic cells with 10^{-8} M testosterone reduced cell cycle time by almost 9 h and the G_1 phase of the cycle was reduced by 20% (Powers and Florini, 1975). They also reported that testosterone induced an increase in DNA labeling index of myogenic cells. In contrast, Gospodarowicz et al. (1976) found that testosterone had no direct effect on enhancing bovine myoblast proliferation. Thompson et al. (1989) also observed no direct effect of the synthetic androgen, trenbolone acetate (TBA), on proliferation of rat satellite cells in culture. However, they found that satellite cells isolated from rats treated with TBA had greater proliferative activity in culture than satellite cells from control rats. Thus, it appears that androgens may not have a direct effect on satellite cell proliferation and DNA accretion.

There were no statistical differences in in vivo FAR, FSR or FBR between the neonatal boars and barrows at either 1, 2 or 4 wk of age (Table 3). Additionally, in vitro protein synthesis rates did not differ between boars and barrows (Figure 4). This is consistent with the observation that no treatment differences were observed in the protein/RNA, protein/DNA or RNA/DNA ratios presented in Table 2. Androgens, whether in gonadally intact postpubertal males or exogeneously administered, have been shown to stimulate protein synthesis. However, most of these studies with pigs have been conducted with peripubertal or postpubertal boars.

TABLE 3. Protein turnover rates of semitendinosus muscle measured in vivo in neonatal boars and barrows[a]

Item	1 wk		2 wk		4 wk		SEM[c]
	B[b]	C[b]	B	C	B	C	
Fractional accretion rate (FAR)	14.7	14.9	11.5	10.7	5.4	5.1	
Fractional synthesis rate (FSR)	28.3	27.2	20.8	25.2	16.7	18.9	1.3
Fractional breakdown rate (FBR)	13.6	12.3	9.3	14.5	11.3	13.8	

[a] Expressed as protein % per day.

[b] B = boars and C = boars castrated at birth.

[c] Standard error for treatment means. Means for boars and castrates did not differ at 1, 2 or 4 wk of age (P>.05). SEM and significance was determined for FSR only due to the procedure used to calculate FAR and FBR (see methods).

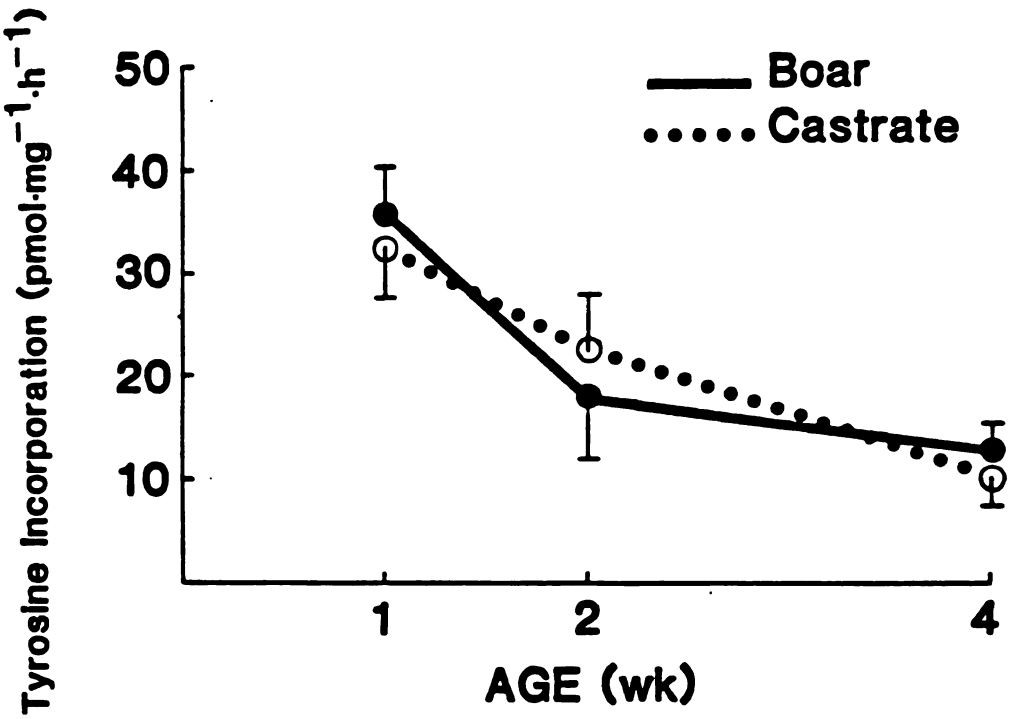


FIGURE 4. In vitro protein synthesis rates for the semitendinosus muscle from neonatal boars and barrows. Muscle strips from the semitendinosus muscle were incubated in 4 mL of Krebs-Ringer bicarbonate buffer containing glucose (10 mM), insulin (.1 U/mL), amino acids (5 x plasma concentrations) and 2.5 μCi/mL [^{3}H]tyrosine. Each data point represents four strips taken from each pig with four pigs per treatment and time point. Protein synthesis is expressed per milligram wet tissue basis as a ratio of ^{3}H tyrosine incorporated (per hour) into the trichloroacetic acid precipitable pool relative to the specific activity of the intracellular pool. No differences (P>.05) were detected between boars and barrows at any age.

Castration at 15 kg had no effect on semitendinosus muscle protein accretion rates, while castration at 75 kg decreased muscle protein accretion rates, and in vitro protein synthesis rates compared with age-paired boars (Mulvaney, 1984).

Barrows implanted with testosterone or dihydrotestosterone had greater protein synthesis rates than control barrows (Mulvaney, 1984; Mulvaney et al., 1985). Administration of testosterone to gonadally intact postpubertal male rabbits also increased incorporation of [^{3}H]-leucine into skeletal muscle proteins (Grigsby et al., 1976). Likewise, muscle protein synthesis was stimulated in rats administered testosterone propionate (Breuer and Florini, 1965) and trenbolone acetate (Vernon and Buttery, 1978). Cardiac muscle protein synthesis rates were decreased in castrated male rats and were stimulated following treatment with testosterone (Kinson et al., 1991). Testosterone propionate administered to castrated male rats increased RNA polymerase activity, ribosomal activity and chromatin template activity (Breuer and Florini, 1965). Abundance of actin mRNA in the rat levator ani muscle was reduced 85% by castration but was restored by injections of testosterone propionate (Boissonneault et al., 1990). Serum from postpubertal boars added to media in which porcine skeletal muscle strips were incubated had 83% greater protein synthesis rates than serum from age-paired barrows (Skjaerlund et al., 1988).

Knudson (1986) compared carcass muscling of boars to

barrows that were castrated either within 24 h of birth or at 6 wk of age. The latter barrows would have been exposed to the neonatal elevation of circulating testosterone, while those castrated at birth would not. No differences in muscling were observed between the two groups of barrows, but both groups had less muscle than boars at 105 kg. These data and those of the present study indicate that neonatal elevated serum testosterone has little effect on skeletal muscle growth or rates of protein turnover. Thus, it appears that the effects of testosterone on muscle growth and protein turnover are manifested only after the onset of puberty. This conclusion is consistent with the observations of Mulvaney (1984).

Despite no significant differences between neonatal boars and barrows in this study, major developmental changes were observed. Semitendinosus weight nearly doubled (92% increase) between birth to 1 wk, did double from 1 to 2 wk (108%) and more than doubled again from 2 to 4 wk of age. Protein concentrations of the semitendinosus muscle increased from 9.7% at birth to 15.8% at 1 wk and to approximately 17.4% at 2 and 4 wk. RNA concentrations, on the other hand, decreased from 10 mg/g at birth to almost half that concentration at 1 wk and then decreased to approximately 3 mg/g at 4 wk of age. As a result of high RNA concentrations at birth, total RNA content of the semitendinosus muscle did not change from birth to 1 wk of age. Because protein concentrations increased and

RNA concentrations decreased between birth and 4 wk postnatally, protein/RNA ratios showed a sixfold increase over these 4 wk. This is consistent with data reported by Gilbreath and Trout (1973). Hakkarainen (1975) stated that the developmental decline in RNA concentrations results from its dilution as protein accumulates. High RNA concentrations present at birth provide the capacity for high rates of protein synthesis that occur immediately after birth.

DNA concentrations also declined from birth to 4 wk of age, although to a lesser extent than RNA concentrations. Protein/DNA ratio, or the DNA unit (Cheek et al., 1971), increased threefold from birth to 4 wk of age, most of which occurred during the first week postnatally during which time it doubled. Cheek et al. (1971) have suggested that protein/DNA ratio is indicative of hypertrophy, with the larger increases in the DNA unit occurring during periods of rapid growth and high rates of fractional protein synthesis. RNA/DNA ratios declined approximately 45% from birth to 4 wk of age. In contrast, Devi et al. (1963) and Winick and Noble (1966) reported an initial increase in the RNA/DNA ratio during the first few weeks of postnatal development of the rat. Millward et al., (1975) and Powell and Aberle (1975) stated that the ratio of RNA to DNA is indicative of the capacity to synthesize protein.

These nucleic acid data are consistent with the high rates of protein synthesis that occurred early postnatally

(Table 3). In the present study, fractional synthesis rates declined from approximately 28% per day at 1 wk of age to approximately 17% per day at 4 wk of age (Table 3). The in vitro rates (Figure 4) showed a similar postnatal decline in protein synthesis. This high rate of protein synthesis immediately after birth is consistent with rates observed in other animals. Fractional synthesis rates of 22% were observed in 3 wk old rat quadriceps muscles (Millward et al., 1975), 34% at 5 d of age (Reeds et al., 1993), 24% in lambs at 1 wk of age (Arnal et al., 1976) and 25% in 2 wk old chickens (Maruyama et al., 1978). Similar to the dramatic postnatal decline in FSR in the present study, FAR rates were one-third lower at 4 wk than at birth. Fractional breakdown rates declined from birth to 4 wk of age but more slowly than FAR or FSR. Fractional accretion rates were halved between 2 and 4 wk, while FBR remained quite constant over this neonatal period.

The developmental decline in in vitro protein synthesis rates (Figure 4) was similar to that observed with the in vivo method. The synthesis rates at 4 wk were half those at 1 wk of age. Thus, the same developmental changes in protein synthesis rates were obtained by both methods and the conclusions are similar. However, the in vitro method cannot be used to predict absolute rates or to calculate net protein accretion rates that actually occur in vivo due to inherent problems with tissue viability. Inadequate diffusion of

oxygen into the core of the muscle strips appears to be the major limiting factor for the in vitro system (see Bergen et al., 1987; Skjaerlund et al., 1988 for complete discussion). However, the in vitro strip system can be used to detect relative changes and responses that occur in vivo (Skjaerlund et al., 1988) as is evident in this direct comparison of in vitro and in vivo methods on the same animals and muscle.

Implications

Castration at birth did not significantly alter neonatal muscle growth or protein turnover of barrows compared with boars. Even though postpubertal concentrations of testosterone stimulate protein synthesis and protein accretion, the high FSR and FAR characteristic of neonates may mask any effect of elevated neonatal testosterone on muscle protein turnover. Dramatic developmental changes in protein, nucleic acid concentrations and ratios are apparent during this neonatal growth period of pigs.

CHAPTER 2

DETERMINATION OF THE RELATIVE ABUNDANCE OF SKELETAL MUSCLE ALPHA ACTIN mRNA IN MUSCLE OF LIVESTOCK SPECIES

Abstract

Three market weight animals were slaughtered in order to obtain tissue samples from each of the meat producing livestock species: porcine (barrows), bovine (steers), ovine (wethers) and avian (cockerals). The four tissues of interest were skeletal muscle, heart, smooth muscle (stomach or gizzard) and liver. Total RNA was isolated from each tissue and then hybridized to a human sk-α-actin [^{32}P]cDNA probe using both dot blot and Northern blot hybridization. No hybridization was observed with RNA from liver or smooth muscle from any of the species suggesting little or no hybridization to nonmuscle and smooth muscle beta and gamma actin isoforms. The human sk-α-actin probe hybridized to RNA from skeletal muscle of pigs, cattle, sheep and chickens although relative hybridization was 75% less with chicken RNA. The hybridization was limited specifically to a band at 1.6 kb, the known length of sk-α-actin mRNA. Hybridization was also observed with RNA from pig heart (1.6 kb) and the relative abundance was consistently 7 to 10% of that observed with porcine skeletal muscle, even as stringency conditions were increased. These results indicate that the human sk-α-actin probe can be used to determine α-actin mRNA expression in skeletal muscle for pigs, cattle and sheep.

Introduction

The accumulation of muscle contractile proteins is a highly synchronous and closely coordinated process during myogenesis (Devlin and Emerson, 1979; Young and Allen, 1979; Affara et al., 1980). Some have suggested a strong correlation between the concentration of mRNA and the synthesis of the respective myofibrillar proteins (Devlin and Emerson, 1979; Shani et al., 1981b). Monitoring the abundance of mRNA would provide an indication of muscle specific gene expression and reflect pretranslational regulation of protein synthesis rates.

Actin represents approximately 22% of the total myofibrillar protein in skeletal muscle (Yates et al., 1983). The skeletal muscle alpha actin isoform (sk-α-actin) represents over 95% of all actin present in adult skeletal muscle (Caravatti et al., 1982; Barton et al., 1987; Garner et al., 1989). No fiber type specific isoforms of actin (i.e., fast and slow muscle) have been found. Thus, actin is an excellent marker for studying the regulation of myofibrillar protein synthesis because there is only one predominant actin isoform in adult skeletal muscle.

To date, little work has been done to construct α-actin probes or to isolate the α-actin gene from livestock species. This study was conducted to further characterize the use of a

human sk-α-actin cDNA probe (Gunning et al., 1983) to measure relative sk-α-actin mRNA abundance in skeletal muscle from cattle, sheep, chickens and pigs.

Materials and Methods

Animals and Sample Collection. Three market weight animals were slaughtered to obtain tissue samples from each of the meat producing livestock species: pigs (barrows), sheep (wethers), cattle (steers) and chickens (cockerals). The four tissues of interest were skeletal muscle, heart, smooth muscle (from stomach or gizzard) and liver. All animals were raised at the Michigan State University farms under normal management and ad libitum feeding practices. Within species, animals of similar weight and genetic makeup were selected before slaughter. The three crossbred (Yorkshire x Hampshire x Duroc) barrows, wethers (Suffolk) and crossbred (British x Continental European breeds) steers averaged 108, 54, and 534 kg body weight, respectively. Immediately after stunning and exsanguination, the tissue samples were removed, dissected free from visible fat and connective tissue, cut into 8 cm^3 pieces and frozen in liquid nitrogen within 5 min. Samples were stored (<30 d) at -80°C until used for RNA isolation. The sternomandibularis muscle was selected for the skeletal muscle sample from pigs, cattle and sheep. Ventricular muscle from the heart apex was also frozen as well as a 15 g sample of liver. Porcine, ovine and bovine smooth muscle was dissected from the exterior wall of the stomach and frozen as described for skeletal muscle. Three male Leghorn chickens

(approximately 1.5 kg body weight) were killed and the entire heart and liver were removed from each and frozen. Avian smooth muscle samples were dissected from the gizzard and skeletal muscle samples were obtained from the breast.

Selection of α-Actin cDNA Probe. A full length cDNA probe coding for human sk-α-actin was obtained from Dr. L. Kedes Laboratory (University of Southern California, School of Medicine, Los Angeles, CA). The probe was characterized by Gunning et al. (1983). The cDNA was inserted into the original Okayama-Berg, modified pBR322 cDNA cloning vector, 2.6 kb in size (Okayama and Berg, 1982). The insert was released from the pHMαA-1 plasmid vector by digestion with Pvu II and Pst I as two fragments of 700 and 800 bp in length due to an internal Pvu II restriction site within actin. There is also a Pst I site located in the ampicillin resistant gene. The cDNA probe was labeled with [^{32}P]dCTP (3,000 Ci/mmol) using the random priming method (Feinberg and Vogelstein; 1983, 1984). The procedures for the purification of plasmid DNA, isolation of cDNA insert and cDNA labeling are included in Appendices A, B and C, respectively.

Isolation of RNA. Total RNA was isolated (Helferich et al., 1990) using a combination of the urea-LiCl procedure (Minty et al., 1982) and the guanidinium thiocyanate-CsCl centrifugation method (Chirgwin et al., 1979). Precipitation of RNA from skeletal muscle using urea:LiCl (4M:2M) is a useful technique, however the preparation is contaminated with

DNA. This contamination is easily removed by separating RNA from DNA using a cesium chloride procedure (Chirgwin et al., 1979). The combination of these two procedures allows for greater yield (typically 200-400 μg) of high purity RNA isolated from skeletal muscle (6 g).

The isolation involves three steps: 1) homogenization and denaturation of protein and precipitation of RNA in 4 M urea and 2 M LiCl, 2) suspension of RNA in 4 M guanidinium thiocyanate and centrifugation of RNA through a 5.7 M CsCl cushion, 3) further purification of RNA through subsequent salt and ethanol precipitations. For skeletal muscle and heart samples, two 3-g portions of frozen tissue were pulverized and then homogenized in two 50-mL centrifuge tubes containing 24 mL of cooled (4°C) 4 M urea and 2 M LiCl. After incubation at 4°C for 48 h, the homogenate was centrifuged in a cooled (4°C) Sorvall RCS at 10,000 x g for 30 min. The two pellets were combined and resuspended in 10 mL of 4 M guanidine thiocyanate, 1 M sodium citrate, 10% N-lauryl sarcosine, .71 % mercaptoethanol, pH 7.0 at room temperature and layered onto a 2 mL filtered 5.7 M CsCl cushion in a 15-mL polyallomer ultracentrifuge tube. For liver and samples for which tissue quantity was limited (chicken heart, stomach muscle), 1 g of tissue was homogenized in a Corex tube containing 8 mL of the 4 M guanidine thiocyanate solution (step 1 was omitted). After centrifugation at 10,000 x g to pellet cell debris, the homogenate was transferred to two 5-mL

ultracentrifuge tubes containing 1 mL 5.7 M CsCl cushion. All samples were centrifuged at 100,000 x g for 18 to 24 h at 17°C using a swinging bucket Beckman 27.1 rotor (Beckman Instruments, Inc., Palo Alto, CA). After centrifugation, the supernatant was decanted, the sides of the tube wiped clean and the clear pellet resuspended in 250 μL of 7 M guanidine hydrochloride, 20 mM sodium acetate, 1 mM dithiothreitol, 10 mM iodoacetic acid, 1 mM EDTA, pH 7.0 at room temperature. After transferring the suspension to a 1.5-mL microfuge tube, 25 μL of 2 M sodium acetate and 150 μL of 100% ethanol (-20°C) were added. After incubation at -20°C overnight and subsequent microcentrifugation at high speed for 10 min, the RNA pellet was washed with 250 μL of 3 M sodium acetate, 10 mM iodoacetamide, pH 5.0 (4°C) and then centrifuged for 5 min. The pellet was then broken up and washed with 250 uL of 33 mM sodium acetate in 66% ethanol, pH 5.0 (-20°C). Following the final 250 μL wash with 100% ethanol (-20°C) and centrifugation, the pellet was allowed to dry briefly before suspension in 25 to 100 μL of TE-8 (10 mM Tris-HCl, 5 mM EDTA, pH 8.0), depending on tissue quantity used and size of pellet. The RNA was stored in the TE-8 buffer at -80°C. RNA solutions were scanned from 320 to 220 nm (included in Appendix D), the A_{260}/A_{280} ratio determined (at least 1.9), and RNA concentrations were calculated from the A_{260}. All RNA samples were size separated on a 1.2% agarose gel and then stained to check any deterioration in the 18S and 28S bands (example in Appendix

E).

Quantification of α-Actin mRNA. Relative abundance of α-actin mRNA was determined by dot blot hybridization methods previously described (Jump et al., 1984; Helferich et al., 1990) using a minifold apparatus (Schleicher and Schuell, Inc., Keene, NH) with 96 wells. Total RNA was blotted onto Zetabind® nylon membrane (CUNO, Inc., Meriden, CT) presoaked in 25 mM sodium phosphate at 1, 2, 3, and 4 μg RNA per dot for nonskeletal muscle samples and at .1, .2, .3, .4 μg RNA per dot for skeletal muscle samples, or as otherwise noted. After prehybridization at 42°C for 2 h in 50% formamide, 5x SSC (1.5 mM sodium citrate and 15 mM sodium chloride), 10x Denhardts (2% BSA, 2% Ficoll 400, 2% polyvinyl pyrolidine-40), 50 mM sodium phosphate, 1 mM EDTA with yeast tRNA (500 μg/mL), the blots were hybridized at 42°C with ^{32}P-labeled human sk-α-actin cDNA overnight. The hybridization solution contained 55% formamide, 5x SSC, 1.2x Denhardts, 50 mM sodium phosphate, 1 mM EDTA and yeast tRNA. The dot blots were washed with 2x SSC, .1% SDS at room temperature and then washed four times with .1x SSC, .1% SDS at 65°C for 45 min each. Dried blots were exposed to X-Ray film (Kodak, Rochester, NY) in cassettes containing two intensifying screens (Dupont, Wilmington, DE) and the extent of hybridization quantified by densitometry.

Northern Blot Hybridization. Total RNA was denatured and electrophoretically separated in 1.2% agarose, 40 mM MOPS, 10 mM sodium acetate, 1 mM EDTA, pH 7.0, and 18% formaldehyde.

The RNA was transferred to nylon membrane (Zetabind®) with 25 mM sodium phosphate buffer. Additional lanes were used for 18S and 28S RNA markers and standards and removed before transfer for staining (ethidium bromide) and visualization of RNA for measurement of migration distances for subsequent size analysis. After hybridization with the ^{32}P-labeled human sk-α-actin cDNA, the Northern blots were washed, dried, and exposed to X-Ray film, as previously described for dot blot hybridization.

Results and Discussion

Electrophoretic studies with isoelectric focusing have resolved six distinct actin isoforms in mammals which include two beta (β), two gamma (γ) and two alpha (α) actin isoforms (Whalen et al., 1976; Vandekerckhove and Weber, 1978a). The beta and gamma isoforms are found in smooth muscle (sm-β-actin, sm-γ-actin) and in nonmuscle cells (nm-β-actin, nm-γ-actin). The two α-actin isoforms correspond to cardiac alpha actin (c-α-actin) and skeletal muscle alpha actin (sk-α-actin) and are the predominant isoform in adult heart and skeletal muscle, respectively (Minty et al., 1982; Vanderkerckhove et al., 1986). Sk-α-actin differs from nm-β-actin and nm-γ-actin in 24 to 25 amino acid subsitutions of the total 374 amino acids that comprise the actin protein (Vandekerckhove and Weber, 1978b). There are 6 to 8 amino acid substitutions in the sm-β-actin and sm-γ-actin when compared to sk-α-actin (Vandekerckhove and Weber, 1979). While the amino acid sequence of sk-α-actin and c-α-actin are highly conserved with only four noncharged amino acid substitutions (99% sequence identity) (Vandekerckhove and Weber, 1978a), there is approximately 15% difference in their nucleic acid sequence (Buckingham et al., 1984; Buckingham, 1985).

Sk-α-actin cDNA clones have been isolated from the rat (Shani et al., 1981a; Shani et al., 1981b; Garfinkel et al.,

1982), mouse (Sassoon et al., 1988), chick (Gordon et al., 1984; Paterson et al., 1984) and human (Gunning et al., 1983; Ponte et al., 1984). There is a high degree of conservation and similarity across species for each isoform of actin (Buckingham et al., 1984; Buckingham, 1985; Buckingham et al., 1987). Several investigators have suggested probes corresponding to the 3′ untranslated region may be more isoform specific and could distinguish between the α-actin mRNA isoforms (Cleveland et al., 1980; Minty et al., 1981; Gunning et al, 1984; Ponte et al., 1984). Some sequences within the 3′ untranslated region are similar across species, whereas other segments are not only isoform specific but also species specific and unique to the species of interest (Gunning et al., 1984).

RNA was isolated from the longissimus muscle, heart and liver from a market weight pig (107 kg). In addition, RNA was isolated from the hind limb muscle of an adult rat (300 g) for comparison. RNA (1,2,3 and 4 μg) from pig and rat skeletal muscle and 5,6,7 and 8 μg RNA from pig liver and heart was hybridized to the human sk-α-actin cDNA probe using dot blot hybridization. As shown in Figure 5, hybridization was detected with pig and rat skeletal muscle RNA.

Liver serves as a control for cross hybridization of the sk-α-actin probe to the nonmuscle β- and γ-actin isoforms. In the preliminary experiment, no hybridization was observed to pig liver mRNA suggesting that the human probe may not

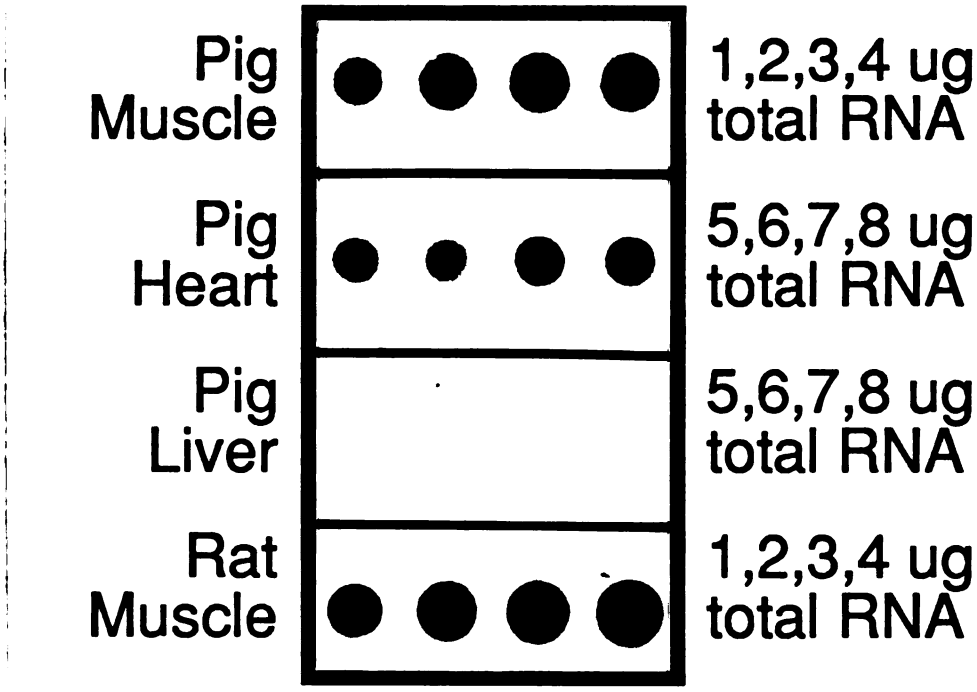


FIGURE 5. Hybridization of human sk-α-actin probe to rat and porcine tissues. RNA from the adult pig (107 kg body weight) heart, liver and longissimus muscle and rat (300 g body weight) hind limb muscle were dot blotted (quantities indicated from left to right) and then hybridized to the human sk-α-actin probe (Gunning et al., 1984). The blot was washed at 65°C with .1x SSC and .1% SDS and subjected to autoradiography for 8 h at -80°C.

hybridize to nm-β- and nm-γ-actin or that their abundance in liver is low and not detectable. In subsequent studies, liver RNA was blotted at 10 times the quantity of skeletal muscle RNA and exposed to X-Ray film for a much longer period of time to determine whether the human sk-α-actin probe will hybridize to liver nm-β and nm-γ-actin mRNA. A small amount of nm-β and nm-γ-actin is present in skeletal muscle but the abundance is very low compared to that of sk-α-actin.

There have been several reports of sk-α-actin expression in the heart. Using isoform specific cDNA probes, expression of sk-α-actin was observed in normal adult rat and mouse hearts by Shani et al. (1981), Minty et al. (1982), Mayer et al. (1984), Buckingham et al. (1987) and Garner et al. (1989). As expected, some hybridization of the human sk-α-actin probe to RNA from the pig heart was observed (Figure 5), although significantly less than for skeletal muscle. The extent of hybridization to heart RNA, based on equivalent quantity of RNA applied, was approximately 10% of that observed with skeletal muscle RNA. This is consistent with observations of Garner et al. (1989) who used mouse isoform specific probes and found the abundance of sk-α-actin in adult mouse heart to be 10.3% of that in adult mouse skeletal muscle. However, because a porcine isoform specific probe was not used, one cannot rule out the possibility that the human sk-α-actin probe may be hybridizing to c-α-actin.

Stringency conditions of the washes following

hybridization can be raised by increasing the temperature or decreasing the concentration of salt (SSC). Under higher stringency conditions, nonspecific hybridization is reduced. In order to determine the optimum conditions for washing, total RNA from pig liver, heart, smooth muscle and skeletal muscle was size separated on 1.2% agarose denaturing gels and transferred to nylon membrane. After hybridization to the full length sk-α-actin probe, the Northern blots were washed at various temperatures (55°C - 75°C) with .1x SSC and .1% SDS in an attempt to determine the specificity of binding (Figure 6). In addition, RNA from the same samples were quantified using dot blot hybridization under conditions identical to Northern blotting (Figure 7).

At none of the temperatures (55°C, 65°C and 75°C) was hybridization to RNA from pig liver observed, even at 2.1 kb, the known size of nm-β- and nm-γ-actin mRNA (Minty et al., 1981). Likewise, no hybridization to RNA from smooth muscle was detected. Since sm-β- and sm-γ-actin are the isoforms expressed in smooth muscle, these results would suggest that the human sk-α-actin probe does not cross hybridize with any of the porcine β or γ-actin mRNA isoforms under these conditions. Hybridization to skeletal muscle RNA was observed and was specific to one band only, approximately 1.6 kb in size, the size of sk-α-actin mRNA (Minty et al., 1981).

Hybridization was also detected with RNA from the heart and corresponded to mRNA approximately 1.6 kb in size. At

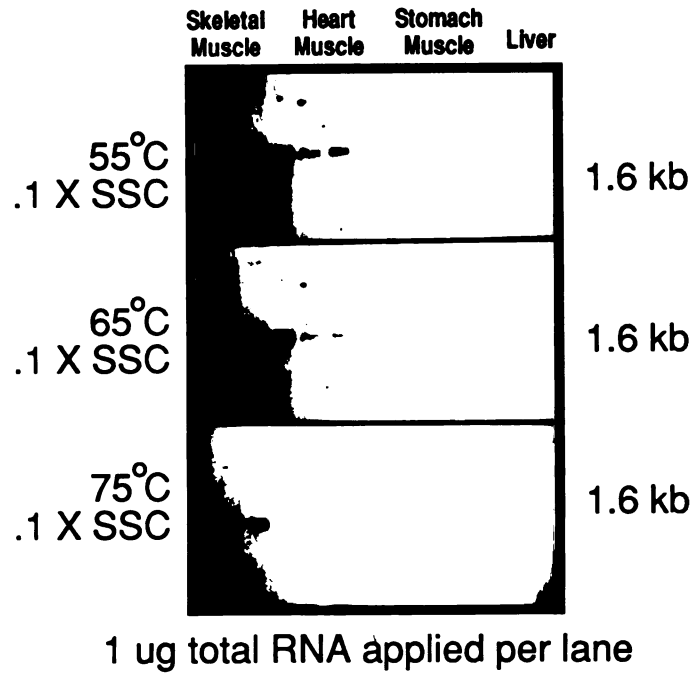


FIGURE 6. The effect of wash temperature on human sk-α-actin hybridization with porcine Northern blots. RNA (1 μg per lane) from liver, heart, smooth muscle (stomach wall) and sternomandibularis muscle of three market weight pigs (average 108 kg body weight) were size-separated (one individual sample per lane) on 1.2% agarose gels and transferred to nylon membrane. After hybridization to the human sk-α-actin cDNA probe (Gunning et al., 1984), the Northern blot was washed at 55°C with .1x SSC and .1% SDS and subjected to autoradiography for 8 h at -80°C. The blot was then subsequently washed at the higher temperatures (65° and 75°C) and again subjected to autoradiography. A single band was observed following hybridization to the full length human skeletal muscle α-actin cDNA. The size of this band was estimated to be approximately 1.6 kb using the 18S and 28S ribosomal bands as size markers.

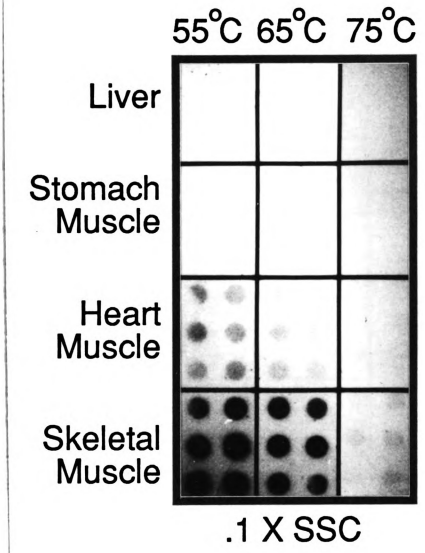


FIGURE 7. The effect of wash temperature on dot blot hybridization. RNA (.2 μg per well) from liver, heart, smooth muscle (stomach) and sternomandibularis muscle of three market weight pigs (108 kg average body weight) were dot blotted in duplicate (individual samples top to bottom, duplicate left to right). The blot was then hybridized to the human sk-α-actin cDNA probe and washed at 55°C with .1x SSC and .1% SDS. Following autoradiography for 14 h at -80°C, the blot was subsequently washed at the higher temperatures (65°C and 75°C) and again subjected to autoradiography.

higher wash temperatures (65°C and 75°C) a hybridization band was still detectable. The higher temperature would allow hybridization of the cDNA only to mRNA of very similar nucleotide sequence. As the temperature increased from 55°C to 75°C, the extent of hybridization with heart RNA relative to hybridization with skeletal muscle RNA remained a consistent 7%. This observation is consistent with mouse and rat data reported by Shani et al. (1981), Minty et al. (1982), Mayer et al. (1984), Vandeherckhove et al. (1986), and Garner et al. (1989). For all future experiments, washing conditions of 65°C and .1x SSC were employed. Again, one cannot rule out the possibility of the human sk-α-actin probe binding to porcine c-α-actin since both α-actin mRNAs are identical in size. The results of Hanauer et al. (1983), Buckingham et al. (1984) and Buckingham et al. (1987) suggest that there is a greater conservation of nucleotide sequence of the same α-actin isoform across species than between actin isoforms within the same species. The consistent relative hybridization to RNA from skeletal muscle and heart, even at the very high stringency conditions, would indicate that the degree of cross-hybridization may be minimal.

In order to determine if the human sk-α-actin probe could be used to measure the relative abundance of α-actin in skeletal muscle of other livestock species, the human probe was also hybridized to bovine, ovine and avian RNA. Total RNA from skeletal muscle, heart muscle, smooth muscle (from

stomach or gizzard) and liver was isolated from cattle, sheep and chickens in addition to pigs and was Northern blotted (consolidated in Figure 8) and dot blotted (consolidated in Figure 9) and hybridized to the human sk-α-actin probe. No hybridization was observed with RNA from liver or smooth muscle from any of the four species even though 10 times more nonskeletal muscle RNA than skeletal muscle RNA was applied and subjected to autoradiography for 48 h at -80°C. The extent of hybridization of the human sk-α-actin cDNA with pig heart RNA at 1.6 kb was approximately 7% of that observed with pig skeletal muscle RNA, consistent with the results of Garner et al. (1989).

A single hybridization band was observed with skeletal muscle RNA for each of the four species, approximately 1.6 kb in size, the size of sk-α-actin mRNA (Minty et al., 1981). The relative abundance of α-actin mRNA in skeletal muscle appears to be approximately the same in pigs, cattle and sheep. Hybridization of the human sk-α-actin probe to chicken breast muscle is only 25% of that observed with the other meat producing species. This reduced hybridization with chicken RNA may be due to lower abundance of α-actin which may be the result of different species, muscle types or to less similarity in mRNA sequences between human and avian α-actin. The later is most likely. Shani et al. (1981) used a full length cDNA probe to rat sk-α-actin and also found that hybridization to chicken muscle was considerably less than

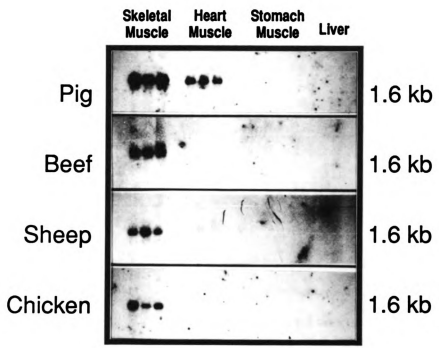


FIGURE 8. Northern blots of liver, heart, smooth and skeletal muscle in pigs, cattle, sheep and chickens. RNA was isolated from the tissues indicated and size separated on 1.2% agarose gels. Three market weight animals represented each species (one sample per lane). Threefold greater amounts (3 μg) of liver, heart and smooth muscle (stomach or gizzard) RNA were applied per lane than skeletal muscle (1 μg). The RNA was transferred to nylon membrane, hybridized to human sk-α-actin cDNA probe (Gunning et al., 1984) and washed at 65°C with .1x SSC and .1% SDS. The Northern blots were subjected to autoradiography for 8 h at -80°C. A single band was observed following hybridization to the full length human skeletal muscle α-actin cDNA. The size of this band was estimated to be approximately 1.6 kb using the 18S and 28S ribosomal bands as size markers.

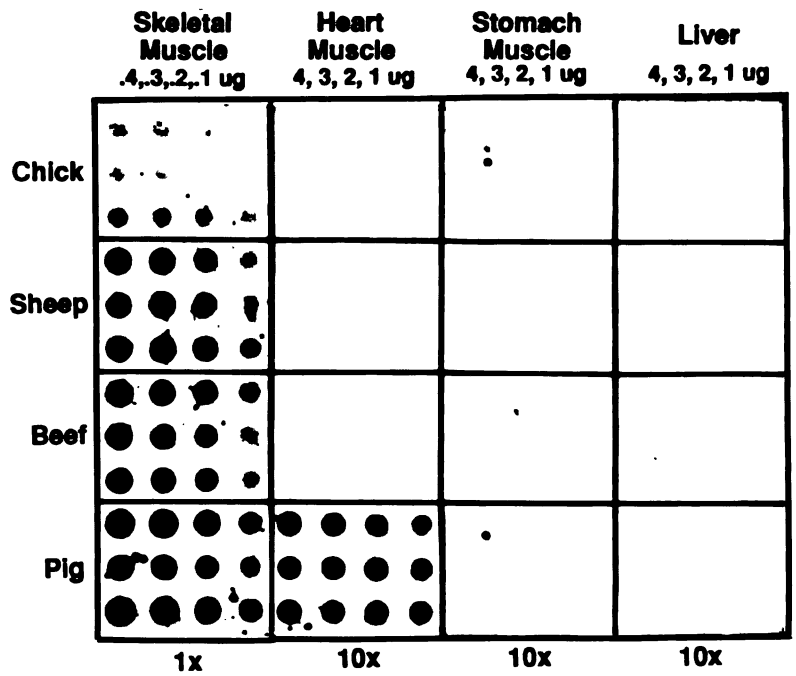


FIGURE 9. Dot blot quantification of α-actin hybridization in pigs, cattle, sheep and chickens. RNA was isolated from the tissues indicated and blotted onto nylon membrane. Three market weight animals represented each species. Tenfold greater amounts of total RNA (1 to 4 μg) were applied for the liver, heart and smooth muscle (stomach or gizzard) than for skeletal muscle (.1 to .4 μg). Replicates are from left to right for each tissue and individual animals are from top to bottom. All blots were hybridized to the human sk-α-actin cDNA probe (Gunning et al., 1984) and washed at 65°C with .1x SSC and .1% SDS. The blots were subjected to autoradiography for 16 h at -80°C.

that observed with rat muscle. When a probe specific to the 3′ untranslated region of rat sk-α-actin was used, no hybridization to chicken muscle RNA was detected. These results indicate greater α-actin sequence identity and conservation within mammals than across phyla, e.g. avian.

Cross hybridization to other actin isoforms appears to be minimal and is insignificant in skeletal muscle where sk-α-actin represents over 95% of all actin (Caravatti et al., 1982; Barton et al., 1987). The human sk-α-actin cDNA probe hybridizes to a specific 1:6 Kb band of skeletal muscle mRNA, the known size of sk-α-actin. These results indicate that the human sk-α-actin cDNA probe does hybridize to sk-α-actin mRNA in meat producing species and can be used for further studies in determining the regulation of α-actin gene expression and mRNA abundance in skeletal muscle from pigs, cattle and sheep.

Implications

These results indicate that the human sk-α-actin probe can be used as a specific marker for α-actin mRNA expression in skeletal muscle for pigs, cattle and sheep. A cDNA probe for α-actin is a valuable research tool in meat animal research. This probe can be used to determine the α-actin mRNA abundance in skeletal muscle and to monitor the changes that occur in actin gene expression due to physiological, developmental, nutritional or hormonal regulation.

CHAPTER 3

SKELETAL MUSCLE GROWTH AND EXPRESSION OF SKELETAL MUSCLE ALPHA ACTIN mRNA IN PIGS DURING FEEDING AND WITHDRAWAL OF RACTOPAMINE

Abstract

Sixty crossbred barrows were used to study the effect of ractopamine (a phenethanolamine/beta-adrenergic agonist) treatment and its withdrawal on muscle growth and on the relative abundance of skeletal muscle alpha actin (sk-α-actin) mRNA. Ractopamine was fed (20 ppm) for periods of 2, 4 and 6 wk (six pigs per group). Additional pigs (four per group) were fed ractopamine (20 ppm) for 6 wk and then slaughtered 1, 3, and 7 d after withdrawal of ractopamine. Ractopamine increased ($P<.05$) longissimus muscle weight and protein content, although protein concentrations were not different. The increased muscle weight and protein content attained by feeding ractopamine for 6 wk was retained when ractopamine was withdrawn. The RNA and DNA concentrations did not change, whereas total DNA and RNA content per muscle was 18 and 26.7% greater, respectively, in ractopamine treated pigs at 4 wk but there were no differences at 2 or 6 wk or among the withdrawal groups. The relative abundance of sk-α-actin mRNA in the longissimus muscle was 41 and 62% greater ($P<.05$) in treated animals at 2 and 4 wk but similar to controls at 6 wk and during the withdrawal period. These results indicate that the ractopamine-enhanced muscle growth may result from increased myofibrillar gene expression at the pretranslational level which is maximal with short-term treatment of ractopamine.

Introduction

Administration of the phenethanolamine, ractopamine, to finishing pigs increases muscle mass, total muscle protein and RNA content, and fractional protein synthesis rates (Bergen et al., 1989). This is due in part to enhanced fractional synthesis rates of skeletal muscle alpha actin (sk-α-actin) and increased relative abundance of sk-α-actin mRNA (Helferich et al., 1990). A time-course study in which muscle growth and abundance of sk-α-actin is monitored during administration and withdrawal of ractopamine to pigs is necessary to identify possible biological mechanisms mediating ractopamine-induced muscle hypertrophy. The objective of the present study was to monitor changes in skeletal muscle protein metabolism and sk-α-actin mRNA abundance in pigs fed ractopamine during a 6 wk feeding period and a subsequent 7 d withdrawal period.

Materials and Methods

Animals, Treatments, and Sample Collection. Sixty crossbred barrows (Yorkshire x Hampshire x Duroc) with an average initial body weight of 72.5 kg were randomly divided into two groups, one group of 36 pigs for a ractopamine feeding time course experiment and another group of 24 pigs for a ractopamine withdrawal experiment. All pigs were housed at the Swine Research Facility at Michigan State University and given ad libitum access to a 16.6% corn/soybean meal finishing diet (Table 4). The first group (36 pigs) was allotted to four pens (nine pigs/pen) and pigs in two of the pens were fed the phenethanolamine, ractopamine {1-[4-hydroxyphenyl]-2-[1 methyl-3(4 hydroxyphenyl) propylamino] ethanol, Eli Lilly, Indianapolis, IN}. Pigs were fed the control diet or the control diet plus 20 ppm of ractopamine for 2, 4, or 6 wk (three pigs/pen/feeding period). After the designated feeding period, pigs were slaughtered immediately at the Michigan State University Meat Laboratory, tissues collected and carcass measurements obtained. The 24 pigs for the ractopamine withdrawal experiment were assigned to four pens (six pigs/pen) and fed the control diet with or without 20 ppm of ractopamine (two pens/treatment) for 6 wk. Ractopamine was then withheld and the pigs were fed the control

TABLE 4. Finishing diet fed to crossbred barrows

Ingredients	Composition %
Corn grain	79.2
Soybean meal (48% protein)	17.9
Calcium phosphate (dibasic)	1.0
Calcium carbonate	.9
NaCl	.5
Vitamin-mineral premix[a]	.5
Lysine hydrochloride	.1

[a] Premix provided per kg of diet: Vitamin A, 3,300 IU; vitamin D_3, 600 IU; riboflavin, 3.3 mg; nicotinic acid, 17.6 mg; d-panthothenic acid, 13.2 mg; choline, 110 mg; vitamin B_{12} 19.8μg; Zn, 74.8 mg; Fe, 9.4 mg; Mn 37.4 mg; Cu, 9.9 mg; I, .5 mg; Se, 1 mg.

diet for an additional 1, 3, or 7 d (two pigs/pen/withdrawal time) before slaughter and collection of tissue samples and carcass measurements.

Immediately after stunning and exsanguination, samples of the left longissimus muscle and the liver were excised and weighed. The muscle sample was dissected free of visible fat and connective tissue, and both tissues were cut into cubes of approximately 8 cm^3 and frozen by submersion in liquid nitrogen. All samples were collected and frozen within 5 min of stunning and then stored at -80°C for subsequent analysis. The remaining longissimus muscle of the left side was removed, dissected free of fat, weighed, and added to above sample weight to obtain total left longissimus muscle mass. A portion of the frozen longissimus sample was powdered with solid CO_2 at -70°C and used to determine protein, RNA and DNA content. Nucleic acids (DNA and RNA) were assayed according to a modified procedure (Munro and Fleck, 1969) as described by Bates et al. (1985) and protein was determined by the micro-Kjeldahl method (AOAC, 1980).

Determination of Sk-α-Actin mRNA Abundance. Total RNA for quantification of sk-α-actin mRNA was isolated from longissimus muscle by a combination of urea-LiCl precipitation (Minty et al., 1981) and guanidine isothiocyanate-CsCl centrifugation (Chirgwin et al., 1979) as previously described (Helferich et al., 1990; Skjaerlund et al., 1993). Northern blot analysis was also performed for qualitative purposes only

to ensure specificity of hybridization as previously reported by Skjaerlund et al. (1993). Northern blot analysis was performed by denaturing total RNA extracted from longissimus muscle and separated electrophoretically (1 μg per lane) using a denaturing agarose gel (1.2% agarose, 40 mM 3-N-morpholinopropanesulfonic acid, 10 mM sodium acetate, 1 mM EDTA, and 2.2 M formaldehyde). The RNA was transferred to a nylon membrane, Zetabind®(CUNO, Meriden, CT), using 25 mM sodium phosphate buffer. Additional lanes were used for 18S and 28S RNA markers for size determination. Hybridization was carried out as subsequently described for dot-blots. Relative abundance of sk-α-actin mRNA was determined by dot-blot hybridization methods as previously described (Helferich et al., 1990) using a minifold apparatus (Schleicher and Schuell, Keene, NH) with 96 wells. Total RNA was blotted onto nylon membrane (Zetabind®) at .1, .2, .3, .4 μg longissimus muscle RNA per dot. The conditions for prehybridization (50% formamide, 5x SSC, 10x Denhardts, 50 mM sodium phosphate, 1 mM EDTA, 500 μg/mL yeast tRNA, 42°C), hybridization (55% formamide, 5x SSC, 1.2x Denhardts, 50 mM sodium phosphate, 1mM EDTA, 500 μg/mL yeast tRNA, 2 million cpm of ^{32}P-labeled probe/mL, 42°C) and subsequent washings (.1x SSC, .1% SDS, 65°C) were similar to those described by Skjaerlund et al. (1993). The cDNA probe, a full-length probe coding for human sk-α-actin obtained from Dr. L. Kedes' laboratory (University of Southern California, School of Medicine, Los Angeles, CA)

and characterized by Gunning et al. (1983), was labeled with [^{32}P]deoxycytidine triphosphate (3,000 Ci/mmol, Amersham, Arlington Heights, IL). The insert was excised from the plasmid (pHMαA-1) by digestion with *Pvu*II and *Pst*I before labeling. It was previously determined that this probe can be used with livestock species (Skjaerlund et al., 1993). Dried blots were exposed to X-Ray film (Kodak, Rochester, NY) in cassettes containing two intensifying screens (Dupont, Wilmington, DE) and the extent of hybridization was quantified by densitometry. For comparison purposes, all procedures were conducted simultaneously; all were prehybridized, hybridized and washed in the same solution, and exposed together to the same X-Ray film.

Statistical analysis. All data were statistically analyzed using the general linear models procedure of the Statistical Analysis System (SAS, 1987). A split plot design was used. The effect of ractopamine was tested using variation among pens [i.e., pen (treatment)] as the error term. The effects of feeding or withdrawal times and the interaction of treatment with time were tested using pen (treatment x time) as the error term. Due to the low number of pens per treatment and degrees of freedom for the error terms, variation among pens was pooled with variation among pigs and used as the error term in cases in which the F statistic for variation for pens was less than 2F.50 as outlined by Gill (1989); however, there is potential for bias

from pooling the sum of squares (and corresponding degrees of freedom) for pens and animals. Means comparisons were made using the Student-Newman-Kuels procedures in SAS (1987). Sk-α-actin mRNA abundance data were analyzed within each feeding or withdrawal time using the Student's t test (Gill, 1978).

Results and Discussion

Feeding 20 ppm of ractopamine to pigs in a finishing diet increased (P<.05) longissimus muscle weight 17.3% at 2 wk, 19% at 4 wk, and 13.8% at 6 wk relative to control longissimus muscle weights (Table 5). Protein contents of the longissimus muscle from treated pigs were 18, 19, and 17% greater than those of muscle from control pigs at 2, 4, and 6 wk, respectively. Protein concentrations were not affected by treatment. In a similar study, Bergen et al. (1989) found that ractopamine increased semitendinosus muscle mass 25% over controls at 4 wk but only by 9% at 6 wk. Other beta-adrenergic agonists have provided similar responses. In young male rats, clenbuterol increased weights of selected muscles by 18 to 39% over controls after 11 d of feeding, but only 0 to 6% after 25 d (Reeds et al., 1986). Cimaterol has also been demonstrated to increase muscle weight 27 to 33% after 7 wk of treatment, but the difference between controls and cimaterol-fed lambs was less after 12 wk (Beermann et al., 1987). Kim et al. (1992) found increased weight gain in skeletal muscles of rats fed cimaterol for up to 2 wk, but no further increase occurred with feeding cimaterol for an additional 2 wk. Rats fed cimaterol had the greatest accleration in gain within 1 wk and rats no longer gained at an accelerated rate after 1 wk (Eadara et al., 1989).

TABLE 5. Longissimus muscle weight, nucleic acid and protein content of finishing pigs fed ractopamine for 2, 4 or 6 weeks[a]

Variable	2 weeks		4 weeks		6 weeks		SEM
	C	R	C	R	C	R	
Longissimus wt, g	1542[b]	1808[c]	1842[c]	2192[de]	2001[cd]	2277[e]	68
Protein, g/muscle	321[b]	380[c]	397[cd]	472[ef]	433[de]	507[f]	15
Protein, %	20.8[b]	20.9[b]	21.6[cd]	21.5[bc]	21.7[cd]	22.3[d]	.2
RNA, mg/muscle	2860[b]	3086[bc]	3074[bc]	3896[d]	3564[cd]	3641[cd]	238
RNA, mg/g	1.87[b]	1.70[b]	1.67[b]	1.78[b]	1.78[b]	1.60[b]	.11
DNA, mg/muscle	768[b]	873[bc]	766[b]	906[c]	822[bc]	885[bc]	60
DNA, mg/g	.50[d]	.48[cd]	.42[bc]	.41[bc]	.41[bc]	.39[b]	.03
RNA/DNA	3.73[b]	3.64[b]	4.04[b]	4.32[b]	4.40[b]	4.19[b]	.33
Protein/DNA	424[b]	448[bc]	521[cd]	532[cd]	542[cd]	582[d]	33
Protein/RNA	114[b]	124[bc]	132[bc]	124[bc]	129[bc]	141[c]	9.3

[a] C = control diet; R = control diet supplemented with 20 ppm ractopamine; within a feeding time, there were 6 pigs/treatment.

[b,c,d,e,f] Means within a row lacking a common superscript letter differ ($P<.05$).

Likewise in their study, fractional accretion rates increased up to 120% in response to cimaterol at 1 wk but no changes in fractional accretion rates were detected thereafter. It appears that beta-adrenergic agonists enhance muscle mass early on and that prolonged feeding of them does not continue to increase muscle weight, but the early increment of gain is maintained. Withdrawal of ractopamine for up to 7 d after the 6 wk feeding period did not diminish the previously increased longissimus muscle weight or protein content (Table 6). This further suggests that the effect of beta-adrenergic agonists on skeletal muscle accretion occurs early on and that the net increase is maintained at least for 7 d after withdrawal of the agonist. The ractopamine-induced gain also has been shown to be maintained in steers even after a 78 d withdrawal period (Schiavetta et al., 1990).

Content of RNA was increased 26.7% compared with the controls at 4 wk (Table 5). There were no differences between treatment groups in muscle RNA content at 2 or 6 wk or between the withdrawal groups (Table 6). There were no significant differences in muscle RNA concentrations although RNA concentrations tended to be greater in pigs fed ractopamine for 4 wk and lower in pigs fed ractopamine for 6 wk, including those after withdrawal of ractopamine. These results are similar to those reported by Bergen et al. (1989) in which

TABLE 6. Longissimus muscle weight, nucleic acid and protein content of finishing pigs after withdrawal of ractopamine for 1, 3, or 7 days[a]

Variable	1 day		3 days		7 days		SEM
	C	R	C	R	C	R	
Longissimus wt, g	2093[bc]	2379[cd]	2036[b]	2456[d]	2072[bc]	2468[d]	103
Protein, g/muscle	456[b]	550[c]	443[b]	571[c]	452[b]	577[c]	24
Protein, %	21.8[b]	23.1[c]	21.8[b]	23.2[c]	21.7[b]	23.4[c]	.3
RNA, mg/muscle	3710[b]	3922[b]	3543[b]	3811[b]	3626[b]	4101[b]	312
RNA, mg/g	1.76[b]	1.65[b]	1.74[b]	1.57[b]	1.76[b]	1.66[b]	.14
DNA, mg/muscle	891[b]	855[b]	809[b]	1042[b]	859[b]	1122[b]	137
DNA, mg/g	.42[b]	.38[b]	.40[b]	.42[b]	.43[b]	.46[b]	.06
RNA/DNA	4.21[b]	4.40[b]	4.58[b]	3.99[b]	4.48[b]	3.81[b]	.51
Protein/DNA	529[b]	660[b]	584[b]	578[b]	554[b]	557[b]	78
Protein/RNA	127[b]	144[b]	128[b]	152[b]	126[b]	143[b]	13

[a] C = Control diet fed for 6 weeks; R = ractopamine-supplemented diet (20 ppm) fed for 6 weeks; within a withdrawal time, there were 4 pigs/treatment.

[b,c,d] Means within a row lacking a common superscript letter differ ($P<.05$).

ractopamine-fed pigs had increased RNA content by 36% at 4 wk but not at 2 or 6 wk. The RNA concentrations also showed the same trend but were not statistically different. Feeding cimaterol also increased RNA concentrations initially in rat skeletal muscle (Kim et al., 1988). The RNA content in rat hindlimb muscle increased greatly after 1 wk of cimaterol feeding but the increase was less pronounced after 2 and 4 wk (Eadara et al., 1989). Feeding cimaterol to lambs for 7 and 12 wk increased RNA content 36 and 37%, respectively, although RNA concentrations were not altered (Beerman et al., 1987). The differences in RNA content between controls and clenbuterol-fed rats were greatest at 11 d, paralleling the differences in muscle mass (Reeds et al. 1986).

Longissimus muscle DNA concentration was not altered by ractopamine at any sampling period. Feeding ractopamine for 4 wk increased muscle DNA content 18% above that of control pigs, but muscle DNA content did not differ significantly between treatment groups at other sampling times. Due to the trends in nucleic acid and protein contents, ratios of protein/DNA, protein/RNA and RNA/DNA were not changed by feeding ractopamine. Even though ractopamine increased the proliferation of cultured chick breast muscle satellite cells (Grant et al., 1990), the recruitment of additional satellite cell nuclei does not seem to be a prerequisite for accelerated accretion of skeletal muscle as DNA concentrations are not enhanced with the feeding of ractopamine. The increased

protein accretion and RNA accounts for the majority of the ractopamine-induced muscle hypertrophy. The fact that DNA concentration remained relatively constant over the treatment period implies that DNA increases proportionally with the ractopamine-induced muscle hypertrophy. In rats fed cimaterol, DNA content did not differ at 1 wk but increased after 2 and 4 wk following the early induced hypertrophy evident at 1 wk (Eadara et al., 1989). Longissimus muscle weight, protein content, and RNA content in pigs fed ractopamine for 2 and 4 wk were similar to those in control pigs at 4 and 6 wk, respectively; thus, ractopamine is similar to cimaterol in that it accelerates muscle growth to attain more mature stages sooner as suggested by Beermann et al. (1987).

Total RNA was isolated from the longissimus muscle and subjected to Northern blotting for qualitative purposes only, in order to determine the specificity of hybridization. The RNA from three randomly selected samples per treatment group were applied (1 μg/lane) and hybridized to the sk-α-actin cDNA probe (autoradiograms consolidated in Figure 10). Hybridization of the sk-α-actin cDNA probe was observed as a single band, approximately 1.6 kb in size, the size of sk-α-actin mRNA (Minty et al., 1981) and corresponds with hybridization bands observed previously (Skjaerlund et al., 1993). In order to quantify differences in the relative abundance of sk-α-actin mRNA, longissimus RNA (.1, .2, .3, and

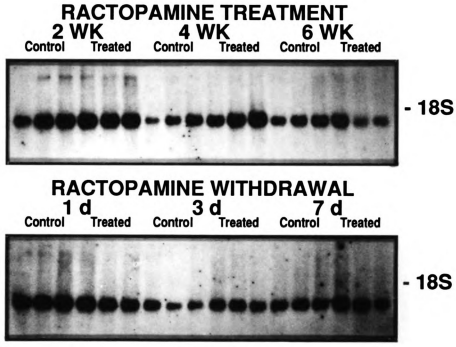


FIGURE 10. Northern blots of sk-α-actin mRNA in longissimus muscle isolated from ractopamine-treated pigs and after withdrawal. RNA was isolated from longissimus muscle and size-separated on 1.2% agarose/18% formaldehyde gels. One μg of RNA was applied to each lane (one animal per lane). The RNA was transferred to nylon membrane, hybridized to a human sk-α-actin cDNA probe and washed at 65°C with .1x SSC and .1% SDS. The single band observed after autoradiography at -80°C was estimated to be approximately 1.6 kb using the 18S and 28 S ribosomal bands as size markers.

.4 μg RNA/dot) from each animal was hybridized to the sk-α-actin probe using dot-blot hybridization. The results, quantified by densitometry, are shown in Figure 11. At 2 and 4 wk, pigs fed ractopamine had 41 and 62%, respectively, greater (P<.05) sk-α-actin mRNA abundance than control pigs. Helferich et al. (1990) demonstrated approximately a twofold increase in sk-α-actin mRNA abundance in pigs fed ractopamine for 4 wk and Smith et al. (1989) observed an increase in myosin light chain mRNA abundance in cattle fed ractopamine. Koohmaraie et al. (1991) showed a 30% increase in sk-α-actin mRNA abundance in lambs fed the beta-adrenergic agonist $L_{644,969}$ for 6 wk. The increase in sk-α-actin mRNA abundance is consistent with ractopamine-enhanced fractional synthesis rates of α-actin observed in pigs (Bergen et al., 1989; Helferich et al., 1990) and with the increased fractional accretion rates in hindlimb muscle following feeding cimaterol to rats (Eadara et al., 1989). McElligott and Chaung (1987) observed that serum from rats treated with clenbuterol and serum from normal rats had similar effects on protein synthesis and degradation and cell proliferation in cultures of L8 myoblasts. Ractopamine has been shown to directly increase protein synthesis in cultures of L6 myotubes (Adeola et al., 1989; Anderson et al., 1990) suggesting that beta-adrenergic agonists may act directly on skeletal muscle in vivo to increase muscle growth. The increased muscle mass observed with feeding beta-adrenergic agonists appears to be

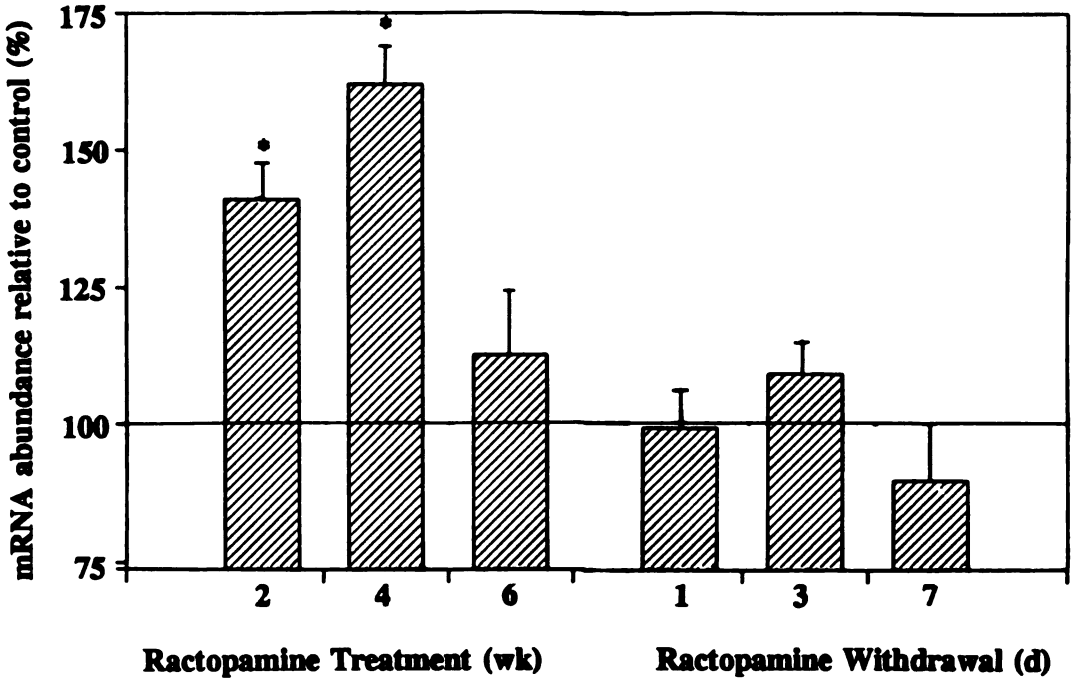


FIGURE 11. Abundance of sk-α-actin mRNA in longissimus muscle of ractopamine-treated pigs relative to controls. The abundance of sk-α-actin mRNA was quanitified using dot-blot hybridization. RNA (.1, .2, .3, .4 μg/dot for each sample) isolated from longissimus muscle of each animal were blotted onto nylon membrane. All samples were hybridized at one time to the human sk-α-actin cDNA probe and washed at 65°C with .1x SSC and .1% SDS. The blots were subjected to autoradiography at -80°C simultaneously. Extent of hybridization was quantified by densitometry. Abundance of sk-α-actin mRNA in ractopamine-treated pigs is expressed relative to the respective controls. An asterisk (*) indicates $P<.05$.

due in part to pretranslational enhancement of myofibrillar protein synthesis. The increased abundance of sk-α-actin mRNA could result from several pretranslational events, i.e., increased transcription, enhanced processing of the pre-mRNA or greater stability of the mature message.

Beta-adrenergic agonists most likely elevate mRNA abundance of muscle proteins sooner than 2 wk. In rats fed cimaterol, RNA concentration in skeletal muscle increased after 3 d of administration and the effect was lost at approximately 2 wk of treatment (Kim et al., 1988). In our study, the relative abundance of sk-α-actin mRNA was not significantly different between pigs fed ractopamine for 6 wk and control pigs and no differences were detected after 1, 3, or 7 d of ractopamine withdrawal (Figure 11). The RNA content or concentration also did not differ after feeding ractopamine for 6 wk, which corroborates data reported by Bergen et al. (1989). The increase in muscle mass and relative abundance of sk-α-actin mRNA resulting from feeding ractopamine may occur before 6 wk. However, the additional muscle mass attained at 6 wk is maintained after withdrawal of ractopamine for at least 7 d. Stimulation of muscle accretion by feeding ractopamine seems to wane with chronic administration. This attenuation effect with prolonged treatment may be due in part to a change in beta-adrenoreceptor density. Kim et al. (1992) and Rothwell et al. (1987) reported a reduction of up to 50% in the number of skeletal muscle beta-receptor binding sites

that preceded the attenuation of muscle gain and receptor affinity was not altered at any time point. Intermittent feeding, rather than continuous feeding, of beta-adrenergic agonists may prevent the attenuation in muscle gain that occurs over time as suggested by McElligott et al. (1989).

Implications

The results of this study indicate that the ractopamine-enhanced muscle growth is partly due to increased gene transcription or other pretranslational events that increase mRNA abundance of α-actin and probably other myofibrillar proteins as well. Continuous feeding of ractopamine may have a greater short-term effect rather than a sustained, long-term effect on α-actin gene expression.

SUMMARY

Even though postpubertal concentrations of testosterone stimulate protein synthesis and protein accretion, elevated neonatal testosterone concentrations seem to play a less significant role in skeletal muscle protein metabolism. Castration at birth did not significantly alter neonatal muscle growth or protein turnover of barrows compared to those of boars. Developmental changes in protein, nucleic acid concentrations and ratios are apparent during this neonatal growth period of pigs and these changes are probably the most dramatic during the entire growth and development period following birth.

The human sk-α-actin probe can be used as a specific marker for α-actin mRNA expression in skeletal muscle for pigs, cattle and sheep. This cDNA probe for α-actin is a valuable research tool in meat animal research for further determining pretranslational control of protein synthesis. This human probe can be used to determine the α-actin mRNA abundance in skeletal muscle and to monitor the changes that occur in actin gene expression due to physiological, developmental, nutritional or hormonal regulation.

The ractopamine-enhanced muscle growth is partly due to increased gene transcription or other pretranslational events that increase mRNA abundance of α-actin and probably other

myofibrillar proteins as well. Continuous feeding of ractopamine may have a greater short-term effect rather than a sustained, long-term effect on α-actin gene expression and muscle growth, although short-term withdrawal of ractopamine did not reduce the previous gains. This attenuation effect of beta-adrenergic agonists may perhaps be prevented by intermittent feeding rather than long-term continuous feeding.

APPENDICES

APPENDIX A

GROWTH OF BACTERIA AND PURIFICATION OF PLASMID DNA

The alpha actin cDNA was received inserted into the Okayama-Berg, modified pBR322 and transformed into E. coli. A colony was used to inoculate 10 mL of culture medium, L-broth (Bacto tryptone and yeast extract with sodium chloride), .2% glucose, ampicillin in a 50 mL Erlenmeyer flask and grown overnight at 37°C in shaking water bath. A 1 mL aliquot of the overnight culture was added to 250 mL of culture medium containing ampicillin and grown at 37°C in a shaking water bath until a density of .8 A600 was obtained. Chloramphenicol was then added (final .2 mg/mL) and the cells grown overnight at 37°C in a shaking water bath. The medium was then transferred to 250 mL polypropylene bottles and centrifuged for 15 min at 5,000 rpm. The pellet was resuspended in 25 mL of cold 10 mM sodium chloride and centrifuged in 30 mL glass Corex tubes for 15 min at 5,000 rpm. The cells were drained and stored at -80°C until plasmid DNA was extracted.

The sedimented cells were then resuspended in 4 mL of 25 mM Tris-HCl, 10 mM EDTA and 1.5% RNase-free sucrose. After complete resuspension, an additional 2 mL were added with 6 mg/mL lysozyme. After cells were lysed at 4°C for 15 min, 12 mL of .8% sodium hydroxide and 1% SDS were added and mixed thoroughly until the solution clarified. The solution was centrifuged for 10 min at 5,000 rpm after the addition of 7.5 mL 3 M sodium acetate, pH 5.0. The supernantant was transferred to a 150 mL Corex bottle and incubated for 20 min at 37°C with RNase A (20 μg/mL) to digest RNA.

The nucleic acids were extracted with an equal volume (25 mL) of phenol:chloroform:isoamyl alcohol (24:24:1). After vigorous shaking for 5 min and centrifugation at 3,000 rpm for 5 min, the supernatant was further extracted with an additional volume of chloroform:isoamyl alcohol (24:1). After shaking and centrifugation, 2 volumes of 100% ethanol was added to the supernatant and stored at -20°C for 1 hour or overnight. The precipitated DNA was recovered by centrifugation at 5,000 rpm for 10 min and the pellet rinsed with 10 mL of 70% ethanol. The pellet was then dissolved in 1.6 mL sterile water and transferred to a 15 mL Corex tube with the addition of .4 mL 4 M sodium chloride (1 M final) and 2 mL 13% polyethylene glycol (6.5% final concentration), mixing after each addition. DNA was precipitated for 60 min on ice and the plasmid DNA pelleted by centrifugation at 5,000 rpm for 10 min. The cellular RNA remained in the supernatant. The pelleted DNA was washed with 70% ethanol and then resuspended in 1 mL sterile TE-8 and stored at 4°C. DNA

concentration was determined by UV absorbance at 260 nm and a quality control check was performed by digesting 1 μg of DNA with the appropriate restriction enzyme and then analyzed on a 1% agarose gel with ethidium bromide.

APPENDIX B

RESTRICTION DIGESTS AND ISOLATION OF INSERT cDNA

The full length alpha actin plasmid DNA (200μg) was digested first with *Pst* I for 8 h at 37°C. The DNA was precipitated with 25 μL 4 M sodium chloride and 1 mL ethanol and pelleted by centrifugation in a microfuge. After the addition of water, restriction buffer and *Pvu* II, the DNA was cut a second time overnight. The digestion with both *Pst* I and *Pvu* II allows the removal of the cDNA insert but also cuts the cDNA in two yielding a 700 bp and an 800 bp segment. The 3′ untranslated alpha actin probe was digested for 8 h with *Eco* R1 at 37°C and a 136 bp fragment was released.

The DNA was precipitated with 4 M sodium chloride and ethanol and resuspended after centrifugation in 60 μL TE-8. After the addition of 60 μL of 2x loading buffer, the digest was loaded onto a 1% agarose gel and the cDNA inserts separated from the plasmid DNA. The cDNA insert bands were cut out and put in a dialysis bag with 1 mL TE-8 and dialyzed for 3 h with 90 V. After dialysis, the gel was removed and the TE-8 - DNA suspension transferred to a microfuge tube. After centrifugation to remove any agarose, the supernatant was transferred to a new tube and filled to the top with butanol. After vortexing the bottom layer was transfered and more butanol added. This procedure was repeated several times until the bottom layer was approximately 450 μL. This layer was then separately extracted with an equal volume of phenol:chloroform:isoamyl alcohol and chloroform:isoamyl alcohol. The DNA was then precipitated with 1/20 volume (22 μL) 4 M sodium chloride and 1 volume isopropyl alcohol and placed at -20°C for 1 h. After centrifugation and washing with 70% ethanol, the pellet was dried and resuspended in 25 μL TE-8. DNA concentration was determined by microtiter plates with ethidium bromide and UV illumination using pBR322 DNA as standards.

The following page shows the 700 and 800 bp restriction fragments following 1.2% agarose gel electrophoresis.

The restriction cut (Pst I, Pvu II) plasmid DNA was separated from the insert DNA on 1.2% agarose gels. The insert bands were then excised and electroluted. Size of the purified fragments was confirmed on 1.2% agarose gel stained with wthidium bromide. Lane 1 contains the full length 700 and 800 bp fragments. Lane 2 contains a 136 bp 3' untranslated insert cut with Eco R1. Lane 3 is a Hind III cut lambda for size markers of 23.1, 9.4, 6.6, 4.4, 2.3, 2.0, 1.0 and .6 kb. Lane 4 is a Hae III phiX digest for size markers of 1353, 1078, 872, 603, 310, 281, 271 and 234 bp.

APPENDIX C

RANDOM PRIMING OF cDNA PROBES

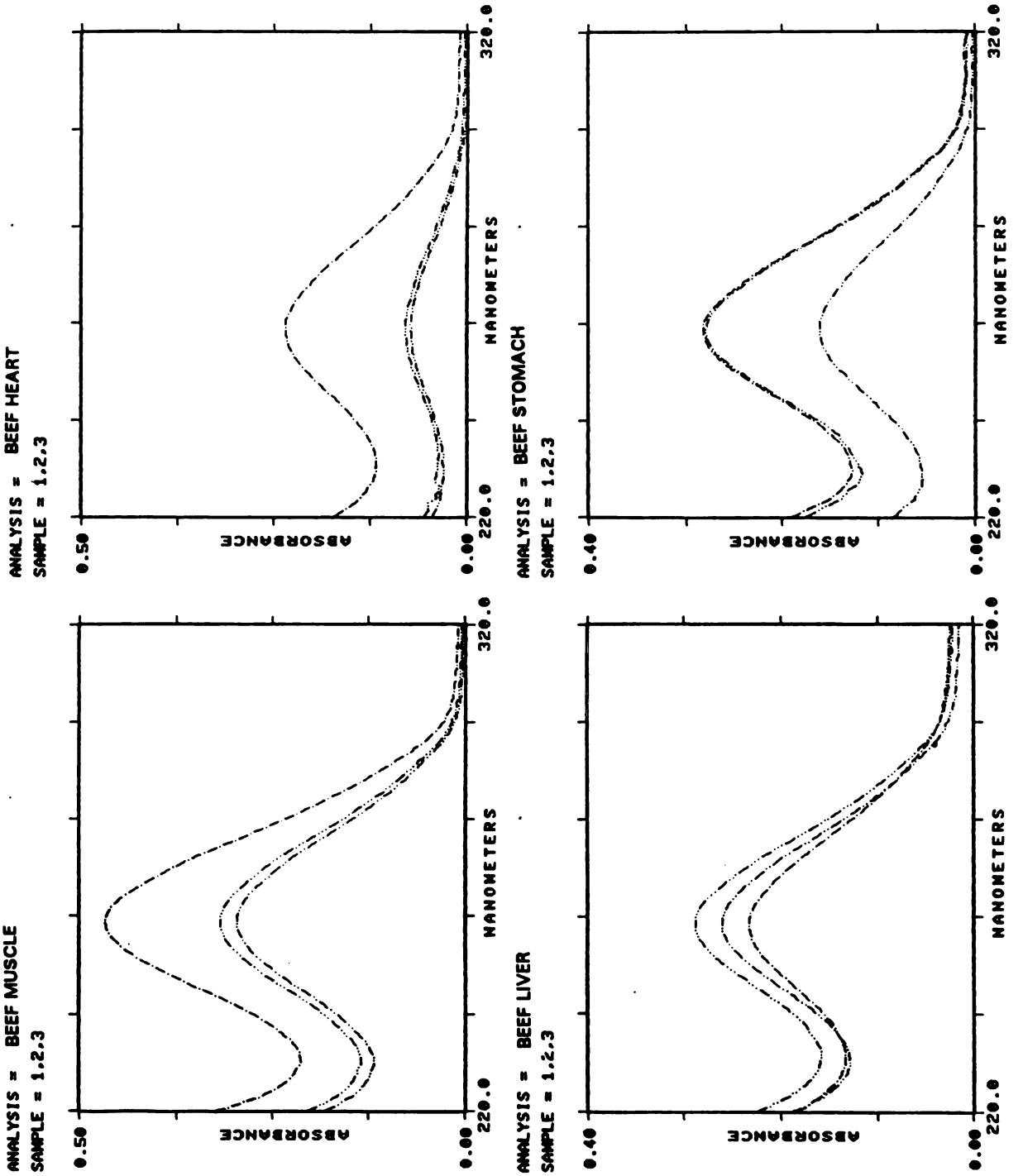
The method of random priming for labeling DNA as developed by Feinberg and Vogelstein (1983, 1984) was used for the cDNA probes. A complementary strand is synthesized from the 3′ OH terminal of a random hexanucleotide primer using the Klenow enzyme. A Boehringer-Mannheim DNA labeling kit was used to random prime DNA with [^{32}P]cytidine (3,000 Ci/mmol). Incorporation was monitored by precipitating a 1 μL aliquot with TCA and ethanol and washing through a glass fiber filter. The filter was then placed in scintillation cocktail and counted. Incorporation was typically 400,000,000 cpm per μg DNA. The length of the fragments are typically 80 to 200 nucleotides in length.

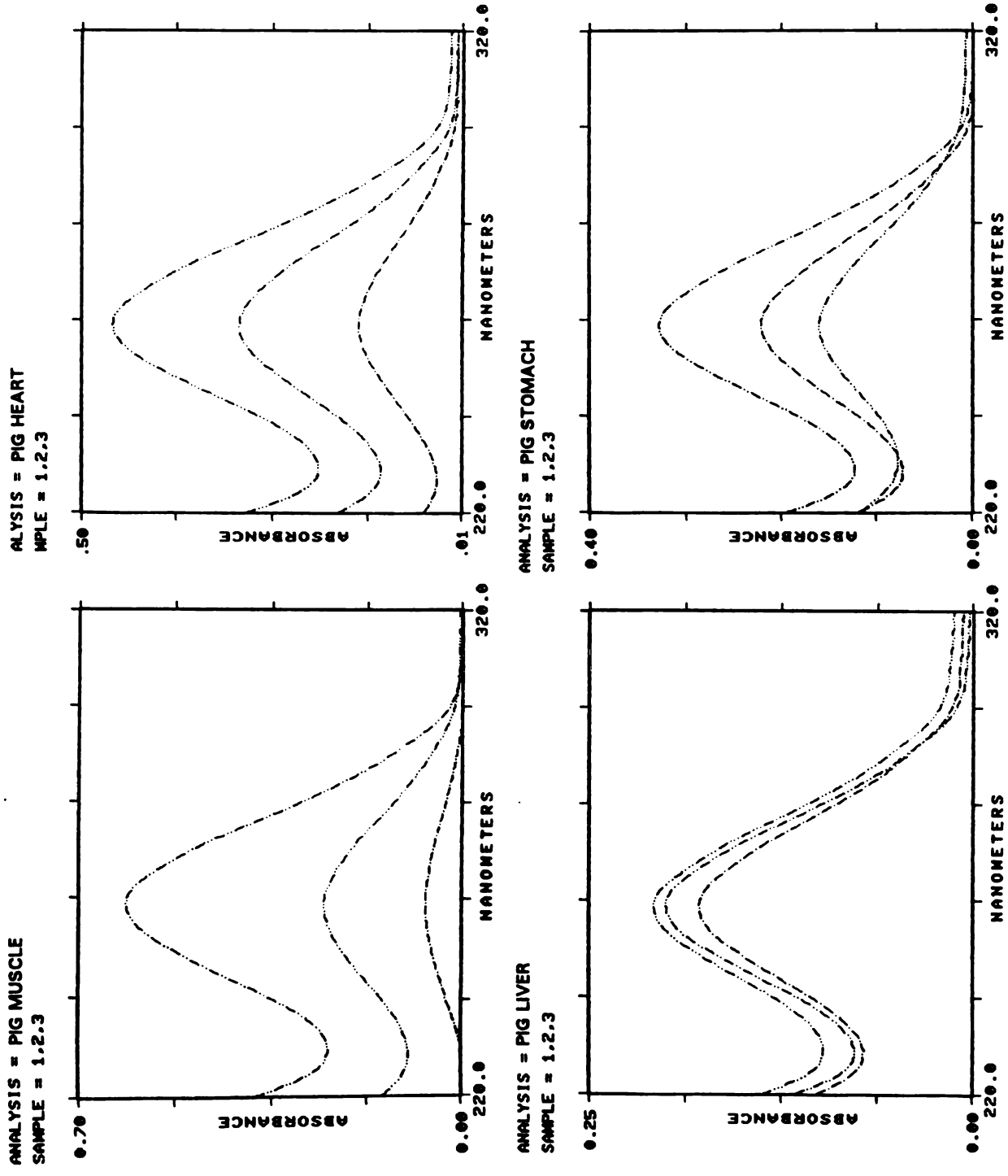
The isolated insert DNA, approximately 125 ng, was incubated at 37°C for 30 min in the presence of dATP, dTTP, dGTP, [^{32}P]dCTP, hexanucleotide primer and Klenow fragment. The reaction was stopped by the addition of 2 μL of .2 M EDTA and heating for 10 min at 65°C. The DNA was purified (i.e. removal of nonincorporated nucleotides) by precipitation after the addition of 10 μL tRNA (10 μg/μL), 10 μL 5 M ammonium phosphate and 100 μL isopropanol. The DNA was pelleted by centrifugation, washed with 70% ethanol and then resuspended in 100 μL TE-8. An 40 μL aliquot of 5 M ammonium acetate was added followed by 300 μL of isopropanol to reprecipitate the DNA. After centrifugation and washing the pellet with 100 μL of 70% ethanol, the DNA was resuspended in 100 μL of TE-8 and the specific activity determined before adding to the hybridization solution.

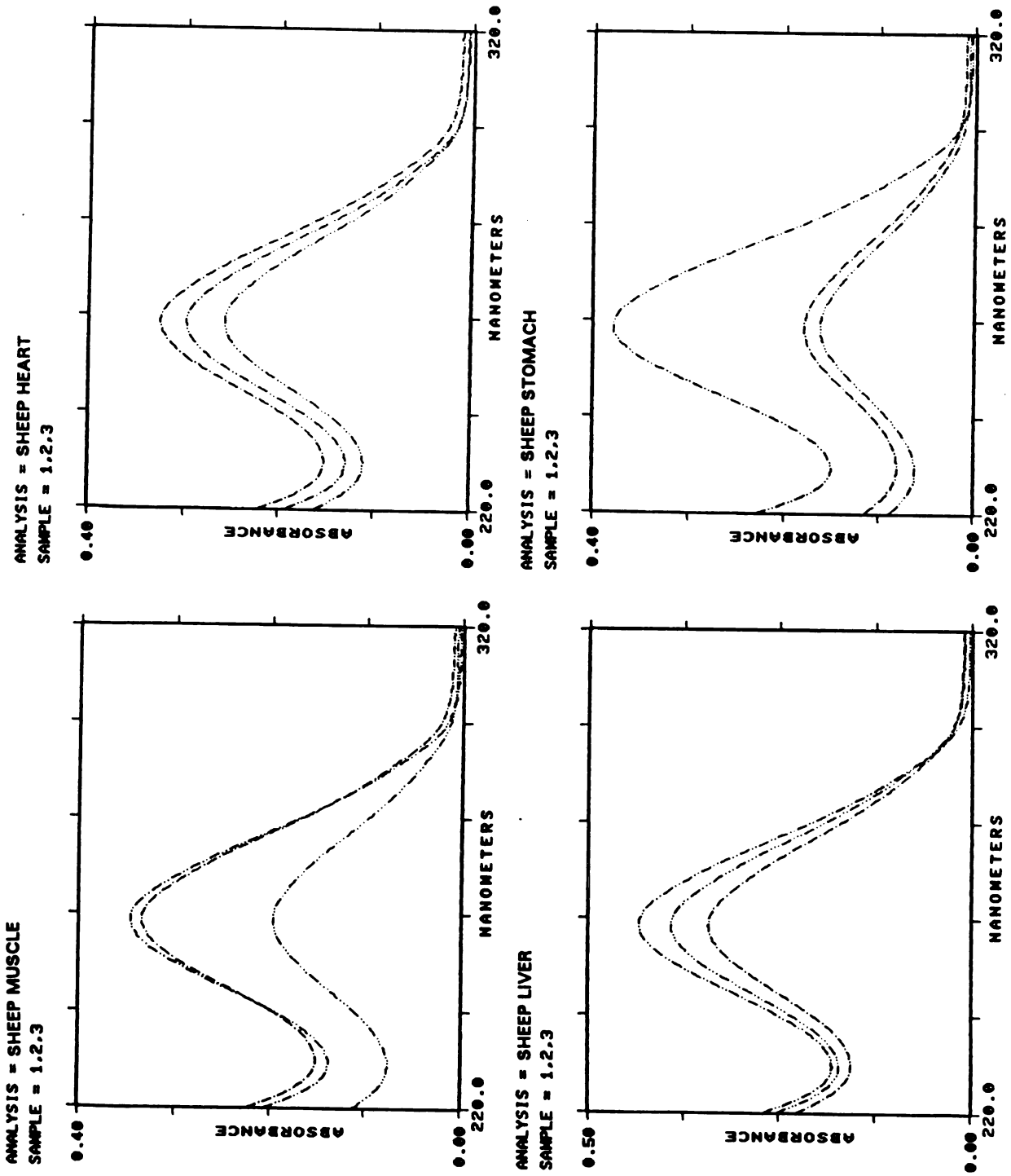
APPENDIX D

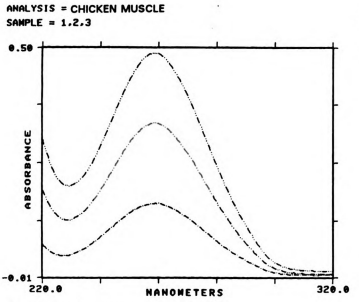
SPECTROPHOTOMETRIC SCANS OF RNA SAMPLES USING WAVELENGTHS FROM 320 TO 220 NANOMETERS

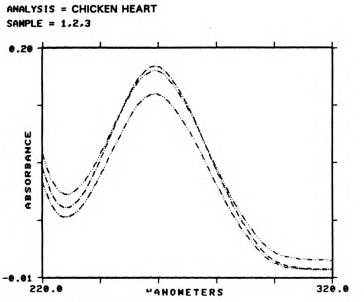
All samples were scanned from 320 nm to 220 nm and the concentration of RNA determined at 260 nm. 280/260 ratios were also recorded as a quality check. The following scans are representative of the RNA samples used in chapter 2.

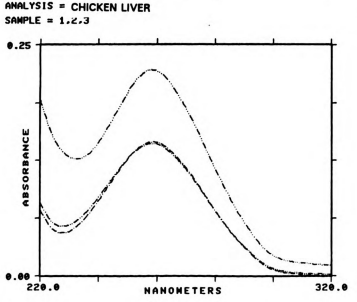

ANALYSIS = BEEF MUSCLE
SAMPLE = 1.2.3
ANALYSIS = BEEF HEART
SAMPLE = 1.2.3
ANALYSIS = BEEF LIVER
SAMPLE = 1.2.3
ANALYSIS = BEEF STOMACH
SAMPLE = 1.2.3
ABSORBANCE
NANOMETERS
0.50
0.40
0.00
220.0
320.0

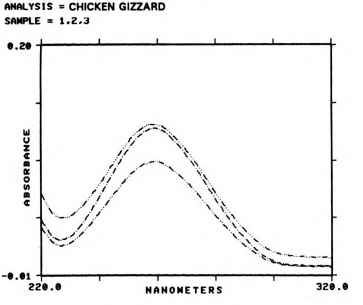

ANALYSIS = PIG MUSCLE
SAMPLE = 1,2,3
ALYSIS = PIG HEART
MPLE = 1,2,3
ANALYSIS = PIG LIVER
SAMPLE = 1,2,3
ANALYSIS = PIG STOMACH
SAMPLE = 1,2,3
ABSORBANCE
NANOMETERS
220.0
320.0
0.70
0.00
.50
.01
0.40
0.25


ANALYSIS = SHEEP MUSCLE
SAMPLE = 1,2,3
ANALYSIS = SHEEP HEART
SAMPLE = 1,2,3
ANALYSIS = SHEEP LIVER
SAMPLE = 1,2,3
ANALYSIS = SHEEP STOMACH
SAMPLE = 1,2,3
ABSORBANCE
NANOMETERS
220.0
320.0
0.00
0.40
0.50


ANALYSIS = CHICKEN MUSCLE
SAMPLE = 1,2,3
0.50
ABSORBANCE
-0.01
220.0
NANOMETERS
320.0


ANALYSIS = CHICKEN HEART
SAMPLE = 1,2,3
0.20
ABSORBANCE
-0.01
220.0
NANOMETERS
320.0

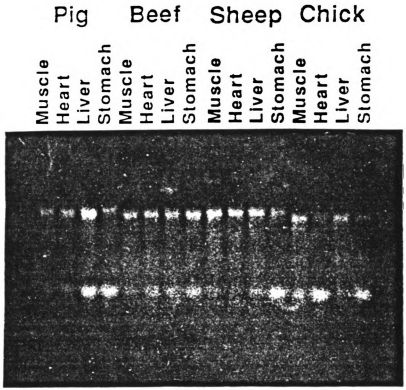

ANALYSIS = CHICKEN LIVER
SAMPLE = 1,2,3
0.25
ABSORBANCE
0.00
220.0
NANOMETERS
320.0


ANALYSIS = CHICKEN GIZZARD
SAMPLE = 1,2,3
0.20
ABSORBANCE
-0.01
220.0
NANOMETERS
320.0

APPENDIX E

SIZE SEPARATION OF RNA SAMPLES ON AGAROSE GELS

For quality control checks, RNA was separated with 1.2% agarose gel electrophoresis and then stained with eithidium bromide. This is a representative gel showing the 18S and 28S ribosomal bands.



LITERATURE CITED

Adeola, O., R.O. Ball and L.G. Young. 1989. Ractopamine stimulates porcine myofibrillar protein synthesis. J. Anim. Sci. 67(Suppl. 1.):191(Abstr.).

Affara, N., P. Baubas, A. Weydert and F. Gros. 1980. Changes in gene expression during myogenic differentiation. J. Mol. Biol. 140:459.

Airhart, J.A., A. Vidrich and E.A. Khairallah. 1974. Compartmentation of free amino acids for protein synthesis in rat liver. Biochem. J. 140:539.

Alemany, N. 1976. Effect of amino acid reutilization in the determination of protein turnover in mice. Horm. Metab. Res. 8:70.

Alexis, S.D., S. Basta and V.R. Young. 1972. Dietary protein intake and skeletal muscle protein metabolism in rats: Studies with salt-washed ribosomes and transfer factors. Biochem. J. 128:521.

Allen, R.E., R.A. Merkel and R.B. Young. 1979. Cellular aspects of muscle growth: Myogenic cell proliferation. J. Anim. Sci. 49:115.

Ambrose, J.A. 1974. Flourometric measurement of tyrosine in serum and plasma. Clin. Chem. 20:505.

American Instrument Co. 1961. The determination of nitrogen by the Kjeldahl procedure including digestion, distillation and titration. Reprint No. 104.

Anderson, P.T., W.G. Helferich, L.C. Parkhill, R.A. Merkel, A.L. Grant and W.G. Bergen. 1990. Ractopamine increases total and myofibrillar protein synthesis in cultured rat myotubes. J. Nutr. 120:1677.

Arnal, M., M. Ferrara and G. Fanconneau. 1976. Nuclear techniques in animal production and health. Int. Atomic Energy Agency Rep. No. 205:393.

Association of Official Analytical Chemists. 1980. Published by Assoc. of Official Analytical Chemists. (13th Ed.) George Bantam Comnpany, Inc. Menasha, WI.

Aub, M. and J. C. Waterlow. 1970. Analysis of a five compartment system with continuous infusion and its application to the study of amino acid turnover. J.

Theor. Biol. 26:243.

Bains, W., P. Ponte, H. Blau and L. Kedes. 1984. Cardiac actin is the major actin gene product in skeletal muscle cell differentiation in vitro. Mol. Cell. Biol. 4:1449.

Barton, P.J.R., S. Alonso, A. Cohen, P. Daubas, I. Garner, C. Pinset, B. Robert, A. Weydert and M. Buckingham. 1987. Actin and myosin gene expression in mouse striated muscle. J. Anim. Sci. 65(Suppl. 2):157.

Bates, P.C. and D.J. Millward. 1983. Myofibrillar protein turnover. Biochem. J. 214:587.

Bates, D.B., J.A. Gillett, S.A. Barao and W.G. Bergen. 1985. The effect of specific growth rate and stage of growth on nucleic acid-protein values of pure cultures and mixed ruminal bacteria. J. Anim. Sci. 61:713.

Beermann, D.H., D.E. Hogue, V.K. Fishell, R.H. Dalrymple and C.A. Ricks. 1986. Effects of cimaterol and fishmeal on performance, carcass characteristics and skeletal muscle growth in lambs. J. Anim. Sci. 62:370.

Beermann, D.H., W.R. Butler, D.E. Hogue, V.K. Fishell, R.H. Dalrymple, C.A. Ricks and C.G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. J. Anim. Sci. 65:1514.

Berg, R.T. and R.M. Butterfield. 1976. New Concepts of Cattle Growth. Sydney, Australia: Sydney University Press.

Bergen, W.G. 1974. Effect of dietary protein level on translation in rat liver. Fed. Proc. 33:695.

Bergen, W.G. and R.A. Merkel. 1991. Body composition of animals treated with partitioning agents: implications for human health. FASEB J. 5:2951.

Bergen, W.G., D.R. Mulvaney, D.M. Skjaerlund, S.E. Johnson and R.A. Merkel. 1987. In vivo and in vitro measurements of protein turnover. J. Anim. Sci. 65(Suppl. 2):8.

Bergen, W.G., S.E. Johnson, D.M. Skjaerlund, A.S. Babiker, N.K. Ames, R.A. Merkel and D. B. Anderson. 1989. Muscle protein metabolism in finishing pigs fed ractopamine. J. Anim. Sci. 67:2255.

Berger, F.G., D. Loose, H. Meisner and G. Watson. 1986.

Androgen induction of mRNA concentrations in mouse kidney is posttranscriptional. Biochemistry. 25:1170.

Bergsma, D.J. J.M. Grichnik, L.M.A. Gossett and R.J. Schwartz. 1986. Delimitation and characterization of cis-acting DNA sequences required for the regulated expression and transcriptional control of the chicken skeletal alpha-actin gene. Mol. Cell. Biol. 6:2462.

Bohorov, O., P.J. Buttery, J.H.R.D. Correia and J.B. Soar. 1987. The effecet of the β_2-adrenergic agonist clenbuterol or implantation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. Br. J. Nutr. 57:99.

Boissonneault, G., P. Chapdelaine and R. Tremblay. 1990. Actin and creatine kinase mRNAs in rat levator and vastus muscles as a function of androgen status. J. Appl. Physiol. 68:1548.

Breuer, C.B. and J.R. Florini. 1965. Amino acid incorporation into protein by cell free systems from rat skeletal muscle IV. Effects of animal age, androgens and anabolic agents on activity of muscle ribosomes. Biochemistry 4:1544.

Buckingham, M.E. 1985. Actin and myosin multigene families: their expression during the formation of skeletal muscle. Essays in Biochem. 20:77.

Buckingham, M.E., S. Alonso, P. Barton, G. Bugaisky, A. Cohen, P. Daubas, I. Garner, A. Minty, B. Robert and A. Weydert. 1984. Actin and myosin genes and their expression during myogenesis in the mouse. In: Molecular Biology of Development. Alan R. Liss, Inc., New York. pp. 275-292.

Buckingham, M., S. Alonso, P. Barton, A. Cohen, P. Daubas, I. Garner, B. Robert and A. Weydert. 1987. The regulation of actin and myosin genes during myogenesis in the mouse. Molecular Approaches to Developmental Biology. Alan R. Liss, Inc., New York. pp. 585-597.

Caravatti, M., A. Minty, B. Robert, D. Montarras, A. Weydert, A. Cohen, P. Daubas and M. Buckingham. 1982. Regulation of muscle gene expression: The accumulation of messenger RNAs coding for muscle-specific proteins during myogenesis in a mouse cell line. J. Mol. Biol. 160:59.

Carmon, Y. H. Czosnek, V. Nudel, M. Shami and D. Yaffe.

1982. DNAase I sensitivity of genes expressed during myogenesis. Nucl. Acids Res. 10:3085.

Cavadore, J.C., C. Rovstan, Y. Benyamin, M. Boyer and J. Haiech. 1985. Structural variations in actins: Biochemical and immunological tools for probing the structure of rabbit skeletal muscle and bovine aortic actins. Biochem. J. 231:363.

Cheek, D.B., A.B. Holt, D.E. Hill and J.L. Tolbert. 1971. Skeletal muscle cell mass and growth: the concept of the deoxyribonucleic acid unit. Pediatr. Res. 5:312.

Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294.

Claeys, M.C., D.R. Mulvaney, F.D. McCarthy, M.T. Gore, D.N. Marple and J.L. Sartin. 1989. Skeletal muscle protein synthesis and growth hormone secretion in young lambs treated with clenbuterol. J. Anim. Sci. 67:2245.

Cleveland, D.W., M.A. Lopata, R.J. MacDonald, D.J. Cowan, W.J. Rutter and M.S. Rirschner. 1980. Number and evolutionary conservation of alpha and beta tubulin and cytoplasmic beta and alpha actin genes using specific cloned cDNA probes. Cell 20:95.

Cole, D.J.A., M.R. White, B. Hardy and J.R. Carr. 1976. Tissue growth in the pig. Anim Prod. 22:341.

Colenbrander, B., F.H. de Jong and C.J.G. Wensing. 1978. Changes in serum testosterone concentrations in the male pig during development. J. Reprod. Fert. 53:377.

Colenbrander, B. Th., A.M. Kruip, S.J. Dieleman and C.J.G. Wensing. 1977. Changes in serum LH concentrations during normal and abnormal sexual development in the pig. Biol. Reprod. 17:506.

Condeelis, J. 1974. The identification of F-actin in the pollen tube and protoplast of Amaryllis belladonna. Exp. Cell. Res. 88:435.

Czosnek, H., V. Nudel, Y. Mayor, P.E. Barker, D.D. Pravtcheva, F.R. Ruddle and D. Yaffe. 1983. The genes coding for the cardiac muscle actin and the cytoplasmic beta-actin are located on three different mouse chromosomes. EMBO J. 2:1977.

Devi, A., M.A. Mukunda, V. Srivastara and N.K. Sarkar. 1963. The effect of age on the variations of deoxyribonucleic acid, ribonucleic acid and total nucleotides in liver, brain and muscle of rat. Exp. Cell. Res. 32:242.

Devlin, R.B. and C.P. Emerson, Jr. 1978. Coordinate regulation of contractile protein synthesis during myoblast differentiation. Cell 13:599.

Devlin, R.B. and C.P. Emerson, Jr. 1979. Coordinate accumulation of contractile protein mRNAs during myoblast differentiation. Develop. Biol. 69:202.

Dvorak, M. 1981. Effect of neonatal pig administration of testosterone on piglet growth. Vet. Med. 26:65.

Eadara, J.K., R.H. Dalrymple, R.L. Delay, C.A. Ricks and D.R. Romsos. 1989. Effects of cimaterol, a β-adrenergic agonist, on protein metabolism in rats. Metabolism 38:883.

Elzinga, M., J.H. Collins, W.m. Kuehl and R.S. Adelstein. 1973. Complete amino-acid sequence of actin of rabbit skeletal muscle. Proc. Natl. Acad. Sci. USA. 70:2687.

Emery, P.W., N.J. Rothwell, M.J. Stock and P.D. Winter. 1984. Chronic effects of β_2-adrenergic agonists on body composition and protein synthesis in the rat. Biosci. Rep. 4:83.

Enesco, M. and C.P. Leblond. 1962. Increase in cell number as a factor in the growth of the young male rat. J. Embryol. Exp. Morphol. 10:530.

Enesco, M. and D. Puddy. 1964. Increase in the number of nuclei and weight in skeletal muscle of rats of various ages. Am. J. Anat. 114:235.

Engel, J.N., P.W. Gunning and L.N. Kedes. 1981. Isolation and characterization of human actin genes. Proc. Natl. Acad. Sci. USA. 78:4674.

Engel, J., P. Gunning and L. Kedes. 1982. Human cytoplasmic actin proteins are encoded by a multigene family. Mol. Cell. Biol. 2:674.

Feinberg, A.P. and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6.

Feinberg, A.P. and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity: Addendum Anal. Biochem. 137:266.

Field, R.A. 1971. Effect of castration on meat quality and quantity. J. Anim. Sci. 32:849.

Ford, J.J. 1983. Serum estrogen concentrations during postnatal development in male pigs. Proc. Soc. Exp. Biol. Med. 174:160.

Fornwald, J.A., G. Kuncio, I. Peng and C.P. Ordahl. 1982. The complete nucleotide sequence of the chick alpha-actin gene and its evolutionary relationship to the actin gene family. Nucl. Acids Res. 10:3861.

Forsberg, N.E. and G. Merrill. 1986. Effects of cimaterol on protein synthesis and degradation in monolayer cultures of rat and mouse myoblasts. J. Anim. Sci. 63(Suppl. 1):222.

Fulks, R.M., J.B. Li and A. L. Goldberg. 1975. Effects of insulin, glucose, amino acids on protein turnover in rat diaphragm. J. Biol. Chem. 250:290.

Gallwitz, D. and I. Sures. 1980. Structure of split yeast gene: commplete nucleotide sequence of the actin gene in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA. 77:2546.

Gan, J.C. and H. Jeffay. 1967. Origins and metabolism of the intracellular amino acid pools in rat liver and muscle. Biochem. Biophys. Acta 148:448.

Garber, A.J., I.E. Karl and D.M. Kipnis. 1976. Alanine and glutamine synthesis and release from skeletal muscle. IV. β-adrenergic inhibition of amino acid release. J. Biol. Chem. 251:851.

Garfinkel, L.I., M. Periasamy and B. Nadal-Ginard. 1982. Cloning and characterization of cDNA sequences corresponding to myosin light chains 1, 2 and 3, troponin-C, troponin-T, alpha- tropomyosin and alpha-actin. J. Biol. Chem. 257:111078.

Garlick, P.J., T.L. Burk and R.W. Swick. 1976. Protein synthesis and RNA in tissues of the pig. Am. J. Physiol. 230:1108.

Garlick, P.J., D.J. Millward and W.P.T. James. 1973. The

diurnal response of muscle and liver protein synthesis in vivo in meal-fed rats. Biochem. J. 136:935.

Garner, I., A.J. Minty, S. Alonso, R.J. Burton and M.E. Buckingham. 1986. A 5′ duplication of the alpha cardiac actin gene in BALB/c mice is associated with abnormal levels of alpha cardiac and alpha skeletal actin mRNAs in adult cardiac tissue. EMBO. J. 5:2559.

Garner, I., D. Sassoon, J. Vandekerckhove, S. Alonso and M.E. Buckingham. 1989. A developmental study of the abnormal expression of alpha cardiac and alpha skeletal actins in the striated muscle of a mutant mouse. Develop. Biol. 134:236.

Garrels, J.I. and W. Gibson. 1976. Identification and characterization of multiple forms of actin. Cell 9:793.

Gilbreath, R.L. and J.R. Trout. 1973. Effects of early postnatal dietary restruction and repletion on porcine muscle growth and composition. J. Nutr. 103:1637.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Vol. I. Iowa State University Press, Ames, Iowa.

Gill, J.L. 1989. Statistical aspects of design and analysis of experiments with animals in pens. J. Anim. Breed. Genet. 106:321.

Goldberg, A.L. and T.W. Chang. 1978. Regulation and significance of amino acid metabolism in skeletal muscle. Fed. Proc. 37:2301.

Goldberg, A.L., S.M. Martel and M. J. Kushmerick. 1975. In vitro preparations of the diaphragm and other skeletal muscles. Methods in Enzymology. 39(Part D):82.

Goldspink, G. 1972. Postembryonic growth and differentiation of striated muscle. In: G.H. Bourne (Ed.) The structure and Function of Muscle, Volume 1, 2nd edition, p. 179. Academic Press, Inc., New York.

Goldspink, D.F. 1980. Physiological factors influencing protein turnover and muscle growth in mammals. In: D.F. Goldspink (Ed.) Development and Specialization of Skeletal Muscle. pp. 65-90. Cambridge University Press, New York.

Gospodarowicz, D.J. Weseman, S. Moran and J. Londstrom.

1976. Effect of fibroblast growth factor on the division and fusion of bovine myoblasts. J. Cell Biol. 70:395.

Grant, A.L., W.G. Helferich, R.A. Merkel and W.G. Bergen. 1990. Effects of phenethanolamines and propranolol on the proliferation of cultured chick breast muscle satellite cells. J. Anim. Sci. 68:652.

Grichnik, J.M., D.J. Bergsma and R.J. Schwartz. 1986. Tissue restricted and stage specific transcription is maintained within 411 nucleotides flanking the 5′ end of the chicken alpha-skeletal actin gene. Nucl. Acids Res. 14:1683.

Griffin, G.E., P.E. Williams and G. Goldspink. 1971. Region of longitudinal growth in striated muscle fibers. Nature 232:28.

Grigsby, J.S., W.G. Bergen and R.A. Merkel. 1976. The effect of testosterone on skeletal muscle development and protein synthesis in rabbits. Growth 40:303.

Gunning, P., E. Hardeman, R. Wade, P. Ponte, W. Bains, H. Blau and L. Kedes. 1987. Differential patterns of transcript accumulation during human myogenesis. Mol. Cell. Biol. 7:4100.

Gunning, P., T. Mohun, S. Nq, P. Ponte and L. Kedes. 1984. Evolution of the human sarcomeric-actin genes: evidence for units of selection within the 3′ untranslated regions of the mRNAs. J. Mol. Evol. 20:202.

Gunning, P., P. Ponte, H. Blau and L. Kedes. 1983. Alpha-skeletal and alpha-cardiac actin genes are coexpressed in adult human skeletal muscle and heart. Mol. Cell. Biol. 3:1985.

Gunning, P., P. Ponte, L. Kedes, R. Eddy and T. Shows. 1984. Chromosomal location of the co-expressed human skeletal and cardiac actin genes. Proc. Natl. Acad. Sci. USA. 81:1813.

Gunning, P., P. Ponte, H. Okayama, J. Engel, H. Blau and L. Kedes. 1983. Isolation and characterization of full-length cDNA clones for human alpha, beta and gamma actin mRNAs: Skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. Mol. Cell. Biol. 3:787.

Hakkarainen, J. 1975. Developmental changes of protein, RNA, DNA, lipid and glycogen in the liver, skeletal muscle and brain of the piglet. A methodological and experimental study with special reference to protein synthesis. Acta Veterin. Scand. Suppl. 59:1.

Hamada, H., M.G. Petrino and T. Kakunaga. 1982. Molecular structure and evolutionary origin of human cardiac muscle actin gene. Proc. Natl. Acad. Sci. USA. 79:5901.

Hanaver, A., M. Levin, R. Heilig, D. Daegelen, A. Kahn and J.L. Mandel. 1983. Isolation and characterization of cDNA clones for human skeletal muscle alpha-actin. Nucl. Acids Res. 11:3503.

Hanrahan, J.P. 1987. Beta-agonists and their effects on animal growth and carcass quality. Elsevier Applied Sci., New York.

Hansson, I., K. Lundstrom and B. Malmfors. 1975. Effect of sex and weight on growth, feed efficiency and carcass characteristics of pigs. 2. Carcass characteristics of boars, barrows and gilts slaughtered at four different weights. Swed. J. Agric. Res. 5:69.

Hayward, L.J., Y.Y. Zhu and R.J. Schwartz. 1988. Cellular localization in chicken primary myogenic cultures: the induction of alpha-skeletal actin mRNA is regulated independently of alpha- cardiac actin gene expression. J. Cell Biol. 106:2077.

Helferich, W.G., D.G. Jump, D.M. Skjaerlund, W.G. Bergen, R.A. Merkel and D.B. Anderson. 1990. Skeletal muscle alpha-actin synthesis is increased pretranslationally in pigs fed the phenethanolamine ractopamine. Endocrinology 126:3096.

Helferich, W.G., D.G. Jump, D.M. Skjaerlund, R.A. Merkel and W.G. Bergen. 1988. Alpha actin mRNA abundance in skeletal muscle of boars and barrows. J. Anim. Sci. 66(Suppl. 1):122.

Henshaw, E.C., C.A. Hirsch, B.E. Morton and H.H. Hiatt. 1971. Control of protein synthesis in mamalian tissues through changes in ribosome activity. J. Biol. Chem. 246:436.

Hider, R.C., E.B. Fern and D.R. London. 1969. Relationship between intracellular amino acids and protein synthesis in the extensor digitorum longus muscle of rats.

Biochem. J. 114:171.

Hider, R.C., E.B. Fern and D.R. London. 1971. Identification in skeletal muscle of a distinct extracellular pool of amino acids and its role in protein synthesis. Biochem. J. 121:817.

Holtzer, H. 1970. Myogenesis. In: O. Schjeide (Ed.) Cell Differentiation. pp. 476-503. Van Nostrand-Reinhold, New York.

Hu, M.C.T., S.B. Sharp and N. Davidson. 1986. The complete sequence of the mouse skeletal alpha-actin gene reveals several conserved and inverted repeat sequences outside of the protein- coding region. Mol. Cell. Biol. 6:15.

Humphries, S.E., R. Whittall, A. Minty, M. Buckingham and R. Williamson. 1981. There are approximately 20 actin genes in the human genome. Nucl. Acids Res. 9:4895.

Irvine, S., B. Nadal-Ginard and V. Mahdavi. 1988. Protooncogene induction and repreprogramming of cardiac gene expression produced by pressure overload. Proc. Natl. Acad. Sci. USA. 85:339.

Irvine, J.M., J.H. Ottaway and C.L. Saunderson. 1990. Turnover of sarcoplasmic proteins in the breast muscle of rapidly growing chicks. Int. J. Biochem. 22:187.

Jackson, W.C. and B.G. Doyle. 1977. Characterization of actin from root tops of Phaseolus vulgaris. J. Cell. Biol. 75:268a.

Jockusch, H., U. Mueller and D.M. Jockusch. 1984. Beating heart muscle in a skeletal muscle bed. Exp. Biol. Med. 9:121.

Jump, D.B., P. Naragan, H. Towle and J.H. Oppenheimer. 1984. Rapid effects of triiodothyroxine on hepatic gene expression: hybridization analysis of tissue-specific triiodothyroxine regulations of $mRNA_{s14}$. J. Biol. Chem. 259:2789.

Kattesh, H.G., E.T. Kornegay, F.C. Guazdauskas, J.W. Knight and H.R. Thomas. 1979. Peripheral plasma testosterone concentration and sexual behavior in young prenatally stressed boars. Theriogenology 12:289.

Kelly, A.M. 1978. Satellite cells and myofiber growth in the rat soleus and extensor digitorum longus muscles. Develop. Biol. 65:1.

Kim, Y.S., Y.B. Lee and C.R. Ashmore. 1988. Cimaterol-induced growth in rats: Growth pattern and biochemical characteristics. Growth Dev. Aging 52:41.

Kim, Y.S., Y.B. Lee and R.H. Dalrymple. 1987. Effects of the repartitioning agent cimaterol on growth, carcass and skeletal muscle characteristics in lambs. J. Anim. Sci. 65:1392.

Kim, Y.S., R.D. Sainz, R.J. Summers and P. Molenaar. 1992. Cimaterol reduces β-adrenergic receptor density in rat skeletal muscles. J. Anim. Sci. 70:115.

Kinson, G.A., R.A. Layberry and B. Herbert. 1991. Influences of anabolic androgens on cardiac growth and metabolism in the rat. Can. J. Physiol. Pharmacol. 69:1698.

Kiser, T.E., R.A. Milvae, H.D. Hafs, W.D. Oxender and T.M. Louis. 1978. Comparison of testosterone and androstenedione secretion in bulls given prostaglandin F_2 or luteinizing hormone. J. Anim. Sci. 46:436.

Knudson, B.K., M.G. Hogberg, R.A. Merkel, R.E. Allen and W.T. Magee. 1985a. Developmental comparisons of boars and barrows: I. Growth rate, carcass and muscle characteristics. J. Anim. Sci. 61:789.

Knudson, B.K., M.G. Hogberg, R.A. Merkel, R.E. Allen and W.T. Magee. 1985b. Developmental comparisons of boars and barrows: II. Body composition and bone development. J. Anim. Sci. 61:797.

Knudson, B. 1986. Comparison of performance, composition and energy partitioning between maintenance, protein and fat in boars and barrows. PhD Dissertation, Michigan State University, East Lansing.

Kochakian, C.D., J. Hill and G. Costa. 1964. Amino acid compositions of the proteins of the muscles and organs of the normal, castrated and testosterone treated guinea pig. Acta Endocrinologica 45:613.

Kochakian, C. D. 1976. Anabolic-Androgenic Steroids. Springer-Verlag, New York.

Koohmaraie, M., S.D. Shackelford, N.E. Muggli-Cockett and R.T. Stone. 1991. Effect of the β-adrenergic agonist $L_{644,969}$ on muscle growth, endogenous proteinase activities and postmortem proteolysis in wether lambs. J. Anim. Sci. 69:4823.

Laurent, G.J. and D.J. Millward. 1980. Protein turnover during skeletal muscle hypertrophy. Fed. Proc. 39:42.

Laurent, G.J., M.P. Sparrow and D.J. Millward. 1978. Changes in rates of protein synthesis and breakdown during hypertrophy of the anterior and posterior lattisimus muscle. Biochem. J. 176:407.

Leavitt, J., P. Gunning, L. Kedes and R. Jariwalla. 1985. Smooth muscle alpha-actin is a transformation-sensitive marker for mouse NIH3T3 and Rat-2 cells. Nature 316:840.

Leblond, C.P. 1972. Growth and renewal. In: R.J. Goss (Ed.) Regulation of Organ and Tissue Growth, pp. 13-39. Academic Press, Inc., New York.

Lewis, S.E.M., F.J. Kelly and D.F. Goldspink. 1984. Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow and fast-twitch skeletal muscles of the rat. Biochem. J. 217:517.

Li, J.B., R.M. Fulks and A.L. Goldberg. 1973. Evidence that the intracellular pool of tyrosine serves as precursor for protein synthesis in muscle. J. Biol. Chem. 248:7272.

Li, J.B. and L.S. Jefferson. 1977. Effects of isoproterenol on amino acid levels and protein turnover in skeletal muscle. Am. J. Physiol. 232:E243.

Lobley, G.E., A. Walker, A. Connell and H. Galbraith. 1983. The effects of trenbolone acetate on growth rate and carcass composition of young female rabbits. Anim. Prod. 36:111.

MacLennan, P.A. and R.H.T. Edwards. Effects of clenbuterol and propranolol on muscle mass: evidence that clenbuterol stimulates muscle β-adrenoceptors to induce hypertrophy. Biochem. J. 264:573.

Maniatis, T., E.F. Fritsch, J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory, New York.

Martin, T.,E., B. Baker, Jr., H.W. Miller and T.F. Kellogg. 1984. Circulating androgen levels in the developing boar. Theriogenology 21:357.

Maruyama, K., M.L. Sunde and R.W. Swick. 1978. Growth and muscle protein turnover in the chick. Biochem. J.

176:573.

Mathis, D. P. Oudet and P. Chambon. 1980. Structure of transcribing chromatin. Prog. Nucl. Acid Res. Mol. Biol. 24:1.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493.

Mayer, Y., H. Czosnek, P.E. Zeelon, D. Yaffe and V. Nudel. 1984. Expression of the genes coding for the skeletal muscle and cardiac actins in the heart. Nucl. Acids Res. 12:1087.

McElligott, M.A., A. Barreto, Jr., and L.Y. Chaung. 1989. Effect of continuous and intermittent clenbuterol feeding on rat growth rate and muscle. Comp. Biochem. Physiol. 92C:135.

McElligott, M.A. and L.Y. Chaung. 1987. Effects of serum from rats treated with a beta adrenergic agonist on protein turnover in L8 muscle cells. FASEB J. 46:1020A.

McKeown, M. and R. Firtel. 1981. Differential expression and 5′ end mapping of actin genes in Dicytostelium. Cell 24:799.

McKnight, G.S. and R.D. Palmitten. 1979. Transcriptional regulation of the ovalbumin and canalbumin genes by steriod hormones in chick viduct. J. Biol. Chem. 254:9050.

Melloul, D., B. Aloni, J. Calvo, D. Yaffe and V. Nudel. 1984. Developmentally regulated expression of chimeric genes containing muscle actin DNA sequences in transfected myogenic cells. EMBO J. 3:983.

Millward, D.J. 1980. Protein turnover in skeletal and cardiac muscle during normal growth and hypertrophy. In: K. Wildenthal (Ed.) Degradative Processes in Heart and Skeletal Muscle, pp 161- 199. Elsevier/North Holland Biomedical Press, New York.

Millward, D.J., P.C. Bates and S. Rosochacki. 1981. The extent and nature of protein degradation in the tissues during development. Reprod. Nutr. Develop. 21:265.

Millward, D.J., P.J. Garlick, W.P.T. James, P.M. Sender and J.C. Waterlow. 1975. Protein turnover. In: Protein Metabolism Nutrition, p. 49. Proc. Int. Sym.,

Butterworth, London.

Millward, D.J., P.J. Garlick, D.O. Nnanyelugo and J.C. Waterlow. 1976. The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. Biochem. J. 156:185.

Millward, D.J. and J.C. Waterlow. 1978. Effect of nutrition on protein turnover in skeletal muscle. Fed. Proc. 37:2283.

Minty, A.J., S. Alonso, M. Caravatti and M.E. Buckingham. 1982. A fetal skeletal muscle actin mRNA in the mouse and its identity with cardiac actin mRNA. Cell 30:185.

Minty, A.J., S. Alonso, J.L. Guenet and M.E. Buckingham. 1983. Number and organization of actin-related sequences in the mouse genome. J. Mol. Biol. 167:77.

Minty, A., H. Blau and L. Kedes. 1986. Two-level regulation of cardiac actin gene transcription: muscle-specific modulating factors can accumulate before gene activation. Mol. Cell. Biol. 6:2137.

Minty, A.J., M. Caravatti, B. Robert, A. Cohen, P. Daubas, A. Weydert, F. Gros and M.E. Buckingham. 1981. Mouse actin messenger RNAs: Construction and characterization of a recombinant plasmid molecule containing a complementary DNA transcript of mouse alpha-actin mRNA. J. Biol. Chem. 256:1008.

Minty, A. and L. Kedes. 1986. Upstream regions of the human cardiac actin gene that modulate its transcription in muscle cells: presence of an evolutionarily concerned repeated motif. Mol. Cell. Biol. 6:2137.

Mirsky, A.E. and H. Ris. 1949. Variable and constant components of chromosomes. Nature 163:666.

Miwa, T., L. Boxer and L. Kedes. 1987. CArG boxes in the human cardiac actin gene are core binding sites for positive trans- acting regulatory factors. Proc. Natl. Acad. Sci. USA. 84:6402.

Miwa, T. and L. Kedes. 1989. Duplicated CArG box domains have positive and mutually dependent regulatory roles in the expression of the human cardiac actin gene. Mol. Cell. Biol. 7:2803.

Moos, M. and D. Gallwitz. 1983. Structure of two human

beta actin related processed genes, one of which is located next to a simple repetitive sequence. EMBO J. 2:757.

Morgan, J.L., C.R. Holloday and B.S. Spooner. 1980. Immunological differences between actins from cardiac muscle, skeletal muscle and brain. Proc. Natl. Acad. Sci. USA. 77:2069.

Morgan, J.B., S.J. Jones, and C.R. Calkins. 1988. Cimaterol-fed broiler chickens: influence on muscle protein turnover. J. Anim. Sci. 66(Suppl. 1):278.

Morrison, P.R., G.W. Muller and F.W. Booth. 1987. Actin synthesis rate and mRNA level increase during early recovery of atrophied muscle. Am. J. Physiol. 253:C205.

Moss, F.P. 1968a. The relationship between the dimensions of the fibers and the number of nuclei during normal growth of skeletal muscle in the domestic fowl. Am. J. Anat. 122:555.

Moss, F.P. 1968b. The relationship between the number of nuclei during restricted growth, degrowth and compensatory growth of skeletal muscle. Am. J. Anat. 122:565.

Moss, F.P. and C. P. Leblond. 1970. Nature of dividing nuclei in skeletal muscle of growing rats. J. Cell. Biol. 44:459.

Moss, F.P. and C.P. Leblond. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170:421.

Mostafavi, M.S. 1978. The effect of growth rate, sex and age on skeletal muscle and adipose tissue growth and development. Ph.D. Dissertation, Michigan State University, East Lansing.

Mulvaney, D.R. 1981. Protein synthesis, breakdown and accretion rates in skeletal muscle and liver of young growing boars. Master Thesis, Michigan State University, East Lansing.

Mulvaney, D.R. 1984. Effects of castration and administration of androgens to castrated male pigs upon growth and carcass composition. Ph. D. Dissertation, Michigan State Univ., East Lansing.

Mulvaney, D.R. and D.N. Marple. 1987. Effects of testosterone, estrogen and progesterone on growth of neonatal pigs. J. Anim. Sci. 65(Suppl 1):62.

Mulvaney, D.R., D.N. Marple and R.A. Merkel. 1988. Proliferation of skeletal muscle satellite cells after castration and administration of testosterone propionate. Proc. Soc. Exp. Biol. Med. 188:40.

Mulvaney, D.R., R.A. Merkel and W.G. Bergen. 1985. Skeletal muscle protein turnover in young male pigs. J. Nutr. 115:1057.

Mulvaney, D.R., R.A. Merkel, D.M. Skjaerlund and W.G. Bergen. 1983. Skeletal muscle protein synthesis and degradation rates of growing boars and castrates measured in vitro. J. Anim. Sci. 57(Suppl. 1):201.

Munro, H.N. and A. Fleck. 1969. The determination of nucleic acids. Methods Biochem. Anal. 14:113.

Muscat, G.E., T.A. Gustafson and L. Kedes. 1988. A common factor regulates skeletal and cardiac alpha-actin gene transcription in muscle. Mol. Cell. Biol. 8:4120.

Muscat, G. and L. Kedes. 1987. Multiple 5'-flanking regions of the human alpha-skeletal actin gene synergistically modulate muscle-specific expression. Mol. Cell. Biol. 7:4089.

Noll, H. 1969. Polysomes: Analysis of structure in function. In: P.N. Campbell and J.R. Sargent (Eds.) Techniques in Protein Biosynthesis, Volume 2, p. 101. Academic Press, New York.

Nudel, V., D. Greenberg, C.P. Ordahl, O. Saxel, S. Neuman and D. Yaffe. 1985. Developmentally regulated expression of a chicken muscle-specific gene in stably transfected rat myogenic cells. Proc. Natl. Acad. Sci. USA. 82:3106.

Nudel, V., R. Zakut, D. Katcoff, Y. Carmon, H. Czosnek, M. Shani and D. Yaffe. 1983. Isolation and structural analysis of the genes coding for rat skeletal-muscle actin and for cytoplasmic actin. In: Muscle Development: Molecular and Cellular Control, pp 177-188.

Obinata, T., K. Maruyama, H. Sugita, K. Kohama and S. Ebashi. 1981. Dynamic aspects of structural proteins in vertebrate skeletal muscle. Muscle Nerve 4:456.

Ogata, E.S., S.K.H. Fourg and M.A. Holliday. 1978. The effects of starvation and refeeding on muscle protein synthesis and catabolism in the young rat. J. Nutr. 108:759.

Okayama, H. and P. Berg. 1982. High efficiency cloning of full-length cDNA. Mol. Cell. Biol. 2:161.

Okazaki, K. and H. Hultzer. 1966. Myogenesis: Fusion, myosin synthesis and the mitotic cycle. Proc. Natl. Acad. Sci. USA. 56:1484.

Ordahl, C.P. 1986. The skeletal and cardiac alpha actin genes are coexpressed in early embryonic striated muscle. Develop. Biol. 117:448.

Ordahl, C.P. and T.A. Cooper. 1983. Strong homology in promoter and 3′ untranslated regions of chick and rat alpha-actin genes. Nature 303:348.

Ordahl, C.P., S.M. Tilghman, C. Ovitt, J. Fornwald and M.T. Largen. 1980. Structure and developmental expression of the chick alpha-actin gene. Nucl. Acids Res. 8:4989.

Paul, J. 1974. Macromolecular synthesis in sea urchin development. In: J. Paul (Ed.) Biochemistry of Cell Differentiation, pp 85-94. Butterworth, London.

Paterson, B.M. and J.D. Eldridge. 1984. Alpha cardiac actin is the major sarcomeric isoform expressed in embryonic avian skeletal muscle. Science 224:1436.

Paterson, B.M., A. Seiler-Tuyns and J.D. Eldridge. 1984. Expression and regulation of chicken actin genes in avian and murine myogenic cells. In: Molecular Biology of Development, pp 383-394. Alan R. Liss, Inc., New York.

Pollard, T.D. and R.C. Weihing. 1974. Actin and myosin in cell movement. CRC Crit. Rev. Biochem. 2:1.

Ponte, P., P. Gunning, H. Blau and L. Kedes. 1983. Human actin genes are single copy for alpha-skeletal and alpha-cardiac actin but multicopy for beta and gamma cystoskeletal genes: 3′ untranslated regions are isotype specific but are conserved in evolution. Mol. Cell. Biol. 3:1783.

Ponte, P., S.Y. Ng, J. Engel, P. Gunning and L. Kedes. 1984. Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta

actin cDNA. Nucl. Acids Res. 12:1687.

Powell, S.E. and E.D. Aberle. 1975. Cellular growth of skeletal muscle in swine differing in muscularity. J. Anim. Sci. 40:476.

Powers, M.L. and J.R. Florini. 1975. A direct effect of testosterone on muscle cells in tissue culture. Endocrinology 97:1043.

Prescott, J.H.D. and G.E. Lamming. 1967. The influence of castration on the growth of male pigs in relation to high levels of dietary protein. Anim. Prod. 9:535.

Reeds, P.J. 1987. Metabolic control and future opportunities for growth regulation. Anim. Prod. 45:149.

Reeds, P.J., D.G. Burrin, T.A. Davis and M.L. Fiorotto. 1993. Postnatal growth of gut and muscle: competitors or collaborators. Proc. Nutr. Soc. 52:57.

Reeds, P.J., S.M. Hay, P.M. Dorwood and R.M. Palmer. 1986. Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis. Br. J. Nutr. 56:249.

Reeds, P.J. and G.E. Lobley. 1980. Protein synthesis: are there real species differences? Proc. Nutr. Soc. 39:43.

Reeds, P.J. and R.M. Palmer. 1985. Intracellular control of muscle protein turnover: A potential site for the manipulation of muscle growth. Rowett Res. Rep. pp 9-24.

Reznik, M. 1969. Thymidine-^{3}H uptake by satellite cells of regenerating skeletal muscle. J. Cell Biol. 40:568.

Richmond, R.J. and R.T. Berg. 1982. Relative growth patterns of individual muscles in the pig. Can. J. Anim. Sci. 62:575.

Richter, H.E., R.B. Young and D.M. Moriarity. 1989. Regulation of myofibrillar protein gene expression. In: D.R. Campion, G.J. Hausman and R.J. Martin (Eds.) Animal Growth Regulation, pp 103- 122. Plenum Press, New York.

Robert, B., P. Barton, A. Minty., P. Daubas, A. Weydert, F. Bonhomme, J. Catalan, D. Chazottes, J.L. Guenet and M.

Buckingham. 1985. Investigation of genetic linkage between myosin and actin genes using an interspecific mouse back cross. Nature 314:181.

Robinson, D.W. 1969. The cellular response of porcine skeletal muscle to prenatal and neonatal nutritional stress. Growth 33:231.

Rogozkin, V. 1975. Anabolic and androgenic effects of methandrostenlone ("Nerobol") during systemic physical activity in rats. Br. J. Sports Med. 9:65.

Rothwell, N.J., M.J. Stock and D.K. Sudera. 1987. Changes in tissue blood flow and β-receptor density of skeletal muscle in rats treated with the β2-adrenoceptor agonist clenbuterol. Br. J. Pharmacol. 90:601.

Rubenstein, P.A. and J.A. Spudich. 1977. Actin microheterogeneity in chick embryo fibroblasts. Proc. Natl. Acad. Sci. USA. 74:120.

Rugh, R. 1968. The Mouse: Its Reproduction and Development. Burgess Publishing, Minneapolis, MN.

SAS. 1987. SAS User's guide: statistics. SAS Inst. Inc., Cary, North Carolina.

Santidrian, S., M. Moreyra, H.N. Munro and V.R. Young. 1982. Effect of testosterone on the rate of myofibrillar protein breakdown in castrated and adrenalectomized rats measured by urinary excretion of 3-methylhistidine. Metabolism 31:1200.

Sassoon, D.A., I. Garner and M. Buckingham. 1988. Transcripts of alpha-cardiac and alpha-skeletal actins are early markers for myogenesis in the mouse embryo. Development 104:155.

Scarpulla, R.C. and R. Wu. 1983. Nonallelic member of the cytochrome multigene family of the rat may arise through different messenger RNAs. Cell 32:473.

Scheller, R., L. McAllister, W. Cram, D. Durica, J. Posnkony, T. Thomas, R. Britten and E. Davidson. 1981. Organization and expression of the multiple actin genes in the sea urchin. Mol. Cell. Biol. 1:609.

Schiaffino, S., J.L. Samuel, D. Sassoon, A.M. Lompre, I. Garner, F. Marotte, M. Buckingham, L. Rappaport and K. Schwartz. 1989. Nonsynchronous accumulation of alpha skeletal actin and beta myosin heavy chain mRNAs during

early stages of pressure overload-induced cardiac hypertrophy demonstrated by in situ hybridization. Circulat. Res. 64:937.

Schiavetta, A.M., M.F. Miller, D.K. Lunt, S.K. Davis and S.B. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the β-adrenergic agonist for clenbuterol for 50 days and after 78 days of withdrawal. J. Anim. Sci. 68:3614.

Schimke, R.T. 1970. Regulation of protein degradation in mammalian tissues. In: H.W. Munro (Ed.) Mammalian Protein Metabolism, Vol. 4, p. 177. Academic Press, New York.

Schimke, R.T. 1977. Why is there protein turnover? Proc. Second Intl. Sym. Protein Metab. Nutr., Flevohof, Netherlands.

Schreurs, V., H.A. Bockholt, R.E. Koopmanschap and W. VanRotterdam. 1985a. Relative synthesis rate of individual muscle proteins: a new approach. Netherlands J. Agric. Sci. 33:297.

Schreurs, V., G. Mensink, H.A. Bockholt and R.E. Koopmanschap. 1985b. Relation of protein synthesis and amino acid oxidatin: effects of protein deprivation. Netherlands J. Agric. Sci. 33:318.

Schwartz, K., D. DeLaBastie, P. Bouveret, P. Oliviero, S. Alonso and M. Buckingham. 1986. Alpha skeletal muscle actin mRNA's accumulate in hypertrophied adult rat hearts. Circulat. Res. 59:551.

Schwartz, R.J. and K.N. Rothblum. 1981. Gene switching in myogenesis: Differential expression of the chicken actin multigene family. Biochemistry 20:4122.

Seiler-Tuyns, A., J.D. Eldridge and B.M. Paterson. 1984. Expression and regulation of chicken actin genes introduced into mouse myogenic and nonmyogenic cells. Proc. Natl. Acad. Sci. USA. 81:2980.

Shani, M., V. Nudel, D. Zevin-Sonkin, R. Zakut, D. Givol, D. Katcoff, Y. Carmon, J. Reiter, A.M. Frischauf and D. Yaffe. 1981a. Skeletal muscle actin mRNA: Characterization of the 3′ untranslated region. Nucl. Acids Res. 9:579.

Shani, M., D. Zevin-Sonkin, Y. Carmon, H. Czosnek, V. Nudel

and D. Yaffe. 1983. Changes in myosin and actin gene expression and DNAase-I sensitivity associated with terminal differentiation of myogenic cultures. In: Muscle Development: Molecular and Cellular Control, pp. 189-200.

Shani, M., D. Zevin-Sonkin, O. Saxel, Y. Carmon, D. Katcoff, V. Nudel and D. Yaffe. 1981b. The correlation between the synthesis of skeletal muscle actin, myosin heavy chain, and myosin light chain and the accumulation of corresponding mRNA sequences during myogenesis. Develop. Biol. 86:483.

Sharp, S.B., T.A. Kost, S.H. Hughes, C.P. Ordahl and N. Davidson. 1987. Regulation of intact and hybrid beta and alpha actin genes inserted into myogenic cells. In: Molecular Approaches to Developmental Biology, pp. 609-618. Alan R. Liss, Inc., New York.

Shimizu, N. and T. Obmata. 1980. Presence of three actin types in skeletal muscle of chick embryos. Develop. Growth Different. 22:789.

Slagboom, P.E., W.J.F. DeLeeuw and J. Vijg. 1990. Messenger RNA levels and methlation patterns of GAPDH and beta actin genes in rat liver, spleen and brain in relation to aging. Mech. Aging Develop. 53:243.

Skjaerlund, D.M., A.L. Grant, W.G. Helferich, W.G. Bergen and R.A. Merkel. 1993. Determination of the relative abundance of skeletal muscle alpha actin mRNA in muscle of livestock species. J. Anim. Sci. 71:393.

Skjaerlund, D.M., A.L. Grant, W.G. Helferich, W.G. Bergen and R.A. Merkel. 1993. Skeletal muscle growth and expression of skeletal muscle alpha actin mRNA and IGF-I mRNA in pigs during feeding and withdrawal of ractopamine. J. Anim. Sci. (In press).

Skjaerlund, D.M., D.R. Mulvaney, R.H. Mars, A.L. Schroeder, M.A. Stachiw, W.G. Bergen and R.A. Merkel. 1988. Measurement of protein turnover in skeletal muscle strips. J. Anim. Sci. 66:687.

Skjaerlund, D.M., D.R. Mulvaney and R.A. Merkel. 1984. Muscle protein synthesis and degradation measured in vitro in neonatal boars and barrows. J. Anim. Sci. 59(Suppl. 1):206.

Smith, S.B., D.K. Garcia and D.B. Anderson. 1989. Elevation of a specific mRNA in longissimus muscle of

steers fed ractopamine. J. Anim. Sci. 67:3495.

Srirastava, V. and K.D. Chaudary. 1969. Effect of age on protein and ribonucleic acid metabolism in mouse skeletal muscle. Can. J. Biochem. 47:231.

Stockdale, F.E. and J.B. Miller. 1987. The cellular basis of myosin heavy chain isoform expression during development of avian skeletal muscles. Develop. Biol. 123:1.

Stromer, M.H., D.E. Goll, R.B. Young, R.M. Robson and R.C. Parrish, Jr. 1974. Ultrastructural features of skeletal muscle differentiation and development. J. Anim. Sci. 38:1111.

Swaneck, G.E., J.L. Nordstrom, F. Kreuzaler, M.J. Tsai and B.W. O'Malley. 1979. Effect of estrogen on gene expression in chicken oviduct: Evidence for transcriptional control of ovalbumin gene. Proc. Natl. Acad. Sci. USA. 76:1049.

Swatland, H.J. 1976. Recent research on postnatal muscle development in swine. Proc. Recip. Meat Conf. 29:86.

Swick, R.W. 1982. Growth and protein turnover in animals. CRC Crit. Rev. Food Sci. Nutr., pp. 117-126.

Taylor, A., H.P. Erba, G.E. Muscat and L. Kedes. 1988. Nucleotide sequence and expression of the human skeletal alpha actin gene: evolution of functional regulatory domains. Genomes 3:323.

Thompson, S.H., L.K. Boxhorn, W. Kong and R.E. Allen. 1989. Trenbolone alters the responsiveness of skeletal muscle satellite cells to fibroblast growth factor and insulin-like growth factor I. Endocrinology 124:2110.

Thorton, R.F. and R.K. Tume. 1988. Manipulation of growth in domestic animals. Proc. Int. Cong. Meat Sci. Tech. 34:6.

Umbreit, W.W., R.H. Burris and J.F. Stauffer. 1964. Manometric Techniques, 4th Ed. pp 132-133. Burgess Publishing Co., Minneapolis, MN.

Vandekerckhove, J., G. Bugaisky amd M. Buckingham. 1986. Simultaneous expression of skeletal muscle and heart actin proteins in various striated muscle tissues and cells. J. Biol. Chem. 261:1838.

Vandekerckhove, J. and K. Weber. 1978a. At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. J. Mol. Biol. 126:783.

Vandekerckhove, J. and K. Weber. 1978b. Mammalian cytoplasmic actins are the products of at least two genes and differ in primary structure in at least 25 identified positions from skeletal muscle actins. Proc. Natl. Acad. Sci. USA. 75:1106.

Vandekerckhove, J. and K. Weber. 1978c. Actin amino-acid sequences: Comparison of actins from calf thymus, bovine brain and SV40-transformed mouse 373 cells with rabbit skeletal muscle actin. Eur. J. Biochem. 90:451.

Vandekerckhove, J. and K. Weber. 1979. The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fast skeletal muscle and rabbit slow skeletal muscle. Differentiation 14:123.

Van Straaten, H.W.M. and C.J.G. Wensing. 1977. Histomorphometric aspects of testicular morphogenesis in the pig. Biol. Reprod. 17:467.

Van Straaten, H.W.M. and C.J.G. Wensing. 1978. Leydig cell development in the testis of the pig. Biol. Reprod. 18:86.

Vernon, B.G. and P.J. Buttery. 1976. Protein turnover in rats treated with trienbolone acetate. Br. J. Nutr. 36:575.

Vernon, B.G. and P.J. Buttery. 1978. Protein metabolism of rats treated with trienbolone acetate. Anim. Prod. 26:1.

Walsh, K. and P. Schimmel. 1987. Two nuclear factors compete for the skeletal muscle actin promotor. J. Biol. Chem. 262:9429.

Ward, L.C., P.J. Buttery and K.N. Boorman. 1984. Protein synthesis in isolated perfused rat skeletal muscle: contribution of intra- and extracellular amino acid pools. Int. J. Biochem. 16:1077.

Waterlow, J.C., P.J. Garlick and D.J. Millward. 1978. Protein turnover in mammalian tissues and in the whole body. North- Holland Publishing Company, New York.

Waterlow, J.C. and J.M.L. Stephen. 1967. The measurement of

total lysine turnover in the rat by intravenous infusion of L-[U-^{14}C) lysine. Clin. Sci. 33:489.

Waterlow, J.C. and J.M.L. Stephen. 1968. The effect of low protein diets on the turnover rates of serum, liver and muscle proteins in the rat, measured by continuous infusion of L-[^{14}C] lysine. Clin. Sci. 35:287.

Wemberg, E.S. 1977. Programmed information flow in the sea urchin embryo. In: J. Paul (Ed.) Biochemistry of Cell Differentiation II, pp. 157-197. University Park Press, Baltimore, MD.

Weisbrod, S. 1982. Active chromatin. Nature 297:289.

Whalen, R.G., G.S. Butler-Browne and F. Gros. 1976. Protein synthesis and actin heterogeneity in calf muscle cells in culture. Proc. Natl. Acad. Sci. USA 73:2018.

Williams, P.E.V. 1987. The use of beta-agonists as a means of altering body composition in livestock species. Nutr. Abst. Rev. 57:453.

Winick, M. and A. Noble. 1966. Cellular response in rats during malnutrition at various ages. J. Nutr. 89:300.

Yang, Y.T. and M.A. McElligott. 1989. Multiple actions of beta-adrenergic agonists in skeletal muscle and adipose tissue. Biochem. J. 261:1.

Yates, L.D. and M.L. Greaser. 1983. Quantitative determination of myosin and actin in rabbit skeletal muscle. J. Mol. Biol. 168:123.

Young, R.B. and R.E. Allen. 1979. Transitions in gene activity during development of muscle fibers. J. Anim. Sci. 48:837.

Young, V.R. 1970. The role of skeletal and cardiac muscle in the regulation of protein metabolism. In: H.N. Munro (Ed.) Mammalian Protein Metabolism, Volume 4, pp 587-679. Academic Press, Inc., New York.

Young, V.R. 1974. Regulation of protein synthesis and skeletal muscle growth. J. Anim. Sci. 38:1054.

Young, V.R. and M.G. Pluskal. 1977. Mode of action of anabolic agents, with special reference to steroids and skeletal muscle: a summary review. Proc. Second Intl. Sym. Protein Metab. Nutr., Fleuhof, Netherlands.

Young, R.B., D.M. Moriarty, C.E. McGee, W.R. Farrar and H.E. Richter. 1990. Protein metabolism in chicken muscle cell cultures treated with cimaterol. J. Anim. Sci. 68:1158.

Zak, R., A.F. Martin and R. Blough. 1979. Assessment of protein turnover by use of radiosotopic tracers. Physiol. Rev. 59:407.

Zakut, R., M. Shani, D. Gival, S. Neuman, D. Yaffe and V. Nudel. 1982. Nucleotide sequence of the rat skeletal muscle actin gene. Nature 298:857.