EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGENS TO CASTRATED MALE PIGS UPON GROWTH AND CARCASS COMPOSITION

By

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ABSTRACT

EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGENS TO CASTRATED MALE PIGS UPON GROWTH AND CARCASS COMPOSITION

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Two 5 wk studies involving prepubertal (15 kg) and pubertal (74 kg) pigs were conducted. Pigs at each weight were allotted to the following groups with four pigs in each: initial slaughter group, boars, castrates and castrates implanted with either a low or high number of silastic capsules filled with either testosterone or dihydrotestosterone. All pigs were fed ad libitum an 18% CP corn soybean diet from weaning to slaughter. At initiation of the study all pigs were given oxytetracycline intravenously to serve as an intravital bone marker. Three pigs of each treatment group were individually penned and feed intake was monitored. At slaughter, right sides of carcasses were dissected into soft tissues and bone. Subcutaneous and perirenal adipose tissue was assessed for fatty acid synthesis, lipoprotein lipase and hormone sensitive lipase activities. Several muscles were dissected, weighed and analyzed for composition. Semitendinosus muscle strips were used to assess protein

synthesis and degradation rates by an in vitro assay. Feed intake was 14 to 23% lower ($P < .05$) in boars and 12 to 38% ($P < .05$) lower in testosterone implanted pigs compared to castrates and dihydrotestosterone implanted castrates. Gain was unaffected by treatment but feed to gain ratios favored boars and testosterone implanted pigs by 20% compared to castrates and dihydrotestosterone implanted pigs. Pubertal boars and high testosterone implanted pigs had 1.1 to 1.2-fold more total bone ($P < .05$) than castrates. Testosterone was more effective ($P < .05$) in stimulating tibia linear growth than dihydrotestosterone. Androgen administration increased bone thickening compared to castrates. Total carcass fat was reduced ($P < .05$) in boars and testosterone implanted pigs compared to castrates and dihydrotestosterone implanted pigs. Castration increased ($P < .05$) fatty acid synthesis and lipiprotein lipase activities, and testosterone but not dihydrotestosterone was effective ($P < .05$) in lowering these activities expressed on a gram of tissue basis. Hormone sensitive lipase activities were higher ($P < .05$) in boars and high testosterone implanted pigs relative to castrates. These data support the hypothesis that aromatization of testosterone is required to mediate effects upon fattening. Total muscle mass did not differ ($P > .05$) between treatments but selected muscles were heavier in boars and androgen treated pigs than castrates.

Testosterone was more effective ($P < .05$) in stimulating fat free semitendinosus muscle growth than dihydrotestosterone. Protein synthesis and degradation rates were higher ($P < .05$) in boars than castrates. Androgens increased protein synthesis and decreased ($P. < 05$) degradation rates compared to castrates.

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INTRODUCTION

The ultimate goal of modern animal agriculture is to maximize production of lean tissue and minimize waste. Concerns about efficiency of production of desired products has never been keener. Minimal improvements in efficiency can be expected from traditional nutritional and genetic approaches. A clearer understanding of the mechanisms and their regulation of proliferation and differentiation of muscle, bone and adipose tissues and their interrelationships during growth is needed. Several key questions pertaining to growth need answers before the growth process can be modulated more precisely. Some of these questions are: (1) What controls hyperplasia of myoblasts, adipoblasts and osteoblasts in early growth?; (2) What are the triggers that arrest cell division and differentiation?; (3) What role does hyperplasia have in fully differentiated tissues?; (4) What are the controls for protein and fat accretion?; (5) Can we manipulate hyperplasia and/or hypertrophy in vitro and in vivo? and (6) How does the partitioning of nutrients vary during growth? (Mersmann, 1982).

Growth of domestic animals can be characterized by sigmoidal curves as shown in figure 1. This graph shows three phases of growth: (1) a brief lag period for

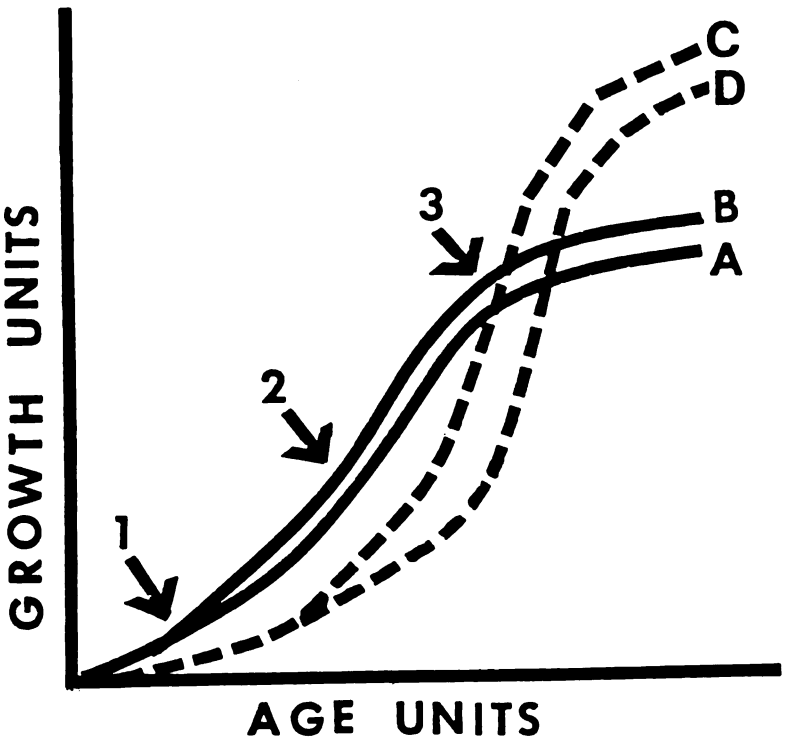


FIGURE 1. Idealized growth curves of muscle (A and B) and adipose tissue (C and D). Three phases of growth are represented: 1) a brief lag phase, 2) an acceleration phase and 3) a deceleration phase. If curves are shifted from A to B and C to D, more efficient lean production would be obtained.

(modified after Bergen, 1974).

synthesis of nuclear material or ultimate cellular machinery, (2) a steep portion representing and acceleration phase and period of most rapid growth and (3) a deceleration phase or period of reduced growth rate. The broken lines represent fat rates and illustrate that the most rapid deposition of fat occurs during the deceleration phase of growth (Bergen, 1974). Since the most efficient lean gains are made during the acceleration phase, it is desirable to lengthen the duration of this phase or in essence shift curve A to curve B. Likewise, if the curves for fat deposition could be shifted from C to D a postponement of the rapid accretion of fat would occur. These types of growth manipulation have been successfully achieved through selection for late maturing or large mature size animals (Trenkle and Marple, 1983). The search for growth stimulants or exogenous agents that achieve the aforementioned shifting is of much interest (Heitzman, 1980). However, through castration of male animals, the curves are in essence shifted in the opposite direction of that described. In other words, muscle deposition is effectively decreased and fat deposition is increased by castration. Figure 2 illustrates expected differences of bulls, rams and boars relative to castrated males (Field, 1971, Galbraith and Topps, 1981; Seideman et al., 1982). Variations in these expected differences between intact males and castrates arise from genetic, nutritional and

managerial (time of castration) differences between studies. While the intact male of the three species shown in figure 2 have advantages in growth rate and lean, the largest commonality appears to be in feed efficiency and percentage fat.

If ablation of the testis results in reduced muscle and increased fat deposition, then testosterone becomes a prime candidate for playing a significant role in regulating protein and fat metabolism. To effectively enhance protein deposition rates, testosterone would have to alter protein synthesis and/or protein degradation rates to result in greater net synthesis of protein. Likewise, to reduce fat accretion, testosterone may be involved in reducing lipogenesis and/or uptake and reesterification of circulating lipids and/or increased lipolysis and mobilization of lipid components.

It was the primary objective of this investigation to examine the effects of castration and administration of testosterone and dihydrotestosterone to castrated male pigs upon changes in body composition. Muscle, bone and adipose tissue accretion, skeletal muscle protein synthesis and degradation rates, muscle proteolytic activity and adipose tissue lipogenic and lipolytic activities were also studied. These studies are hopefully the first phase of subsequent investigations which will ascertain mechanisms of action of testosterone upon muscle, bone and adipose tissue.

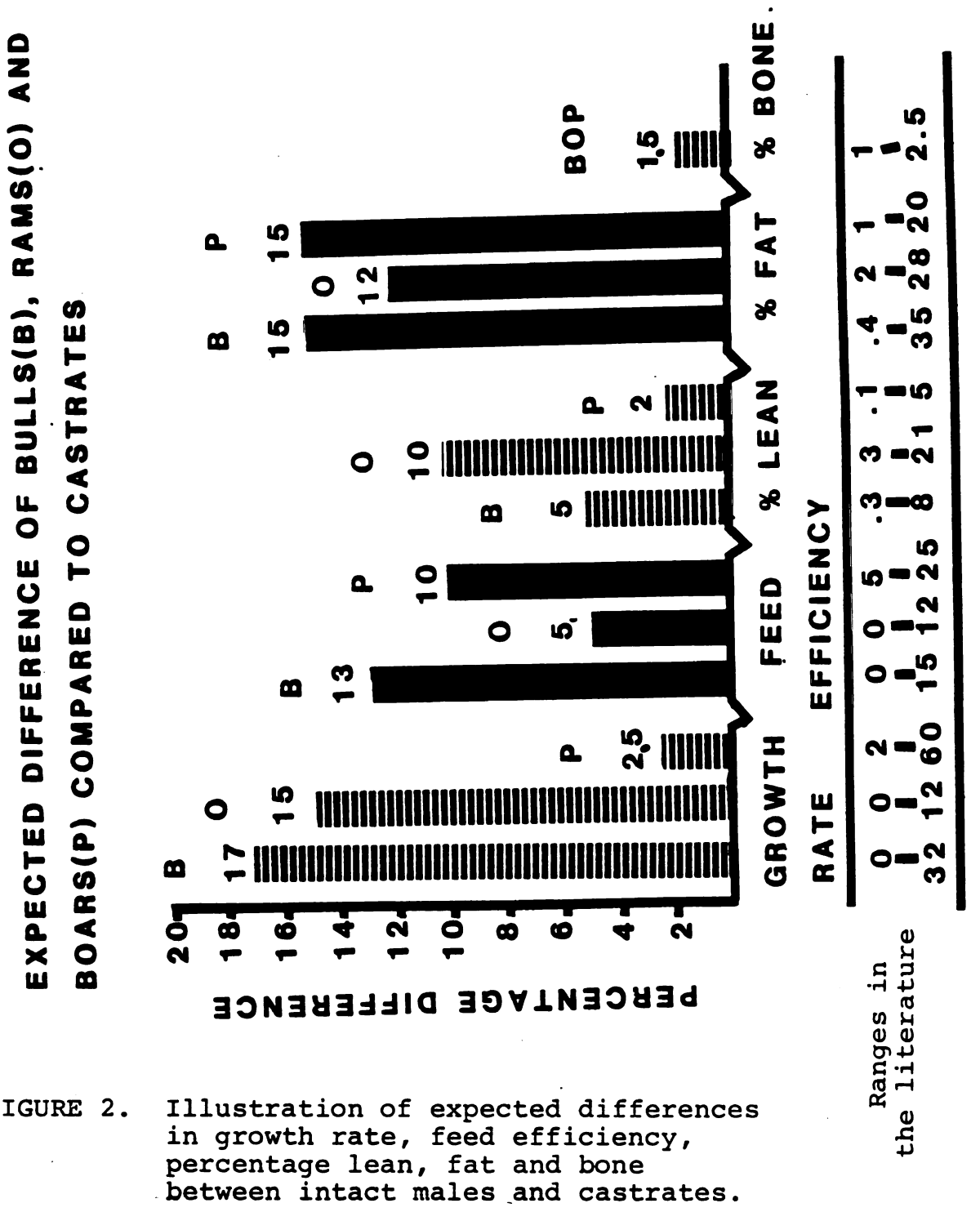


FIGURE 2. Illustration of expected differences in growth rate, feed efficiency, percentage lean, fat and bone between intact males and castrates.

LITERATURE REVIEW

Intact Male Versus Castrated Male Pigs

The merits of intact male pigs were reviewed by Walstra and Kroeske (1968), Wismer-Pedersen (1968), Martin (1969), Turton (1969), Field (1971), Galbraith and Topps (1981) and Seideman et al. (1982). Considering the traits of major economic significance, intact male pigs present many advantages over the castrated male. The ensuing discussion will primarily address growth rate, feed efficiency and body composition differences between boars and castrates. The largest disadvantage of commercially rearing and marketing boars for meat is that of objectional meat odors and boar taint. The latter is believed to be caused by 5α - androst-15-en-3-one deposited in the fat of boars (Patterson, 1968). Measures to reduce the incidence of boar taint are currently being investigated throughout the world (Conference Report, 1981 Symposium of Boar Taint, Zeist, Netherlands). Assuming the boar taint problem will eventually be solved,the growth advantages of boars can be exploited and perhaps greater interest in the mechanisms of testosterone action upon growth in pigs will be stimulated. The literature to date involving comparisons of boars to castrated males has been complicated by variations in breed, age and weight at castration or

slaughter and the level of dietary protein fed and the mode of feeding (Kay and Houseman, 1975; Fuller, 1980).

Boars generally grow slightly (0 to 10%) faster than the castrated counterpart when feed intake is restricted but castrates grow faster (0 to 10%) when fed ad libitum (Kay and Houseman, 1975). Generally, there is a sex x protein interaction such that boars grow faster than castrates at higher dietary protein concentrations. Another confounding factor in comparisons of boars and castrated males has been the observation that castrates have larger appetites which increases the daily food and energy intake (Pay and Davies, 1973). The effect of castration of boars upon growth rate is unique since it is less than the 10 to 20% growth rate depression observed for castrated cattle and sheep (Prescott and Lamming, 1964). While a number of investigations have found no differences in growth rates between boars and castrates (Winters et al., 1942; Kroeske, 1963; Prescott and Lamming, 1964; Hines, 1966; Omtvedt and Jesse, 1968; Hetzer and Miller, 1972; Newell and Bowland, 1972), other reports have shown that boars grow faster than castrates (Bratzler et al., 1954; Blair and English, 1965; Burgess et al., 1966; Siers, 1975; Campbell and King, 1982; Wood and Riley, 1982). Recent reports comparing growth rates of boars and castrates have shown that growth rate varies during different phases of growth. Campbell and King (1982)

observed no difference in average daily gain (ADG) between boars and castrates during a 20 to 45 kg live weight growth period when fed a 17% CP diet. When dietary protein was increased to 21% and restricted fed, ADC and feed efficiency of boars was improved by 14% over those of castrates. When fed ad libitum, differences between boars and castrates during this 20 to 45 kg live weight growth period were small but slightly favored the boar. Similar trends were observed during the 45 to 70 kg live weight growth period. An even more dramatic 53% advantage in ADG was reported by Wood and Riley (1982). Pay and Davies (1973) showed no differences in ADC when boars were compared to castrates. The latter investigators also observed that castrates had a higher (6 to 11%) voluntary food intake than boars from 22 to 55, 55 to 90 and 22 to 90 kg weight intervals. These lower intakes in boars occurred for 16, 18 and 20% CP diets. Campbell and King (1982) found the differences in performance and food intake to be most apparent in boars and castrates weighing more than 45 kg. These latter studies indicated greater potential for utilization of higher protein diets in boars relative to castrates and that higher protein diets are needed for full expression of this added growth potential. Even though Pay and Davies (1973) showed no difference in ADG between boars and castrates over a 22 to 90 kg live weight growth period, there was a trend for boars to have more favorable ADG

responses than castrates per 1% increases in dietary protein concentrations above 16% CP and up to 20% crude protein (3.5% increases in ADG for boars and 2.5% increases in ADG for castrates). Since boars voluntarily consume less feed than castrates, the studies of Campbell and King (1982) become significant. These authors limit-fed boars and castrates equal amounts of isocaloric diets containing 17, 21 and 23% crude protein. Final weights were not different but backfat thicknesses were 10 to 20% lower and dissectable fat in the ham was approximately 15% less in boars relative to castrates. These data indicate that a mechanism other than reduced feed intake is involved in reduced fatness of boars.

In a muscular breed, the Pietrain, no differences in nitrogen (N) retention were observed before 60 kg live weight but by 80 kg, boars had 18% greater N retention relative to castrated males (Rerat, 1976). Piatowski and Jung (1966) found N retention to be 28% higher for 30 to 100 kg live weight boars relative to castrated males. These differences in N retention indicate greater capacity for protein synthesis in boars relative to castrates and may partially explain the increased protein requirements. Boars also have approximately 10% higher heat production than castrates (Fuller et al., 1980). Since boars consume approximately 15% less feed/d than castrates and ADG is equal or higher, boars are superior (10 to 20%) in feed

efficiency (Kay and Houseman, 1975). While feed efficiency advantages exceeding 10 to 20% for boars over castrates have been reported (Rerat, 1976) similar efficiencies have been observed in other studies (Charette, 1961; Hines, 1966; Turton, 1969; Pay and Davies, 1973; Siers, 1975; Wood and Riley, 1982). The efficiency of converting digestible energy into muscle has been reported to be approximately 22% higher in boars than castrated males (Cahill, 1960; Staun, 1963; Walstra, 1969; Newell and Bowland, 1972.)

While boars gain at equal to or faster rates and require less feed to achieve these gains than castrates, boars also are superior in leaness (Carroll et al., 1963; Kay and Houseman, 1975). The amount of muscle in boars has been reported to be about 3% higher (Field, 1971; Wood and Enser, 1982; Wood and Riley, 1982), 15% higher (Prescott and Lamming, 1967) and 20% higher (Hansson et al., 1975) than castrates. Boars reportedly had 2% (Field, 1971), 5% (Wood and Riley, 1982) and 12% (Prescott and Lamming, 1967) greater bone weights than castrates. In addition, boars have been reported to have 15 to 25% less carcass fat than castrates (Prescott and Lamming, 1967; Fuller, 1980; Wood and Enser, 1982). Boars had slightly larger longissimus muscle areas (Siers, 1975) and 20% less average backfat (Turton, 1969) than castrates at traditional market weights. Knudson (1983) found no difference in longissimus muscle area but 31% less tenth rib fat depth in boars

relative to littermate castrates at 105 kg. In the same study, boars had 8% greater fat-free components and 5.4% greater muscle than castrates. Figure 2 illustrates similar differences in lean, bone and fat between boars and castrated males.

In summary of these data, boars have been shown to have equal or greater growth rates than castrates when fed ad libitum and excel in growth rate on restricted dietary regimens. Boars consume about 10% less feed/d than castrates and are 10 to 20% more efficient in conversion of feed into live weight gains. When fed to allow potentials to be maximized, boars have small but significantly greater (about 3 to 5%) fat-free lean, 20% less fat and about 2 to 5% more bone (Walstra and Kroeske, 1968; Field, 1971; Galbraith and Topps, 1981; Seideman et al., 1982).

Growth of Bone, Muscle and Adipose Tissue

The development and accretion of adipose tissue, muscle and bone hinges upon cellular proliferation both embryonically and postnatally, and the balance or net differences between synthesis and degradation of cell products (figure 3). If testosterone is responsible for modulating the development of bone, muscle and adipose tissue, then either cellular proliferation or synthesis and degradation should be affected. The remainder of this review will focus on the growth of bone, muscle and adipose

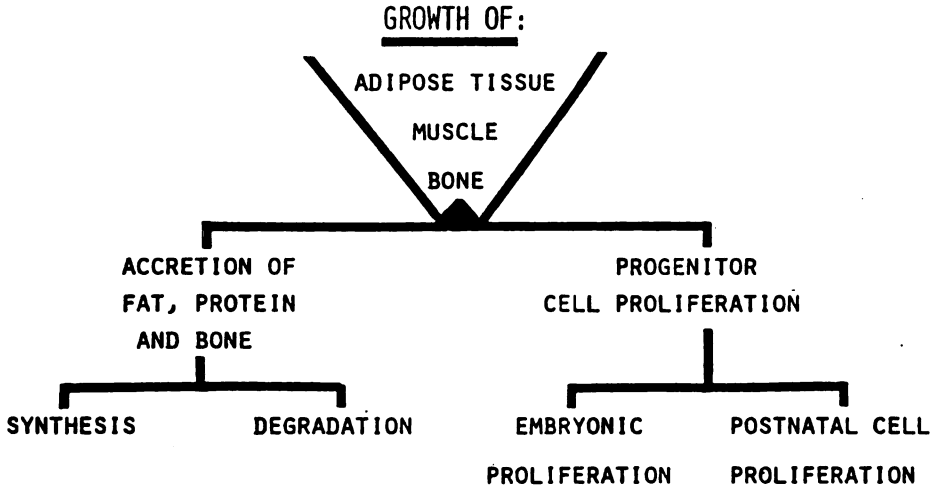


FIGURE 3. Illustration that both cell proliferation and hypertrophy are important to growth.

tissue, the effects of testosterone upon bone, muscle and adipose tissue and identification of potential mechanisms involved in the mediation of these effects.

Bone Growth

Bones grow via cell proliferation and differentiation and are influenced by adjoining tissues through forces applied by these tissues. Bone growth results from the multiple interaction of gene products of cells associated with bone and the responsiveness of the cells to nutrition, growth promoting and inhibiting factors of both hormonal and humoral origin, specific cell differentiation factors, environmental and mechanical factors (Petrovic, 1982). Many of these factors influencing bone growth are yet to be identified (Petrovic, 1982).

Petrovic (1982) described four theories of bone growth control mechanisms. The genetic control theory indicates that bone growth is genetically determined and that general, regional and local factors modulate gene expression. The second theory involves cartilage directed growth applicable to flat bones and craniofacial bones. Adjoining bone growth is secondary or compensatory to the primary growth centers or cartilage. The functional matrix or third theory suggests that bone growth is dependent upon functional demands imparted from muscle to the functional matrix. Finally, the servosystem theory centers around the physiological processes controlling bone growth.

Accordingly, the control of cell division of the epiphyseal cartilages are exerted primarily through extrinsic factors such as growth hormone-somatomedin, estrogens, androgens and thyroid hormones. Local mechanical factors modulate direction of growth but not the amount of growth. In this latter theory, the signal for growth is the coupled effect of muscle activity and the rate and location of cell multiplication of the cartilage. Coupling mechanisms include growth stimulating factors such as growth hormone-somatomedin, thyroid hormones, insulin androgen-estrogens in low doses, glucocorticoids, local mediators such as calmodulin, prostaglandin $F_{2\alpha}$ and other growth promoting peptides and local regulators which have negative feedback on cell division such as cAMP, prostaglandin E_2, contact inhibition or restraining signals and potentially somatostatin and somatostatin-like substances (Vaughn, 1981; Petrovic, 1982).

Bones do not grow interstitially but grow by apposition (Van Sickle, 1982). Bone matrices become calcified almost as soon as they are formed and therefore cannot expand from within its substance (Ham and Cormack, 1979).

There are basically two types of bone formation or ossification in the body: intramembranous and endochondral. Intramembranous or dermal bone formation is typical of the skull which is formed by ossification of connective tissues other than cartilage. Endochondral

ossification begins in cartilaginous bone models and is typical of most long bones (Goss, 1978; Ham and Cormack, 1979). Endochondral bone formation is the mechanism by which long bones grow in length (Van Sickle, 1982).

Once a mesenchymal derived cartilage model is formed, it is transformed to an ossified structure by two processes: (1) a primary osseous collar is formed and vascularized which becomes the diaphysis and metaphyses and (2) vascular mediated ossification in the epiphysis to form the secondary ossification center. The latter occurs postnatally. Epiphyseal growth plates develop between the primary and secondary ossification processes (Ogden, 1980). The epiphyseal plate is organized into zones through which a chondroblast proceeds, changing biochemically, first undergoing division, then differentiation, hypertrophy and finally death followed by vascular invasion.

Bone is comprised of cells and an organic intercellular matrix which is made up of collagen fibrils embedded in an amorphous component (Ham and Cormack, 1979). There are three primary bone cell types: osteoblasts, osteocytes and osteoclasts (Aurbach et al., 1981). Owen (1978) suggested that in postnatal animals the stem cells of the osteogenic (osteoblastic and chondroblastic) cell lineage are a part of the stromal system of marrow. Cells playing a resorptive role in the matrix are derived from the

hemopoeitic stem cell primarily through the monocyte-macrophage cell line (Owen, 1982).

Osteoblasts are located at the bone forming surfaces and produce procollagen and the organic constituents of the extracellular matrix. Mineralization occurs in this matrix. The junction between mineralized bone and nonmineralized osteoid is called the calcification front. These regions incorporate tetracycline and can be used to measure rate of bone or mineral deposition. Osteoblasts eventually become surrounded by the matrix and after morphological and biochemical differentiation, they become osteocytes. Osteoclasts are involved in bone catabolism and are localized at areas of bone resorption (Pritchard, 1972; Aurback et al., 1981). Along the shaft of the cartilaginous bone model, osteoblasts deposit a "collar" of bone until periosteum is formed. During early growth endosteal resorption occurs but at a slower rate than periosteal apposition. Primary spongiosa or trabeculae of calcified cartilage form at the metaphyseal ends of long bones. These trabeculae are resorbed and replaced with bony trabeculae in the secondary spongiosa. An epiphyseal ossification center develops at each bone end.

According to Vaughn (1981), the cells of the calcifying zone enlarge, lose their transverse walls and their longitudinal walls calcify. Capillaries carrying osteoblasts, osteoclasts and other bone resorbing cells

push up and invade the dying cells. Osteoblasts deposit osteoid on the calcified spicules of cartilage which become calcified, as well as, eroded by bone resorbing cells. At the same time, dying hypertrophic cartilage cells are replaced by proliferating and maturing chondroblasts on the upper end of the plate. The whole process is repeated continuously until growth in length of bone is completed.

Models of all long bones are able to grow in length only due to interstitial growth occurring in their cartilaginous epiphyses. From the epiphyseal plate to the diaphyseal aspect, there are four zones: resting cartilage, proliferating cartilage, maturing cartilage and calcifying cartilage. The epiphyseal plate does not become thicker because it is constantly being resorbed and ossified on the diaphyseal interface. The new bone or trabeculae makes the diaphysis longer. Once these epiphyseal centers ossify, linear growth ceases. Bone mass can continue to increase primarily because of growth in width or greater periosteal apposition rates relative to endosteal resorption (Ham and Cormack, 1979; Aurback et al., 1981) After an epiphyseal plate has developed, the cartilage of the epiphyseal plate is no longer replaced with bone on the epiphyseal interface but only on the diaphyseal interface.

Physical Factors. Bones are designed for mechanical support and perturbations of the "local stresses" applied to bones affect their metabolism. Redden (1970) applied centrifugation to chick embryos and observed enhanced linear growth of long bones. McMaster and Weinert (1970) applied tension to long bones in culture and observed similar results. Lambs which were weighted to increase the force of gravity developed thicker metacarpals but the rate of linear bone growth was unchanged (Tulloh and Romberg, 1963). Stubbs (1970) reported loss of bone mass under conditions of weightlessness. Goss (1978) reported that combinations of denervation, tenotomy and mechanical immobilization to effect contralateral limbs to carry greater loads caused bones of the contralateral limb to be thicker but not longer. Immobilization of bones enhanced bone atrophy (Burkhart and Jowsey, (1967). Experiments in which the supraspinatus muscle was cut or when the infraspinatus and trapezius muscles were removed showed reductions in the scapular spine and infraspinous fossa (Wolffson, 1950). In addition, use of pulsed electrical currents appeared to enhance bone deposition. This technique has been used medically to aid in fracture healing (Levy, 1974). Manipulations of blood supply through ligation, arteriole fistulas and application of tourniquets caused bone growth distal to the blockage and some long bones even attained greater lengths and were

thicker than controls (Kelly, 1971). Hall-Craggs and Lawrence (1969) stapled the diaphysis and epiphysis and halted long bone growth in rabbit tibias. In other work, natural physical constraints of bones were negated by transplantation of fetal rat phlanges to the spleen, brain, renal capsule and subcutaneous tissues, the bones continued to develop and almost attained normal size (Felts, 1959; Noel and Wright 1972).

Endocrine Effects on Bone. According to a review by Burrows (1949), probably one of the first to observe that the gonads affected the symmetry of the skeleton of birds was Yarrell (1827; see Burrows, 1949). Godard (1859; see Burrows, 1949) reported that males which appeared to have been born without testes had characteristics similar to females and males castrated as babies. As a result of these observations, Poncet (1877; see Burrows, 1949) experimented with animals. In early experiments, littermate rabbits were left intact or castrated at 3 mo of age. After 3 mo, all rabbits were killed and the skeletons examined. Femurs, tibias, fibulas and ilia from castrates were longer, stronger and straighter than noncastrates. Bouin and Ancel (1906; see Burrows, 1949) extracted testes and administered the extracts daily to 2 to 4 wk old guinea pigs. Although no statistical analyses were reported, the castrated untreated guinea pigs had longer femurs and tibias than noncastrates or noncastrates injected with the

extract. In conflict with these results are the data of Steinach (1916) who grafted whole testes into castrated females, and ovaries into castrated males. Bones from castrated guinea pigs weighed less than those from noncastrates and were heaviest in castrated females grafted with testes. At this early date, he explained that testosterone may have no effect on longitudinal bone growth but counteracts the effects of estrogens. Later work by Aschkenasy-Lelu and Aschkenasy (1959) showed that castrated male rats grew more slowly than noncastrates. Bergstrand (1950) reported that the variable results of androgen effects on bone growth were a result of the dose of androgen. When testosterone propionate was given at small doses to female rats, an acceleration of bone growth was reported (Joss et al., 1963). Large doses inhibited growth and caused premature closure of epiphysis (Rubinstein and Solomon, 1941a). The effect of large doses of testosterone may not exert its primary effect on bone but as a secondary effect through inhibition of pituitary function (Silberberg and Silberberg, 1971). Simpson et al. (1944) proposed a synergistic effect of testosterone and growth hormone in promoting skeletal growth. In fact, Zachmann and Prader (1970) found that combinations of testosterone and growth hormone were more effective in maximizing growth in hypopituitary boys than growth hormone alone. In other experiments, children with pituitary lesions did not

respond to testosterone treatment which indicate that androgens mediate their effects through inducing growth hormone release (Martin et al., 1968). Martin et al. (1979) demonstrated that young boys with delayed adolescence and short stature had enhanced growth hormone release in response to a hypoglycemia or arginine challenge after being administered testosterone. Similar results are observed after estrogen administration (Merimee et al., 1966). Since testosterone can be converted to estrogen it is possible that the increased growth hormone response to hypoglycemia and arginine infusion after testosterone administration is mediated through estrogens. Martin et al. (1979) tested this possibility by administering a nonaromatizable androgen, methyltestosterone, and still observed an enhanced growth hormone response. Recent experiments by Jansson et al. (1983) examined the effects of different doses of testosterone propionate on linear bone growth in castrated, castrated hypophysectomized and castrated hypophysectomized (hypox) rats given bovine growth hormone. Estradiol was administered to female rats in studies designed similar to that described for males. Oxytetracycline was used as a marker to enable measurement of linear bone growth. Results showed a dose dependent increase in linear bone growth when testosterone was given to castrates but no increases were observed for the castrated-hypox or castrated-hypox-GH treatments. Estrogen

inhibited longitudinal bone growth. These investigators concluded that while testosterone stimulated bone growth by changes in hypothalamo-pituitary function, estrogen does not mediate its inhibitory effects in this manner. Stenstrom et al. (1982) used similar measures of linear bone growth in female rats and found that ovariectomy increased longitudinal bone growth. As mentioned by Raisz and Kream (1981), bone growth appeared to be more dependent on androgen than estrogen. Estrogen prevents maximal bone growth by suppressing somatomedin production (Wiedemann et al., 1976). It is possible that some androgenic effects on bone growth are partially mediated through the more general anabolic effects on muscle longitudinal growth which elicits tension on bone.

The effects of other endocine factors and local growth factors upon bone growth are summarized in table 1.

Muscle Growth

As illustrated in figure 3, muscle growth is dependent upon both the net difference between protein synthesis and degradation as well as cell proliferation. Therefore, factors which influence muscle growth should mediate their effects through myogenic cell proliferation or differentiation and/or muscle protein accretion rates (Allen et al., 1979).

TABLE 1 ENDOCRINE AND LOCALIZED FACTORS AFFECTING BONE

Hormonal regulation of bone and factors affecting bone growth — Reviews	Becker (1981) Canalis (1983) Norman (1980) Raisz and Kream (1981) Raisz et al. (1978) Tonna (1973) Van Sickle (1980) Vaughn (1981)
Parathyroid Hormone (PTH)	
In vivo evidence of both increases (anabolic) and decreases (catabolic) of bone formation.	Canalis (1983) Raisz et al. (1978)
Inhibited bone collagen synthesis and stimulated resorption at 10^{-7} to 10^{-9} M.	Norman (1980) Haltrop and Raisz (1970)
Repeated doses of PTH increased collagen synthesis.	Vaughn (1981)
May have direct effects on osteoclasts.	Halse (1979)
PTH indirectly effects calcitonin release.	
Parathyroid function is not inhibited by acromegaly.	
Calcitonin	
Main effect is a direct inhibition of bone resorption.	Canalis (1983)
Effects are related to decreases in osteoclast cell numbers.	Vaughn (1981)
Has no effects (direct or indirect) on bone formation.	Canalis (1983)
Effects of injected calcitonin are transient.	Vaughn (1981)
Pituitary-parathyroid axis must be health for optimal osteogenesis.	Van Sickle (1982)
Vitamin D	
Is important in maintenance of normal serum and tissue concentrations of calcium.	Canalis (1983)
Increased both bone mineralization and resorption by indirect mechanism.	Canalis (1983) Kream et al. (1977) Raisz et al. (1978)
Bone cytosol has receptors for 1, 25-$(OH)_2D_3$ which inhibits bone collagen synthesis.	

Thyroid Hormone	
Thyroxine controls differentiation and maturation of chondrogenic and osteogenic tissues.	Vaughn (1981)
Treatment of hypox rats with only thyroxine causes premature closure of epiphyses.	Vaughn (1981)
Hyperthyrodism is associated with hypercalcemia.	Norman (1980)
Severe hypothyroidism is associated with arrested linear growth.	Underwood & VanWyk (1981)
Role may be permissive for GH action and synthesis.	
Thyroid hormones are necessary for SM synthesis by liver; have a direct stimulation effect upon cartilage growth and are synergistic with SM.	
In in vitro bone cultures, TH does not increase collagen or noncollagen protein synthesis.	Canalis (1983)
Insulin	
Diabetics have reduced bone mass compared to nondiabetics of similar ages.	Norman (1980)
Serum $1,25(OH)_2D_3$ is reduced in streptozotocin diabetic rats.	Norman (1980)
Insulin effects are difficult to dissociate from insulin like activity of somatomedin.	Vaughn (1981)
Bone cultures treated with insulin increased collagen synthesis.	Raisz et al. (1978)
Only at supraphysiologic levels does insulin act as a primary growth stimulant.	Underwood & VanWyk (1981)
Glucagon	
Simultaneous administration of PTH and glucagon increases hydroxyproline excretion.	Vaughn (1981)
Decreases incorporation of ^3H proline into bone.	Kalu et al. (1982)
May inhibit bone resorption and bone formation.	Vaughn (1981)
Growth Hormone (GH)	
GH stimulates bone growth indirectly through somatomedins.	Underwood & VanWyk (1982)
Localized administration of GH stimulated linear bone growth in rats.	Isaksson et al. (1982)
GH may stimulate production of $1,25(OH)_2D_3$.	Norman (1980)

Growth Hormone (GH) continued	
GH has no direct effects on cartilage or bone based upon in vitro experiments.	Canalis (1983)
In vivo GH stimulates bone formation, intestinal absorption of calcium and bone mineralization but in vitro does not stimulate bone matrix synthesis.	Canalis (1983)
In vivo GH stimulates longitudinal bone growth.	Thorngren & Hansson (1974)
Somatomedin (Sm)	
SM C and A are potent stimulators of collagen, noncollagen protein and DNA synthesis in bone cultures.	Canalis (1983)
SM's have direct growth promoting activity on bone and cartilage and are essential for normal linear growth.	Canalis (1983)
In vitro experiments with cartilage indicate SM increases chondrocyte proliferation and collagen and noncollagen protein synthesis.	Underwood & VanWyk (1981)
Purified SM C infused into hypox rats caused a dose dependent stimulation of body weight, tibia epiphyseal width and ^{3}H thymidine incorporation into cartilage DNA.	Schoenle et al. (1982)
Generally, SM synthesis are under GH control but GH like-hormones (placental lactogens), prolactin and insulin can stimulate SM production. Estrogens and glucocorticoids in excess reduce SM production.	Herington et al. (1983)
Prolactin	
Acromegalic patients often exhibit elevated prolactin concentrations and prolactin may act through somatomedin as a growth factor.	Raisz et al. (1978)
Prolactins effects on bone are indirect based upon in vitro collagen and noncollagen protein synthesis experiments.	Canalis (1983)
Single acute or chronic administration of ovine prolactin to chicks increased vitamin D hydroxylase activity in vitro. In vivo administration is correlated with elevated plasma $1,25(OH)_2D_3$.	Norman (1980)

Glucocorticoids	
Chronic administration arrested endochondial and periosteal bone formation.	Silberman et al. (1979)
Prevented replication and differentiation of osteoblast precursors.	Vaughn (1981)
Directly inhibit bone resorption; preserve cytosolic binding of $1,25(OH)_2D_3$.	Canalis (1983)
Short term effects of cortisol enhances bone collagen stimulating effect of SM and BDGF.	Canalis (1983)
Long term effects of glucocorticoids are inhibitory to bone formdation; decreased DNA and collagen synthesis possibly through impairment of SM effects.	Raisz & Kream (1981)
Reduce gut Ca absorption; probably arrest linear growth by direct action.	Underwood & VanWyk (1981)
Androgens	
Androgen excess (androgen secreting tumors) are associated with increased linear growth.	Underwood & VanWyk (1981)
The presence of GH is essential for maximum effect.	
In vitro studies indicate no receptors or affects on bone collagen synthesis.	Canalis & Raisz (1978)
May affect activity of vitamin D metabolites.	Vaughn (1981)
May be involved in pituitary output of GH.	Jansson et al. (1983)
Estrogens	
Sites of action for estrogen and testosterone are different.	Jansson et al. (1983)
Estrogens may increase serum PTH and $1,25\text{-}(OH)_2D_3$ and increase mineralization.	Canalis (1983)
Pharmacologic doses to excessively tall girls slowed linear growth; generally decreases linear growth and width of epiphysis.	Canalis (1983)
May be mediated by inhibiting SM secretion but not action.	Underwood & VanWyk (1981)
No evidence of direct action.	Canalis (1983)

Other factors	
As yet, other unidentified serum bone factors besides insulin or somatomedin affects bone growth.	Raisz et al. (1978)
OAF-osteoclastic activating factor, released by T-lymphocytes couples bone resorption to bone formation.	VanSickle (1982)
PAA-plasminogen activation activity increases during vascularization of the physis when the interstitial matrix is degraded and resorbed to allow capillary in growth.	
FGF-fibroblast growth factor, increases cell replication in cartilage but retards synthesis of cartilage proteoglycans. Has been shown to increase bone DNA and a subtle stimulation of resorption.	
EGF-epidermal growth factor, role not established. In bone culture, EGF caused a dose dependent stimulated of DNA synthesis and inhibited collagen synthesis. Inhibits osteoblastic function.	
PDGF-platelet derived growth factor, stimulated DNA, collagen and noncollagen protein synthesis in rat bone cultures. Unique effects relative to FGF and EGF since both DNA synthesis and nonspecific protein synthesis are stimulated. Cortisol, PDGF and SM-C are all important in bone fracture healing.	
BDGF-bone derived growth factor, stimulates proliferation of osteoprogenitor cells and may be progression factor. Resembles a somatomedin like activity.	Urist et al. (1983)
BMP-bone morphogenetic protein, may act as a competency factor for BDGF, induces differentiation of progenitor cells.	Urist et al. (1983)
Prostaglandins-at high doses inhibits bone collagen synthesis and stimulates bone resorption; at low doses opposite results are obtained produced by bone in culture.	Raisz & Kream (1982)

Factors	
CDF-cartilage derived factor, local regulator of cartilage growth. Increased DNA, collagen and noncollagen protein synthesis in bone cultures.	
Osteonectin-binds to bone collagen and may be important in initiating mineralization.	
Bone GLa protein or osteocalcin-contains carboxyglutamic acid; binds to bydroxyapatite crystals and inhibits crystallization; opposes effects of osteonectin.	Canalis (1983)

Embryonic Proliferation and Differentiation. Consideration of embryonic muscle cell differentiation is important since this is a time in which muscle cells are programmed (Young and Allen, 1979) and the number of mitotic divisions that occur dictates the number of muscle cells found postnatally and probably to some degree, the growth potential of muscle (Holtzer and Bischoff, 1970; Burleigh, 1980).

During embryonic muscle development, myogenic cells are mononucleated cells with high mitotic activity. Morphologically, these cells feature cytoplasmic constituents similar to other cell types. Because these cells eventually express muscle cell characteristics upon cloning, they are referred to as presumptive myoblasts (Konigsberg, 1963; Fischman, 1972). In early myogenesis, these presumptive myoblasts elongate, eventually cease dividing and begin synthesizing muscle specific proteins (Holtzer, 1970). This transition from replicating myogenic cells to muscle fibers results from activation of new genes and suppression of others (Holtzer and Holtzer, 1976; Young and Allen, 1979). After making the transition to this postmitotic or myoblast stage, the cells fuse to form multinucleated cells referred to as myotubes (Okazaki and Holtzer, 1966). It appears that fusion of myoblasts occurs when a critical cell density is reached (Konigsberg, 1971). Fusion of myoblasts is characterized by the

appearance of muscle specific proteins, namely, myosin, actin, creatine kinase as well as, appearance of cross striations and acetylcholine receptors. In synchrony with these events, DNA replication and nuclear divisions are repressed (Stockdale and Holtzer, 1961; Stockdale, 1970). Subsequent changes that occur are the further accumulation of muscle proteins and assembly of myofibrils which displace the once centrally located nuclei as the nuclei migrate to the periphery of the cell. Cells with these latter characteristics are called muscle fibers or myofibers.

Through clonal analysis of myogenic cells in culture, myogenesis appears to be governed by intrinsic, preprogrammed, time dependent factors as opposed to neural extrinsic factors (Konigsberg, 1963). In general, the cue for synthesis of contractile proteins revolves around two theories. Patterson and Strohman (1972) indicated the critical event is associated with cell fusion; whereas, Holtzer et al. (1974) viewed the withdrawal of myoblasts from the cell cycle as the prerequisite for subsequent myogenesis. Furthermore, Holtzer et al. (1982) indicated that only myogenic cells in G_1 have the option to fuse. Cells in S, G_2 or M do not fuse. Buckley and Konigsberg (1973) stated that cells which were capable of fusion but that do not fuse can reenter the cell cycle. Holtzer et al. (1982) provided evidence that cells with the option to

fuse, withdraw from the cell cycle as a precondition to fusion and cannot be induced to reenter the cell cycle.

Dienstman and Holtzer (1975) proposed a model of myogenic lineage in which cells undergo a quantal mitosis as a requisite of passage from one cell compartment in the myogenic cell lineage to another. Also, the myoblast has the unique capability of fusing and cells in the penultimate compartment do not have the option to fuse. Accordingly, exogenous agents should not be capable of inducing presumptive myoblasts to fuse. Konigsberg (1982) theorized that cells undergo a protracted G_1 period and that fusion is closely related to the time cells remain in this phase of the cell cycle. The time spent in G_1 and protracted G_1 is related to changes in the cell environment also caused by cells themselves (Konigsberg, 1982). It is apparent that differentiation revolves around the G_1 phase of the cell cycle. In theory, a variety of factors could mediate an effect of shortening the G_1 portion of the cell cycle and result in additional passes through the cell cycle and ultimately generate greater potential for subsequent postnatal muscle growth (Allen et al., 1979).

Postnatal Myogenic Cell Proliferation. In reviewing work by Winick and Noble (1966), Allen et al. (1979) determined that 80% or more of the DNA content in rat muscle was accumulated after birth. Other studies indicate

similar trends of increased total DNA and RNA and decreased concentrations during growth (Burleigh, 1980). This was shown in rats (Mendes and Waterlow, 1953; Devi et al., 1963; Enesco and Puddy, 1964; Winick and Noble, 1965, 1966; Cheek et al., 1971; Howarth and Baldwin, 1971), pigs (Gordon et al., 1966; Robinson, 1969; Gilbreath and Trout, 1973; Tsai et al., 1973; Hakkarainen, 1975; Powell and Aberle, 1975; Harbison et al., 1976; Corring et al., 1982; Wigmore and Stickland, 1983), chickens (Moss et al., 1964, Moss, 1968a, 1968b; Hentges et al., 1983), rabbits (Corring et al., 1982) and ruminants (Laflamme et al., 1973; Johns and Bergen, 1976; Mostafavi, 1978).

Lash et al. (1957) and Enesco and Puddy (1964) demonstrated that there was no polyploidy in muscle. Muscle was found to be composed of many closely associated fibers, enveloped by the endomysium and grouped in bundles by the perimysium. Most muscle nuclei belong to muscle fibers and the remaining nuclei are associated with the connective tissue. At various stages of development in the rat, Enesco and Puddy (1964) found that muscle fiber nuclei represented about 65%, endomysium nuclei 25% and those in the perimysium 10% of the total. Cordesse and Nougues (1973) showed that the distribution of nuclei between myofibers and related connective tissue did not vary in rabbits between 30 to 150 d of age. Connective tissue nuclei represented approximately 35% of the total. As

indicated by Rozovski and Winick (1979), estimation of DNA content even if uncorrected for nonmuscule nuclei, proportionately reflects increased muscle cell nuclei.

Through combinations of histometric and chemical techniques, Enesco and Puddy (1964) showed that individual muscle fibers in rats did not increase in number postnatally while the number of nuclei within myofibers and myofiber size did increase. While generally accepted that myofiber number does not increase significantly after birth (Allen et al., 1979), Gordon et al. (1966) observed increased numbers in rats as late as 90 d of age. Other studies have shown increased myofiber numbers in neonatal rats, mice and humans (Chiakulas and Pauly, 1965; Goldspink, 1962; Montgomery, 1962). In the pig, muscle fiber numbers remain constant shortly after birth (Staun, 1963, 1972; Davies, 1972; Stickland and Goldspink, 1973; Hegarty et al., 1973; Ezekwe and Martin, 1975). In contrast, Swatland (1976) analyzed transverse histological sections of pig muscle and found increased fiber numbers between 56 and 168 days of age.

In explanation of the source of the increased muscle DNA during growth, the electron microscopy work by Mauro (1961) and the thymidine incorporation studies by Moss and Leblond (1970, 1971) clearly showed that a population of cells which lies between the plasma membrane and the basement membrane of myofibers called satellite cells are

the source of the new nuclei. Cardasis and Cooper (1975) complemented this theory with results which showed a decrease in the total satellite cell population with age. Kelly (1978) found fewer satellite cells in the extensor digitorum longus muscle than the soleus muscle of both developing and mature rats and these differences in satellite cell number were correlated with myofiber nuclei density. The soleus had a greater rate of increase in myofiber nuclei per myofiber than the extensor digitorum longus as shown by an autoradiographic assessment of ^{3}H thymidine incorporation. Salleo et al. (1980) reported satellite cells were also capable of proliferation and fusion to produce new myofibers in rats.

Moss (1968a, 1968b) and Swatland (1977) indicated a direct relationship existed between myofiber diameter and the number of nuclei. As pointed out by Hakkarainen (1975) and Allen et al. (1979) there appears to be a preprogrammed increase in DNA preceding increases in RNA and protein. As summarized by Allen et al. (1979) the most rapid increase of DNA occurs during rapid growth periods. Also, the number of nuclei is directly related to fiber size and the number of nuclei which may limit the quantity of protein in the myofiber.

Postnatal Muscle Protein Accretion. Postnatal muscle growth is achieved by increased fiber diameter (girth) and length (Goldspink, 1980c). The increased fiber size occurs

gradually and discontinuously, as opposed to continuously, (Goldspink, 1962) and is a result of increased myofibrillar protein accretion within the myofiber (Waterlow et al., 1978). Goldspink (1980c) attributed the increased accretion of protein to increased work loads or demands upon muscle. In addition, Goldspink (1970) indicated that once a particular critical size is achieved, myofibrils split longitudinally. This splitting facilitates development of the sarcotubular system and enables additional proteins to be laid down upon the existing myofilaments. In situations of overloaded muscle where a synergistic muscle was removed, muscle fibers were also observed to split. This splitting was incomplete and localized at the ends of the muscle (Goldspink, 1980c). The extent and significance of muscle fiber splitting during normal growth is not known (Goldspink, 1980c). It appears that myofilaments are added to the periphery of nascent myofibrils (Fischman, 1972) as labeled amino acids administered in vivo are incorporated and localized at the periphery of the myofibrils (Venable, 1969).

The increase in length of myofibers during postnatal growth is largely due to increased sarcomere number in series along the myofibrils and slightly increased sarcomere length (Stromer et al., 1974; Goldspink, 1980a). In mouse soleus muscle, sarcomere numbers increased from 700 to 2200 in the first three weeks of age (Goldspink,

1980a). There are two alternatives for the mechanism of sarcomere addition: interstitial and serial addition. While it appears some insect muscles involve interstitial addition of sarcomeres (Goldspink, 1980a); Griffin et al. (1971) demonstrated strong evidence for serial addition in mammalian muscle. After incorporation of labeled amino acids into protein, individual muscle fibers were dissected and autoradiography performed. These data showed most of the radioactivity was incorporated into the ends of the myofiber adjacent to the tendons. Goldspink (1980a) reported that the addition of sarcomeres could be suppressed by as much as 50% by immobilizing the limbs with casts. When the casts were removed, the rate of sarcomere addition was accelerated and achieved control numbers within a few days. When muscles of adult cats or mice were immobilized into lengthened or shortened positions with casts, adaptive responses were observed (Tardieu et al., 1980; Williams and Goldspink, 1973). Mouse soleus muscles immobilized in a lengthened position produced 20 to 30% more sarcomeres relative to normal muscles and when immobilized in a shortened position, a 25% loss of sarcomeres was observed within a few days. These observations were also similar when muscles were denervated prior to the immobilization (Goldspink et al., 1974).

During postnatal muscle growth, net accumulation of muscle protein is a result of the imbalance between protein

synthesis and degradation rates (Garlick, 1980). With increasing age, the apparent imbalance narrows, and for the adult results in synthesis rates that are completely counterbalanced by degradation rates (Waterlow et al., 1978). Factors which influence either protein synthesis or degradation rates or both are ultimate determinants of skeletal muscle protein mass.

Factors Influencing Muscle Growth and Protein Turnover. For the sake of convenience, the factors influencing muscle growth and protein turnover are classified here as: (1) chemical factors which include hormones and nutrients and (2) mechanical factors which include stretch, physical activity pattern and work induced hypertrophy.

Goldspink (1980a) immobilized a flexor muscle, the extensor digitorum longus (EDL), and an extensor muscle, the soleus, of 50 g growing rats by placing plaster casts on the hind leg ankle such that these muscles, were in a shortened state. The EDL and soleus grew about 10 and 20% less, respectively, relative to controls. In vitro protein synthesis rates measured 2 d after immobilization were 30 and 70% lower for the EDL and soleus, respectively, relative to controls. Corresponding degradation rates were 25 and 50% higher for the EDL and soleus, respectively. The differential protein turnover response between the two muscles was explained on the basis of differential activity of these muscles. The soleus is recruited for greater work

loads relative to the EDL normally and therefore the response to immobilization of the soleus in the shortened state was more evident. In subsequent experiments, Goldspink (1980a) observed that the EDL and soleus returned to normal within 7 d after removal of the casts. In fact, in vitro protein synthesis rates of the soleus were 90% higher than controls within 3 d after cast removal. These data indicated that a reversal of atrophy and loss of sarcomeres as well as compensatory hypertrophy had occurred.

Goldspink (1977) reported opposite trends when the EDL or soleus were immobilized in a lengthened state. In these experiments, the stretch induced growth was accompanied by increased RNA and DNA. This coupling of increased nuclear material and longitudinal growth enabled changes in the nuclear: sarcoplasmic ratios for more efficient maintenance of cellular processes (Goldspink, 1980a). Goldspink (1977) also observed a stimulation of protein synthesis with stretch induced growth even when de novo synthesis of RNA and DNA was blocked. These investigators concluded that any increased nuclear proliferation in stretch induced growth resulted as a secondary response to the primary affect of enhanced translation. Laurent et al. (1978) stretched the anterior latissimus dorsi muscle of chickens with weights and induced muscle hypertrophy. These enhanced growth responses to stretch appear to be

mediated through the muscle cell and are not a consequence of neural effects. Coldspink (1980b) reported that the presence of innervation is not necessary for stretch inducement of sarcomere numbers and enhanced protein synthesis rates. When the EDL was denervated and compared to innervated controls and denervated-immobilized with and without stretch, the denervated and denervated-immobilized with stretch had respectively, 32 and 75% higher synthesis rates and 97 and 124% higher degradation rates relative to innervated controls.

In preliminary studies, Coldspink (1980a) implanted stainless steel electrodes into the soleus muscle at the time of immobilization with plaster casts. Muscles were stimulated electrically for a few hours a day. Electrical stimulation caused sarcomere loss and prevented addition of sarcomeres when muscles were in an immobilized-shortened position. When muscles were in a lengthened but immobilized position, new sarcomeres were added at the ends of the muscles.

Endocrine Factors Influencing Muscle Growth. Kochakian et al. (1956) and Kochakian and Tillotson (1957) investigated the effect of castration upon 48 different muscles of guinea pigs and the effect of twelve androgens upon reversing the slowed growth due to castration. Castration resulted in a 10% reduction in muscle weights relative to noncastrates. Injection of 5.6 mg of

testosterone propionate restored muscles of castrates to that of noncastrates.

Skeletal muscles of male mice are longer and have larger fiber diameters than females (Rowe and Goldspink, 1969) and the greatest rate of increase in fiber diameter is observed in males.

Grigsby et al. (1976) found greater incorporation rates of ^{3}H-leucine into myofibrillar protein fractions when intact male rabbits were treated with testosterone implants.

Powers and Florini (1975) added testosterone to L-6 myoblast cultures and showed direct effects on proliferative activity as indicated by a 25% increased labeling by tritiated thymidine and a decreased G_1 time. These effects were not observed with dihydrotestosterone. Allen et al. (1983) measured α-actin accumulation in muscle cell culture in response to testosterone or growth hormone addition to the medium. Neither testosterone nor growth hormone was effective in selectively stimulating accumulation of this muscle specific protein. Kohama and Ozawa (1978) injected 5 mg of testosterone propionate into chickens three times a week for 6 wk and measured the effects of trophic factors generated in plasma on muscle cell cultures. These investigators found a significant increase in trophic activity from testosterone injected chickens but minimal changes in myoblast multiplication

were observed when triiodothyronine, testosterone or estradiol was added to cultures directly. These trophic factors found in chicken serum have been shown to be transferrin, a necessary component in serum-free culture media (Kimura et al., 1981).

Santidrian et al. (1982) studied the effects of testosterone upon myofibrillar protein breakdown in rats as assessed by N^T-methylhistidine excretion. Castrated or castrated-adrenalectomized rats were injected daily with .2 or 2 mg of testosterone propionate per 100 g body weight, or .8 or 10 mg/100 g body weight of corticosterone. Normal intact rats grew 24% faster than castrated controls which grew at similar rates observed for the testosterone treated rats. The sum of the gastrocnemius, tibialis, EDL and soleus did not differ among treatments. However, castrates excreted 23% less total N^T-methylhistidine relative to intact rats, and castrated-adrenalectomized rats given .2 mg testosterone/100 g body weight excreted 26% less total N^T-methylhistidine. In these studies, adrenalectomy of castrated rats resulted in depressed growth rates which was slightly reversed by testosterone treatment. Large doses of testosterone given to adrenalectomized-castrated rats that also received corticosterone did not prevent depression of growth or increase N^T-methylhistidine output. Interpretation of these data is difficult but it appeared that other factors secreted by the testes may be

more closely associated with N^T-methylhistidine excretion. Additionally, there appears to be an interaction between testosterone and products of the adrenal gland which altered the responsiveness to the testosterone treatment.

Vernon and Buttery (1976, 1978) observed decreased N^T-methylhistidine excretion in rats as well as decreased glucocorticoid output by adrenal slices incubated in vitro after animals were treated with trenbolone acetate. Lobley et al. (1983) concluded that the mechanism of action between testosterone and trenbolone acetate may be different. Rogozkin (1979) found a 16% increased ^{14}C-leucine incorporation into myosin and a 16% increased DNA dependent RNA polymerase activity in gastrocnemius muscles with methandrostenolone administration to rats. Florini (1970a) observed a 50 to 70% enhanced uptake of labeled amino acids into protein and 60% greater mRNA synthesis after intraperitoneal injection of .1 mg of testosterone propionate. It was concluded that no new types of proteins were synthesized and the effect of testosterone was merely to increase availability of existing DNA templates and enhance translational events. In light of the design of that study (Florini, 1970a) and the elevated concentration of circulating insulin accompanying testosterone treatment observed by Grigsby et al. (1976), the direct effects of testosterone upon protein synthetic events is still questioned.

It has not been definitely shown that any anabolic effects of testosterone upon muscle are mediated by direct action upon muscle via binding with intracellular receptors (Michel and Baulieu, 1980). In androgen sensitive reproductive tissues, testosterone enters the cell passively or is aided by an active mechanism. Upon entry testosterone is reduced by 5-α reductases to dihydrotestosterone which binds to a cytosolic receptor which is activated and translocated to the cell nucleus. In the nucleus, the testosterone activated receptor complex associates with acceptor sites on the chromation enhancing transcription and ultimately synthesis of protein (Vermeulen, 1982). However, other data indicate a different mode of action in muscle. There is no strong evidence for the reductase enzymes in muscle (Krieg et al., 1974) nor for the prerequisite transformation of testosterone to dihydrotestosterone (Krieg and Voigt, 1977). Krieg (1976) indicated binding sites of dihydrotestosterone in skeletal muscle were 60 and 7 times lower than in the prostrate or bulbocavernous-levator ani, respectively. Mayer and Rosen (1975, 1977) theorized that the response of muscle to testosterone is due to a blockade of the glucocorticoid pleiotypic catabolic effects and is mediated through competitive inhibition and displacement of glucocorticoids from receptors (Parra and Reddy, 1962; Mayer and Rosen, 1975). The counteractive effects of

glucocorticoids and androgenic steroids such as testosterone lends support to the above theory and would be biologically compatible. Glucocorticoids elicited responses of decreased amino acid uptake (Kostyo, 1965), reduced incorporation of labeled amino acids into skeletal muscle protein (Hanoune et al., 1972), decreased RNA (Peters et al., 1970) and DNA (Goldberg and Goldspink, 1975) synthesis and greater protein degradation (Goldberg, 1969). Contrary to the catabolic effects of glucocorticoids, testosterone has been shown to stimulate both increased amino acid uptake and incorporation of labeled amino acids into skeletal muscle proteins (Arvill, 1967; Breuer and Florini, 1965; Florini, 1970b; Rogozkin, 1975), increased messenger and ribosomal RNA synthesis (Kochakian et al., 1964; Rogozkin, 1975) and decreased amino acid and protein degradation (Bullock et al., 1969; Young, 1970).

Mainwaring (1979) suggested that testosterone may elicit early translational responses as well as time dependent transcriptional responses; however, evidence for nuclear binding has not been indicated. Bicikova et al. (1977) showed that there was rapid transport of radioligands into the nucleus and was dependent upon cytosolic binding. Dionne et al. (1979) demonstrated cytosolic binding but low nuclear binding of testosterone in rat skeletal muscle. These authors also concluded that

dihydrotestosterone was metabolized so rapidly that it would not be a regulatory homone in skeletal muscle. Max (1983) found high affinity (.6nM=K_D) low capacity (3 fmol/mg protein=Bmax) androgen receptors in mouse quadriceps, tibialis, plantaris, soleus, EDL and gastrocnemius muscles. Receptor characteristics were not different between dystrophic and normal mice. Snochowski et al. (1981) provided evidence for testosterone receptors in porcine skeletal muscle. In addition, the receptor complex had dissociation constants twenty times lower than dexamethasone complexes. These data indicated the presence of two separate receptors for androgens and glucocorticoids which is in contrast to the competitive binding theory of Mayer and Rosen (1975) and data of competitive binding between the two hormones provided by Viru and Korge (1979). Michel and Baulieu (1980) found that testosterone and glucocorticoids bind to separate receptors and that the androgen receptor has characteristics much the same as found in other target tissues. Estradiol also bound (Kd=.2 nM) the androgen receptor but diethylstilbestrol did not. Dionne et al. (1979) found specific estrogen binding receptors in rat thigh muscle and reported the presence of nuclear estradiol binding proteins in the levator ani muscle.

The effects of other endocrine factors, nutrients and growth factors upon muscle growth have been studied. Selected actions and interactions are given in table 2.

TABLE 2 ENDOCRINE FACTORS AFFECTING MUSCLE

EXPERIMENTAL APPROACHES	
A variety of approaches are used to study selected muscle responses such as proliferation of progenitor cells in culture and accretion of protein in vitro and in vivo. Typically in vivo administration to normal animals or after ablation of secretory source of endocrine factor being studies. In vitro muscle incubations and myogenic cell cultures have been frequently used. Responses measured are muscle growth, amino acid transport, protein synthesis and degradation rates, myogenic cell differentiation or thymidine incorporation into DNA.	
GROWTH HORMONE	
May be important only from the standpont of being a trophic hormone stimulating somatomedin production.	Riis (1983)
GH administration to hypox rats stimulated muscle DNA accretion.	Beach & Kostyo (1968)
GH included in in vitro diaphragm incubations stimulated AIB transport and protein synthesis only after rats were about 7 to 15 d old.	Nutting (1976)
Lack of GH is responsible for reduced protein synthesis but has no effect on protein degradation.	Goldberg et al. (1980)
Addition to myogenic cell cultures indicated no effect on growth, differentiation or proliferation.	Ewton & Florini (1980) Allen et al. (1983)
Decapitated fetal pigs had normal muscle development.	Campion et al. (1981)
In vivo administration of GH to pigs enhanced protein accretion.	Machlin (1976)

Growth Hormone-Continued

Insertion of hormone GH genes into mice resulted in doubling of growth and size.	Palmiter et al. (1983)
Rats with GH secreting tumors had increased muscle growth and DNA synthesis.	Prysor-Iones & Jenkins (1980)
GH may have some direct effects upon membrane transport of amino acids and glucose effects upon protein synthesis and DNA synthesis are mediated through somatomedins.	Florini (1982) Pain (1980)
SOMATOMEDIN	
Increased incorporation of radioactive label into proteins of isolated diaphram muscle.	Salmon & Duvall (1970)
When added to myogenic cultures, general anabolic effects are observed as well as proliferation and differentiation (fusion).	Florini (1982)
INSULIN	
Increased amino acid "A" transport system.	Goldberg (1980)
Most in vivo and in vitro work correlates to enhanced protein synthesis and reduced protein degradation.	Pain (1980) Florini (1982)
THYROID HORMONE	
Thyroidectomy reduced muscle growth and decreased N -methyl-histine excretion.	Young (1980) Burini et al. (1981)
Administration of thyroxine to ThX rats stimulated protein synthesis.	Flaim & Jefferson (1978)

Thyroid Hormone - Continued

In vitro incubations of muscles from Hypox animals gives either 200 ug or 2 ug thyroxine stimulated protein synthesis rates but degradation rates were 50% higher in animals given the high dose. TH is necessary for normal growth.	Goldberg et al. (1980)
Low doses are anabolic and high doses are catabolic.	Mayer et al. (1980) D. Martino & Goldberg (1981)
Myogenic cell differentiation were stimulated by addition of T_3.	Kumegawa et al. (1980)

GLUCAGON

Remain unclear of effects of muscle.	Pain (1980)
Isolated muscles incubated with glucagon have decreased radioactive label incorporation.	

GLUCOCORTICOIDS

In vitro incubations of muscles from glucocorticoid treated animals have greater degradation rates.	Goldberg (1980)
Required for cell culture systems. Dose dependent effects, at high doses they are catabolic.	Florini (1982)

CATECHOLAMINES

In vitro experiments with diaphragm of diabetic rats, epinephrine at 10^{-7}M stimulated amino acid transport and protein synthesis.	Nutting (1982)
Suggested that in states of depressed protein synthesis or hormonal deprivation, catecholamines have anabolic effects.	

PROSTAGLANDIN	
Protein degradation rates in vitro increases 20 to 40% when arachidonic acid and $P6E_2$ were included in the incubations. PGF_2 but not PGE_2 caused a 35% increase in protein synthesis.	Rodemann & Goldberg (1982)
Indomethacin and meclofenamic acid decreased synthesis rates in intermittently stretched muscle but not in unstretched muscle.	Smith et al. (1983)

Skeletal Muscle Protein Turnover During Growth. Skeletal muscle is comprised largely of proteins and associated water. Total muscle mass represents approximately 60% of the carcass weight and as much as 45% of the live weight of growing pigs (Mulvaney, 1981). The rate of skeletal muscle growth is directly related to the rate of protein deposition. If the rate of skeletal muscle protein deposition could be efficiently enhanced during growth, it follows that there would be increases in weight gain and in the efficiency of lean production. This is not an unrealistic possibility since only 20 to 30% of the skeletal muscle protein that is synthesized per day is deposited in skeletal muscle of young growing boars (Mulvaney, 1981). The reason for these low values is that proteins in skeletal muscle and most other tissue proteins are continually being synthesized and degraded, a process called protein turnover. Muscle protein deposition or growth rate is determined by the difference between protein synthesis and degradation rate. In order to achieve greater net synthesis or deposition of muscle protein, three basic avenues theoretically exist: (1) increase the rate of protein synthesis while keeping degradation the same or even lowering degradation, (2) keep synthesis rate normal but reduce degradation rates and (3) reduce both synthesis and degradation rates while reducing degradation rates more to expand the margin of difference between the two processes.

In order to simplify calculation and interpretation of protein turnover data, the rates of protein synthesis and degradation can be expressed as fractional rates (Swick, 1982). This refers to the percentage or fraction of the total protein in a muscle that is replaced per day (Garlick, 1980). Figure 4 is an idealized depiction of how the fractional protein synthesis rate (FSR, %/d) and fractional protein degradation rate (FDR, %/d) undergo developmental changes. The hatched portion of the figure is the fractional growth rate (FGR, %/d). The FGR is the difference between FSR and FBR and in the adult essentially becomes zero. The developmental decrease in total muscle turnover is due to a combination of the fall in fractional synthesis and degradation rate and the increased amount of muscle protein during growth (Millward, 1980). Quadricep muscles of 3 wk old rats had FSR over 20%/d and by 1 yr values were less than 5%/d (Millward et al., 1975). In lambs, the FSR of over 24%/d was observed at 1 wk which fell to 2%/d at 16 wk (Arnal et al., 1976). Mulvaney (1981) observed a developmental decline in muscle FSR in pigs at 22 to 45 kg but the extent was dependent upon the individual muscle. The work of Maruyama et al. (1978) illustrated a skeletal muscle FSR decrease from 25 to 8%/d in 1 and 2 wk chicks, respectively.

It appears that developmental changes in protein turnover occur in all muscle types. The pattern of change

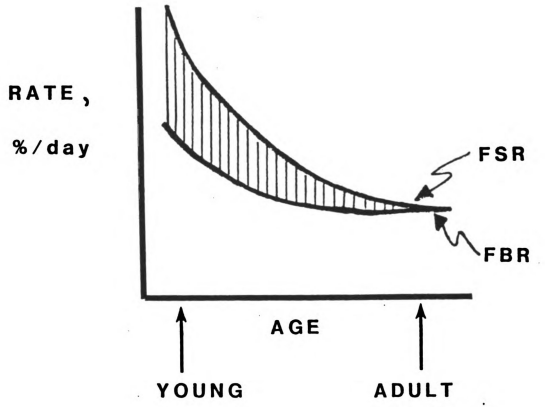


FIGURE 4. Developmental changes in fractional protein synthesis and degradation rates.

varies between muscles such that the hierarcy of relative protein turnover rates change with age. White and red muscles develop such that turnover was highest in white muscle of very young animals but the reverse was true in adults (Arnal et al., 1976; Maruyama et al., 1978). In adult rats, turnover rates of 10 to 12%/d, 8 to 10%/d and 4 to 5%/d have been observed for heart, soleus (red muscle) and quadriceps (white muscle), respectively.

Laurent et al. (1978) estimated protein turnover rates in chick anterior latissimus dorsi (slow tonic) to be 3 times higher than in the posterior latissimus dorsi (fast twitch) and 5 times higher than the breast muscle. Even after the fully grown anterior latissimus muscle was doubled in size through stretch induced hypertrophy for 2 mo the same turnover rate was found but during the early phases of stretch, increases in synthesis and degradation rates were observed (Laurent et al., 1978). Two contentious questions about protein turnover remains: (1) does rapid muscle protein turnover accompany rapid muscle growth? and (2) what determines the rates of protein synthesis and degradation in muscle?

As mentioned in the previous section, muscle grows through hypertrophy of the myofibers and acquisition of new nuclei from satellite cells. This new nuclear material enables the muscle cell to expand its protein to DNA ratio by as much as 300 to 400% (Millward, 1980). Oxidative

muscles were reported to have the lowest DNA unit size or protein to DNA ratio (Millward, 1980). During rapid compensatory muscle growth after nutritional insult in rats (Millward et al., 1975) and stretch induced hypertrophy (Laurent et al., 1978) the RNA to DNA ratio doubled.

Millward et al. (1978) found increased protein degradation rates when growth rates were increased as well as decreased degradation when growth was reduced in rats. Maruyama et al. (1978) indicated rates of protein degradation to decrease to facilitate growth in young chick muscle.

Millward et al. (1981) compared muscle protein degradation rate to cathepsin D activity during normal growth, growth suppression and catch up growth. Degradation rates were higher during rapid growth and decreased shortly after nutritional deprivation. Cathepsin D activities responded similarly. It is uncertain whether normal growth in muscles occurs by the same mechanisms as induced hypertrophy.

Adipose Tissue Development

Cellularity. Development of adipose tissue is accomplished by combinations of cellular hyperplasia and hypertrophy (the imbalance between synthesis and degradation of lipid) and factors which influence ultimate body fat mediate their effects through one or both of these

processes (Anderson, 1972; Leat and Cox, 1980). The contribution of progenitor cell proliferation to fattening during growth and obesity has been a controversial topic (Kirtland and Gurr, 1979).

Bergen (1974) illustrated the relationship of protein and fat accretion during growth. In an idealized portrayal, as the rate of protein deposition decelerates, fat accretion rates are accelerating and at greater rates than protein. The plateau in protein accretion rates occurs because muscle cell numbers are fixed at birth (Staun, 1963) and the maximum muscle cell size is attained during this plateau (Allen, 1976). If adipocyte numbers are also fixed at birth or shortly after and there is a maximum cell size, then fat accretion rates also should plateau. The fact it does not plateau indicates that subpopulations of progenitor cells are reserved for later differentiation and/or lipid filling, or hyperplasia can be induced at some time during growth, or that there is no limitation on the extent of adipocyte cell hypertrophy (Allen, 1976). However, Anderson et al. (1972) and Johnson (1978) indicated adipocytes had maximum cell sizes.

Proliferating preadipocytes have not been identified as a separate and distinguishable cell type (Hausman et al., 1980). Development of adipose tissue embryonically and identification of precursor adipocyte cells is particularly enigmatic because adipocytes are not morphologically

distinguishable until lipid accumulation occurs (Johnson, 1978).

The techniques used for estimating adipose tissue cellularity were reviewed by Gurr and Kirtland (1978). Basically, methods were categorized as those that involve some direct measurements of fat and those that measure DNA content.

A popular method for estimating adipocyte cell size and numbers involves fixation of cells with osmium tetroxide, eventual separation through specified mesh size screens, the counting of cells of varying sizes and integration of cell distribution (Hirsch and Gallian, 1968). Major disadvantages of this method are that cells less than 20 to 25 um in diameter (which would include precursor cells) are not measured and cells swell with osmium tetroxide which overestimates size (Gurr and Kirtland, 1978). Therefore, in situations of increased adipose cell numbers, it is uncertain whether it is due to lipid filling of already differentiated cells or due to newly differentiated cells derived from a pool of dividing precursor cells (Hausman et al., 1980). Another method described by Gurr and Kirtland (1978) is based upon incorporation of ^{3}H-thymidine by proliferating progenitor cells and cells of the stromal fractions. With time after injection of the label, the amount of radioactive DNA in the stromal fraction decreases while that in the nonproliferating fraction (mature fat

cells) increases. Changes in specific activities in the lipid filled fat cell fraction should be a result of newly derived cells. Another method with potential for identifying precursors of adipocytes hinges upon the demonstration of lipoprotein lipase and monoacylglycerol acyltransferase activity in the stromal vascular fraction of developing adipose tissue (Hietanen and Greenwood, 1977; Dodds et al., 1976). Identification of precursor cell types via fluorescent antibody techniques could potentially be used (Hausman et al., 1980).

Based upon the size sieving technique described above, adipose tissue is thought to undergo an early period of hyperplasia followed by a longer and overriding period of lipid filling and cell hypertrophy (Anderson, 1972). However, Johnson (1978) indicated additional hyperplasia occurs during postnatal growth after a critical cell size is reached and this hyperplasia results from recruitment of precursor cells.

At this time, it appears the osmium tetroxide method of estimating increased cellularity by itself offers little understanding or verification about precursor adipose cell proliferation. Klyde and Hirsch (1979a, 1979b) injected ^{3}H-thymidine intraperitoneally into rats and based on labeling of the stromal and adipocyte fractions, suggested the presence of a proliferating cell type which was an adipocyte precursor. Kirtland and Gurr (1980) used

^{3}H-thymidine to assess proliferation in pigs. Rate of proliferation was rapid between 2 and 40 days of age but by d 40 lipid filling was beginning to predominate.

Greenwood and Hirsch (1974) attempted to distinguish between lipid filling and new cell synthesis via osmium fixation and incorporation of ^{3}H-thymidine into DNA. In rat epididymal fat pads, most cell proliferation was completed by 5 wk of age and the observed increases in cell numbers at 12 wk were due to lipid filling. Kirtland and Gurr (1979) suggested that in situations of increased cell number the terminology 'increase in observable fat cell numbers' should be used as opposed to hyperplasia or replication of new fat cells.

Hausman et al. (1980) reviewed white adipose tissue differentitation and development and indicated blood vessels and adipocytes form simultaneously. Through active proliferation, mesenchymal cells result in formation of clusters of spherical basophillic cells (blood islands) connected to each other by strands of elongated cells. The peripheral cells of the blood islands and the strands ultimately form the primitive blood vessels. Preadipocytes could potentially be derived from these mesechymal cells of the blood island as identifiable adipocytes first appear in the perivascular regions (Hausman et al., 1980). Potential adipocyte precursor cells were identified as endothelial, perivascular reticulum, macrophagic fibroblastic and perivascular mesenchymal cells (Hausman et al., 1980).

Napolitano (1963) described the characteristics of stem cells of the epididymal fat pad of rats. These cells were spindle shaped (fibroblast like) with four to five protoplasmic extensions and cells adjacent to capillaries changed to a nearly spherical shapes or like the mature adipocytes. Slavin (1979) indicated similarities of presumptive adipocytes and fibroblasts were more coincidence rather than fibroblasts developing into adipocytes. If the cell diversification and differentiation scheme of Holtzer and Holtzer (1976) is expanded for all cell types, then it would seem fully differentiated fibroblasts could not serve as presumptive adipoblasts unless fibroblasts were capable of undergoing a quantal mitosis.

Leat and Cox (1980) illustrated the remarkable capacity of the adipocyte to store lipid. Since adipocytes have similarities to a sphere (Curr and Kirtland, 1978) and the volume of a sphere is proportional to the cube of the radius, a 2- and 10-fold increase in diameter results in an 8- and 1000-fold increase in volume. A cell with a diameter of 20 um contains about .004 ug lipid, a 100 um cell contains .45 ug lipid and a 200 um cell could contain 3.8 ug of lipid (Leat and Cox, 1980). The factors affecting the observable fat cell numbers (OFCN) and size of OFCN during postnatal growth include species, strain, age, depot, nutrition and endocrine factors.

Differences in postnatal adipocyte proliferation between species appear to be dependent upon the relative degree of maturity of a given depot of the species at birth. For example, rat subcutaneous adipose tissue is less completely developed than in the guinea pig and the epididymal depot is much more developed at birth in the guinea pig at birth relative to the rat (Kirtland and Curr, 1979). The order of maturity of the depots in the meat producing porcine, ovine and bovine species is from earliest to latest developing is: perirenal, subcutaneous, intermuscular and intramuscular (Allen et al., 1976).

Imbalance Between Triglyceride Uptake, Synthesis and Mobilization. The rate of fat deposition in adipose tissue hinges upon the following metabolic processes: uptake of circulating fatty acids, fatty acid synthesis and esterification, lipolysis and mobilization of fatty acids and fatty acid oxidation (Allen et al., 1976). Whether an imbalance favors net storage or net mobilization depends upon multiple interactions of hormones with the adipocyte (Saggerson, 1980).

Uptake of Circulating Fatty Acids. Lipoprotein lipase (LPL) is an enzyme (or closely related enzymes) responsible for extraction of fatty acids from circulating lipoproteins (Allen et al., 1976; Saggerson, 1980; Cryer, 1981; Quinn et al., 1982). LPL functions as an extracellular lipase by

catalyzing the hydrolysis of ester bonds of triacylglycerols releasing nonesterified fatty acids from the lipoprotein carriers, . These fatty acids enter cells leaving a lipoprotein remnant (Quinn et al., 1982). It is currently believed that LPL is secreted by adipocytes and functions at the luminal surfaces of capillary endothelial cells (Saggerson, 1980). LPL is inhibited by high salt concentrations, activated by the serum factor apolipoprotein C-II, heparin and has an alkaline pH (8.0 to 8.5) optimum (Quinn et al., 1982). Kompiang et al. (1976) prepared antibodies to purified chicken adipose tissue LPL and after administration of the antibody, blocked LPL hydrolysis of chylomicra and very low density lipoprotein triglycerides which elevated circulating triglyceride concentrations. In addition to playing a role of triglyceride uptake, LPL appears to direct the uptake of fatty acids to adipose or muscle. LPL activity decreased in adipose tissue and increased in muscle during fasting but refeeding stimulated preferential uptake of triglyceride by adipose tissue (Robinson and Wing, 1970; Nilsson-Ehle, 1981).

Lee and Kauffman (1974) measured subcutaneous adipose tissue LPL activities in Duroc and Hampshire pigs during growth from birth to 8 mo of age. On a wet tissue basis, Durocs had 25% higher LPL activities over the 8 mo period than Hampshire pigs. In another study of crossbred pigs,

LPL activity expressed on a per gram of tissue basis increased 4-fold from birth to 2 wk of age, remained elevated to 70 d and declined between 70 and 150 d of age (Steffen et al., 1978). Lee and Kauffman (1974) observed a similar 2- to 3-fold increase in LPL activity in pigs between 2 and 24 wk of age. In another study, obese Minnesota No. 1 pigs had 4-fold higher subcutaneous LPL activity than lean Hampshire pigs (Weisenburg, 1973). McNamara and Martin (1982) compared LPL activities in fetal and neonatal pigs from lean (Yorkshire) and obese (Ossabaw) pig lines. Even though fetal body composition was not different, obese fetuses had higher adipose tissue LPL activities than lean fetuses. Etherton and Allen (1980) investigated the importance of plasma derived fatty acids relative to fatty acid synthesis in swine adipose tissue. In this latter study, fatty acid synthesis from glucose and esterification of palmitate in adipose tissue was observed in 57, 94 and 124 kg pigs. The ratio of palmitate esterified to palmitate that was synthesized from glucose de novo in adipose tissue increased 4- to 5-fold in pigs weighing 57 to 124 kg.

LPL is an adaptive enzyme (Allen et al., 1976; Cryer et al., 1981). Haugeback et al. (1974) showed that LPL activity was low or nondetectable in subcutaneous, perirenal and intermuscular adipose tissue from lambs fed a maintenance diet. After switching to an ad libitum diet,

LPL activity increased and was highly correlated with increased fat deposition. Activity expressed as nmoles of free fatty acids released per 10^6 adipocytes was 3-fold higher in subcutaneous compared to perirenal adipose tissue. Di Marco et al. (1981) measured a 37% decrease in LPL activity after 10 d of fasting of Holstein steers as well as 100% (of prefasting levels) overshoot upon refeeding. Fasting has been shown to significantly depress adipose tissue LPL activity in pigs (Enser, 1973; Weisenburg, 1973; Steffen et al., 1981).

Hormonal Control of Lipoprotein Lipase. One theory concerning LPL activation revolves around the interaction of insulin and lipolytic agents. Insulin stimulates glucose metabolism which accentuates glycosylation and activiation of lipoprotein lipase. Inhibition of LPL activity by fatty acids and lipolytic agents may involve the interference of the formation of precursors needed for glycosylation by diversion to glyceride-glycerol formation (Fain, 1982).

Insulin has been shown to be intimately involved and positively correlated with the maintenance of adipose tissue LPL activity (Cryer et al., 1976; Garfinkel et al., 1976; Saggerson, 1980). In adipose tissue of alloxan (Robinson and Wing, 1970) and streptozotocin (Ishikawa et al., 1982) induced diabetic rats, LPL activity is lowered.

Others have reported low correlations of LPL activity and insulin when data were expressed on a gram of tissue basis (Kessler, 1963; Redgrave and Snibson, 1977). Saggerson (1980) indicated that insulin plays a primary role in the secretion of an active extracellular form of lipoprotein lipase. Reichl (1972) reported parallel changes in adipose tissue LPL activity with diurnal changes in plasma insulin in meal fed rats. LPL activity in adipose tissue of starved rats also increased during in vitro incubations with insulin and glucose (Salaman and Robinson, 1966; Wing et al., 1966). Ashby and Robinson (1980) reported that glucocorticoids increased the insulin-dependent LPL activity as well as caused subtle independent increases in LPL activity. High concentrations of circulating cortisol are associated with excessive fat deposition in Cushing's syndrome (Rudman and DiGirolamo, 1971). Obesity may involve some increased sensitivity to glucocorticoids which also facilitate insulin stimulation of fatty acid synthesis. Robinson et al. (1983) reported that the synthesis of LPL is enhanced by insulin but further enhanced in the presence of insulin and dexamethasone. It was speculated that dexamethasone stimulated transcription of mRNA and the effects of insulin was a nonspecific translational effect.

Insulin stimulated increases in LPL activity are inhibited or prevented by catecholamines (Robinson et al.,

1983) adrenocorticotrophic hormone (Davies et al., 1974), glucagon, thyroid stimulating hormone (Nestel and Austin, 1969; Robinson and Wing, 1970), dibutyryl cyclic AMP, caffeine and methylxanthines (Robinson and Wing, 1970; Ashby et al., 1978). The aforementioned agents may inhibit or inactivate LPL activity simply through activation of intracellular lipolysis which causes an increase in intracellular fatty acids.

Wilson et al. (1976) measured LPL activity in adipose tissue from 120 to 180 g in male, castrated male and female rats given estradiol cypionate or testosterone enthanate injections. Male rats given weekly doses (for 3 wk) of 5 ug or 500 ug/doses of estradiol had 74 and 91% decreases in LPL activity, respectively. However, weight gain was also depressed by 65% in treated animals indicative of reduced feed consumption (not reported). Female rats given weekly 20 mg doses of testosterone enthanate (for 3 wk) had 12% lower adipose tissue LPL activity. Intact males had 20% lower LPL activity relative to castrated controls and the castrated controls had 7% higher activity than male castrates given weekly injections of 500 ug of testosterone enthanate. These investigators viewed the response to estrogen to be similar to starvation even though insulin concentrations were unchanged from controls. Hamosh and Hamosh (1975) observed similar responses of decreased LPL activity with administration of 17 β-estradiol to castrated

male and female rats. Wade and Gray (1979) and Steingrimsdottir et al. (1981) proposed that gonadal hormone treatment caused changes in both feed intake (sex and species dependent) and body weight which were possibly due to direct effects on adipose tissue. In testing this hypothesis, Ramirez (1981) gave estradiol to rats and concluded that the estradiol induced decrease in LPL activity occurred prior to depression in food intake. Gray and Greenwood (1982) investigated the changes in food intake and adipose tissue metabolism at 1, 2, 3, 7 and 14 d after estradiol benzoate was administered to ovariectomized rats. After estradiol treatment (2 ug/d), LPL activity decreased by 30% the first day and a 50 to 70% suppression was maintained for the following 14 d. When progesterone (2 mg/d) was given along with estradiol, the responses were approximately 50% of those observed for estradiol alone.

Burch et al. (1982) implanted 33 kg sheep with Revalor (17.5 mg trenbolone acetate and 2.5 mg 17 β-estradiol). After 60 days, adipose tissue LPL activity only tended to be lower in implanted relative to nonimplanted sheep, whereas fatty acid synthesis rates were 50% lower. Prior et al. (1983) implanted bulls and steers with estradiol dipropionate. While acetyl coenzyme A carboxylase, ATP citrate lyase, NADP-malate dehydrogenase, aconitate hydratase and NADP-isocitrate dehydrogenase activities were generally half those observed in steer subcutaneous adipose

tissue, estradiol increased lipogenic activities in both bulls and steers.

Lipogenesis. Lipogenesis has been defined as the synthesis of fatty acids from glucose and their esterification to form triglycerides (Allen et al., 1976). Regulation of the metabolic pathways involved in lipogenesis centers around rate limiting steps. Control of metabolic pathways is exerted through (1) modulation of catalytic activities of rate limiting enzymes via allosteric effectors, covalent modification or changes in the synthesis and/or degradation of enzymes and (2) control of rate of passage through cell membranes, changes in availability of precursors from other tissues and flux of substrates and effectors through metabolic pathways (Newsholme and Start, 1973).

A variety of enzymes related to lipogenic activity have been studied. Diffusion of glucose into adipose tissue and subsequent phosphorylation by hexokinase is a key step in lipogenesis (Avruch et al., 1972; Romsos and Leveille, 1974). Glucose is the primary precursor of fatty acids in pig adipose tissue (O'Hea and Leveille, 1968; Martin and Herbein, 1976). Adipose tissue hexokinase activity appears to be adaptive and responds in parallel with changes of plasma glucose and insulin (Romsos and Leveille, 1974). Since fatty acids are synthesized in the cytosolic compartment, the production of cytosolic NADPH by reactions

catalyzed by glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, NADP-isocitrate dehydrogenase and malic enzyme become important considerations (Allen et al., 1976). In addition, phosphofructose kinase, aldolase, pyruvate kinase, pyruvate dehydrogenase, acetyl CoA carboxylase and fatty acid synthetase are involved in the conversion of glucose to fatty acids (Romsos and Leveille, 1974).

Glucose 6-phosphate dehydrogenase catalyzes the first step in the pentose pathway and activity of this pathway is closely associated with fatty acid synthesis (Kather et al., 1972). The reason for this high correlation is that 50% of the NADPH needed for adipose tissue fatty acid synthesis is derived from the pentose-phosphate pathway (O'Hea and Leveille, 1968; Kather et al., 1972). As summarized by Allen et al. (1976), breeds of swine or cattle with low thresholds to fattening and with high lipogenic activities had greater NADPH generating capabilities. As animals grow, this NADPH generating capability increases.

The glycolytic enzymes phosphofructose kinase, aldolase and pyruvate kinase are implicated in the regulation of fatty acid synthesis in adipose tissue (Romsos and Leveille, 1974; Saggerson, 1980). Changes in activity of the pyruvate dehydrogenase enzyme complex are correlated with changes in adipose tissue fatty acid synthesis

(Jungas, 1970). However, Allee et al. (1971a) observed malic enzyme activity in pig adipose tissue to increase up to 50 kg live weight but declined during growth afterwards. It was indicated that the activity of this enzyme merely responds to changes in fatty acid synthesis (Romsos et al., 1971).

The first committed step in fatty acid synthesis is the formation of malonyl CoA via carboxylation of acetyl CoA and is catalyzed by acetyl CoA carboxylase (Numa et al., 1970; Lane et al., 1974). The second step is catalyzed by a multienzyme complex, fatty acid synthetase and involves the condensation of acetyl CoA and malonyl CoA in the presence of NADPH to form palmitic acid (Mayes, 1976).

The primary pathway of triglyceride synthesis in swine adipose tissue is the glycerol 3-phosphate pathway (Stokes et al., 1975). Glycerol 3-phosphate is sequentially acylated by acyl-CoA fatty acids and glycerophosphate acyltransferase to result in phosphatidate. This is followed by hydrolysis of phosphatidate by phosphatidate phosphohydrolase to yield diglyceride. Another acylation of the diglyceride by the activated acyl-CoA fatty acids is catalyzed by diglyceride acyltransferase to yield triglycerides (Hems, 1975; Raju and Six, 1975; Stokes et al., 1975; Mayes, 1976; Fallon et al., 1977). Glycerol 3-phosphate can be generated from glycolysis or gluconeogenesis. Fatty acyl-CoA can be synthesized de novo

in adipose tissue, from plasma free fatty acids or triglyceride via lipoprotein lipase action or from lipolysis of triglyceride stores in the cell (Steffen et al., 1979).

There have been considerable data generated characterizing lipogenic activities in swine adipose tissue. Hood and Allen (1973) assayed malic enzyme (ME), glucose 6-phosphate dehydrogenase (G-6-PDH) and acetyl CoA carboxylase (CBX) in the middle and outer subcutaneous fat and in the perirenal depots of lean and obese pigs. When data were expressed per 10^5 cells, all the above enzyme activities were higher in the perirenal depot than subcutaneous and were higher in the obese line compared to the lean line. The magnitude of the differences varied with live weight. Steele and Frobish (1976) reported higher citrate cleavage enzyme (CCE), G-6-PDH and ME activities in obese Duroc pigs relative to a lean line. In that same study, pigs fed a diet containing 15% lard had suppressed enzyme activities. When meal feeding was compared to ad libitum feeding no significant effects were observed; however, the lean line of pigs appeared to have slightly lowered enzyme activities.

Mersmann et al. (1973a, 1973b) investigated lipogenic activities of neonatal and growing pigs. Around birth and until weaning lipogenic enzymes were generally low. The pentose phosphate dehydrogenase activities increased with

age and G-6-PDH doubled at d 60. Malic enzyme and glycerolphosphate dehydrogenase activities increased with age. Citrate cleavage enzyme (CCE), CBX and fatty acid synthetase activities were low before weaning but by d 60, had increased 10-fold. Similar trends were reported by Allee et al. (1971b). Anderson and Kauffman (1973) measured CBX, G-6-PDH, CCE in adipose tissue of growing pigs and when expressed per 10^6 cells, the activities increased markedly at weaning and again at about 4 to 5 mo of age. Between 5 and 6.5 mo, enzyme activities decreased which indicated some subtle changes in lipolytic and oxidative activities may have occurred. Steele et al. (1982) indicated palmitate oxidation decreased with increasing age of pig adipose tissue.

Anderson et al. (1972) reported CBX, CCE, ME and G-6-PDH enzyme activities in the outer, middle and inner subcutaneous backfat layers, intermuscular, mesenteric, perirenal and hindleg subcutaneous adipose tissue. For all enzymes assayed, the subcutaneous adipose tissue of the lower leg was lowest and the perirenal was the highest in activity. In addition, lower enzyme activity was observed in the outer subcutaneous backfat layer relative to the middle layer.

Scott et al. (1981) investigated the effects of age, breed and line (lean versus obese) on lipogenic and lipolytic activities of female swine adipose tissue. At 3,

4, 5 or 6 mo of age, obese pigs had larger adipocytes than a lean line. In vitro lipogenic activity expressed as nanomoles of ^{14}C glucose incorporated per 10^5 cells was 30 to 40% lower at 3, 4 or 5 mo of age but was 64% lower at 6 mo in the selected lean line. Highest lipogenic activity was observed within a line at 4 mo of age. CBX activity expressed on a cell basis was also dramatically 5-, 8-, 3- and 6-fold lower in lean pigs compared to obese pigs at 3, 4, 5 and 6 mo of age.

Allee et al. (1971a, 1972) fed 12 or 24% CP diets containing .1 or 13% corn oil to pigs and observed a 60 to 50% depression in vitro incorporation of ^{14}C glucose into fatty acids (per 100 mg of tissue) due to feeding fat and 30 to 40% depression due to higher protein. However, in another trial, the protein effect on lipogenesis was not observed. Steffen et al. (1978) also fed 24 or 12.8% fat diets (corn oil) to young pigs for 3 to 4 wk and another group fed 3% fat were fasted 72 h prior to assaying for lipogenesis. Because of greater observable fat cell numbers in the low fat groups, ^{14}C glucose incorporation rates measured in vitro were not different between fat fed groups when data were expressed on a cell basis. However, starvation depressed fatty acid synthesis rates by 85% relative to nonstarved controls regardless of method of data expression.

Martin and Herbein (1976) measured several adipose tissue lipogenic enzymes in vitro in 6 mo old pair fed lean and obese pigs. Activities of G-6-PDH, ME, and CCE (expressed per 10^5 cells) were higher in the obese than lean pigs. Pyruvate kinase and fatty acid synthetase were similar for both groups.

Romsos et al. (1971) showed that alloxan diabetic pigs had reduced lipogenic activity. Restoration of lipid biosynthesis was accomplished after in vivo insulin administration. Insulin added directly to an in vitro lipogenic assay caused minimal changes. These investigators demonstrated that changes in ME and CCE activities are a result of changes in fatty acid synthesis and were not the cause of changes.

Kasser et al. (1983) measured rates of ^{14}C palmitate and ^{3}H water incorporation into fatty acids in adipose tissue slices from intact and decapitated pig fetuses. Decapitation stimulated lipid deposition and lipogenic activity (3-fold) but palmitate esterification only tended to be higher in the intact group. In vitro pancreatic insulin release was also higher in decapitated fetuses relative to intacts.

Lipid Mobilization. Degradation of triglycerides occurs through the action of hormone sensitive triglyceride lipase (HSL), the rate limiting step in adipose tissue lipolysis (Khoo and Steinberg, 1975; Siddle and Hales,

1975). It appears that total triglyceride degradation can be accomplished since HSL includes di- and monoglyceride lipase activity (Khoo et al., 1976).

Fatty acid mobilization has been estimated by measuring release of free fatty acids or glycerol from adipose tissue. Since adipose tissue does not have glycerokinase required for reutilization of glycerol, release of glycerol reflects lipolysis of triglyceride minus the amount of reesterification (Allen et al., 1976; Fain, 1982).

Lipid mobilization has been reviewed by Renold and Cahill (1965), Jeanrenaud and Hepp (1970), Scow and Chernick (1970), Bjorntorp and Ostman (1971), Fain (1973, 1977), Fain et al. (1978), Jungas (1975), Steinberg (1976), Meisner and Carter (1977), Fredholm (1978) and Hales et al. (1978).

Insulin appears to be the only peptide hormone which inhibits triglyceride breakdown. In addition, prostaglandins of the E series and adenosine inhibit lipolysis. Hormones which activate lipolysis include catecholamines, thyroid hormones, thyrotropin, growth hormone, glucocorticoids, glucagon and ACTH (Fain, 1982).

Lipolysis in isolated swine adipocytes was stimulated by epinephrine and norepinephrine, adrenocorticotropin, dibutyryl cyclic AMP but not by glucagon or cyclic AMP (Mersmann et al., 1976). A current working hypothesis is that the above hormones and factors act through binding to

surface membrane receptors, stimulation of adenylate cyclase, activation of cyclic AMP-dependent protein kinase which phosphorylates the lipase (Steinburg and Khoo, 1977).

Catecholamines interact with β_1-adrenergic receptors of adipose tissue to activate adenylate cyclase and to accelerate lipolysis. Binding to α_2 receptors inhibits adenylate cyclase and α_1 receptors increases intracellular calcium for a net effect of lipolytic inhibition (Robinson et al., 1971).

There are dramatic species differences in the sensitivity of adipose tissue to lipolytic hormones (Rudman, 1963) and to presence or absence of an alpha-adrenergic component suppressing lipolysis (Buens et al., 1981).

While lipogenic activity of swine adipose tissue was affected by amount and type of dietary fat (Allee et al., 1971a) and fasting (Mersmann et al., 1973a; Steffen et al., 1977), few studies of the effects of nutrition upon lipolytic activity have been conducted. Martin et al. (1974) reported that a low energy diet fed to neonatal pigs increased the epinephrine stimulated lipolytic response measured at 6 mo of age. Mersmann et al. (1975a) showed that high fat diets fed to 1 mo old pigs imparted greater in vitro lipolytic rates and greater sensitivity to epinephrine compared to low fat diets. Mersmann et al. (1975b) reported that weaning of pigs depressed the

lipolytic rate and sensitivity to epinephrine. Younger pigs were more sensitive to α 1 agonists than older pigs (Mersmann et al., 1976). Steffen et al. (1981) fed 1 mo old pigs isocaloric and isonitrogenous diets with either 12.8 or 2.4% fat. Adipose tissue lipolytic rates were higher in groups fed high fat but adenylate cyclase, phosphodiesterase and hormone sensitive lipase activities were unaffected. Fasting for 72 h elevated lipolysis and hormone sensitive lipase activity. Scott et al. (1981) found no differences in unstimulated lipolysis between lean and obese lines of pigs but the activity per 10^3 cells decreased from 3 to 6 mo of age. Mersmann et al. (1975a) reported a decrease with age in the ratio of fatty acid to glycerol released after epinephrine stimulation of swine adipose tissue. Etherton and Allen (1980) indicated that older swine may have higher rates of esterification. Metz and Dekker (1981) concluded that there were no differences between Large White and Pietrain in fat mobilization as indicated by plasma free fatty acids.

No other hormone may be more important in adipocyte metabolism than insulin. An increased release of fatty acids during fasting may be due to decreased plasma insulin rather than lipolytic hormones. Insulin shifts the adipocyte to lipid storage rather than lipolysis during changes from the fasted to fed states. Insulin inhibits fatty acid release by inhibiting lipolysis as well as

stimulation of reesterification of fatty acid and glycerolphosphate. Insulin plays a role in the uptake of plasma lipids by adipocytes (Fain, 1982).

Insulin inhibits adenylate cyclase and activates cyclic AMP phosphodiesterase which antagonize the effects of catecholamines (Loten and Sneyd, 1970; Hepp and Renner, 1972; Kono et al., 1975). Insulin apparently independently inhibits activation of cyclic AMP-dependent protein kinase (Walkenbach et al., 1978). Insulin also activated pyruvate dehydrogenase (Seals and Jarett, 1980), glycogen synthetase (Larner et al., 1978) and acetyl CoA carboxylase (Halestrap and Denton, 1973) by cyclic AMP independent phosphorylation of proteins or activation of phosphoprotein phosphatases. All the aforementioned effects of insulin appear to be independent of the enhanced glucose uptake even though the insulin stimulated uptake of glucose imparts increased fatty acid synthesis and reesterification (Fain, 1982). Nilsson et al. (1980) showed that insulin and epinephrine antagonize each other through dephosphorylation (inactivation) and phosphorylation (activation) of hormone sensitive lipase.

In opposition of the antilipolytic effects of insulin are growth hormone and glucocorticoids (Rao and Ramachandran, 1977; Fain, 1978). Fain (1982) indicated that both growth hormone and glucocorticoids exert permissiveness by altering adipocyte sensitivity to

activators of lipolysis. Machlin (1972) indicated the lipolytic action of growth hormone may be absent in highly purified forms. Early work showing lipolytic activity associated with growth hormone needs repeating with recombinant DNA produced growth hormone. Nevertheless, it appears that growth hormone and glucocorticoid exert a much greater in vitro lipolytic response when used in combination (Fain, 1982). Vernon (1982) reported that no stimulation of glycerol release was observed when 1 to 10^4 ng/ml concentrations of growth hormone were incubated with cultured sheep adipocytes; however, fatty acid synthesis rates were about 50% lower compared to incubations with insulin and no growth hormone indicating that growth hormone acts as an insulin antagonist for fatty acid synthesis effects.

A potent inhibitor of lipolysis appears to be the feedback of fatty acids (Rodbell, 1965). When ratios of fatty acid to albumin exceeded 2 to 3, basal and hormone stimulated lipolysis was depressed. Another potential regulation of lipolysis is adenosine since removal of it by use of adenosine deaminase elevated cyclic AMP and caused lipolysis in adipocytes (Fredholm, 1981).

Very limited data are available showing specific effects of estrogens or testosterone upon lipid metabolism. Hansen et al. (1980) injected noncastrated 200 g male and female rats intramuscularly with 1 mg of either

estradiol or progesterone and some male rats with 40 mg of testosterone 3 d prior to procurement of epididymal fat pad adipocytes. Female rats that were ovariectomized 15 d prior to slaughter were injected with 1 mg estradiol or 1 mg progesterone 3 h prior to slaughter. Complications due to estrus resulted in the study so that differences in fatty acid synthesis and epinephrine stimulated lipolysis were similar between intact males and females. However, fatty acid synthesis was higher in proestrus than in estrus or diestrus and lipolytic activity was higher in estrus compared to pro- or diestrus. Female rats injected with estradiol 3 d before slaughter had 5-fold lower fatty acid synthesis rates and almost 2-fold greater lipolytic rates on a 10^5 cell basis. Progesterone treated rats had values similar to control values. Ovariectomized female rats given estradiol before isolation of fat cells had similar magnitude of difference as the previously mentioned experiment compared to ovariectomized controls. Treatment of males with estradiol or testosterone depressed fatty acid synthesis by about 65% and increased lipolysis by about 70%. Leszczynski et al. (1982) reported elevated plasma triglycerides after feeding of .05% estradiol to young chickens. Hervey and Hutchinson (1973) indicated that when testosterone doses greater than 1 mg/d are given to castrated females, lean tissue increased but fat decreased. Laron and Kowadlo- Silbergeld (1964) had

demonstrated that after a 10 mg injection of testosterone propionate to 100 g female rats, concentrations of free fatty acids in plasma were 70% higher than control rats.

In order to separate depression of feed intake responses from true metabolic responses due to castration, Hansen et al. (1983) used rats with ventromedial hypothalamic (VMH) lesions. Compared to control rats,VMH lesioned ad libitum fed rats had increased fat deposition, increased fatty acid synthesis and LPL activity. When castrated VMH lesioned rats were compared to VMH lesioned controls, weight gain was increased but fatty acid synthesis rates were not different and lipolysis was significantly lowered by 130%.

Aloia and Field (1976) indicated that synthetic androgens, (norethandiolone) decreased the hyperglycemia induced by glucagon administration in rats but testosterone had only slight effects.

CHAPTER I

GENERAL EXPERIMENTAL DESIGN AND METHODS

The overall experimental design and general methods will be described here but are fundamental to all subsequent chapters.

The effects of testosterone upon growth and composition in pigs was first studied in a pilot study with 26 pigs. In this study, litters with five or more boars were used as one of four replicates. Each replicate was comprised of the following five treatments: boars, castrates and castrates which were implanted with testosterone filled silastic capsules designed to deliver physiologically low (prepubertal or serum concentrations of less than 1 ng/ml), intermediate (approximately 2 to 2.5 ng/ml) or high (pubertal or serum concentrations of 4 to 5 ng/ml) concentrations of testosterone. Specific silastic capsule lengths and serum testosterone concentrations are given in Chapter II. These definitions of prepubertal and pubertal groups and testosterone concentrations will be used throughout subsequent chapters. Each treatment was represented by four pigs and each replicate was comprised of littermates (or two litters of similar dam breeding and same sire mating) randomly assigned to the above five treatments or an initial slaughter group. Only pigs that

were uniform and were representative of others within each replicate were used. This pilot study was initiated at the time of castration and implantation of silastic capsules when pigs weighed 38 kg and continued for 3 wk. The pilot study was used only to evaluate growth rates, feed consumption and growth rates of selected muscles, bones and adipose tissue depots. These pigs were also used to assess treatment differences in fatty acid synthesis rates and lipoprotein lipase activity after 10 d into the study and also at slaughter. Six boars were slaughtered at the initiation of the pilot study so that changes in gross composition, and muscle, fat and bone accretion rates could be evaluated.

There were two major studies identical to each other in overall design that were used to investigate the effects of castration and administration of testosterone (TEST) or dihydrotestosterone (DHT) upon carcass composition, muscle, fat and bone growth, as well as, in vitro protein synthesis and degradation rates, crude muscle proteolytic activity and in vitro adipose tissue lipogenic and lipolytic activities. The first study involved prepubertal pigs (30 kg at initiation) and the second involved pubertal pigs (70 kg at initiation). These studies will be referred to throughout subsequent chapters as prepubertal (PREP) and pubertal (POSTP) studies. For each study, littermate (or two litters with similar dam breeding and same sire mating)

boars were randomly assigned to either be slaughtered at initiation of the study or assigned to one of the following seven treatment groups (table I-1): boars, castrates, castrates implanted with TEST or DHT filled Silastic capsules at two delivery rates designed to mimic physiologically low (prepubertal) or high (pubertal) concentration of testosterone, and lastly, castrates limited fed (the same quantities as boars). Treatments not receiving TEST or DHT were sham implanted with empty Silastic capsules. As shown in table I-1, each treatment had four pigs. Each of these two major studies was continued for 5 wk at which time they were terminated by slaughter of the pigs. In both studies, castration and implantation were performed at the initiation of the study while pigs were under general and local anesthesia. Initial slaughter groups were used to assess the accretion of muscle, fat and bone over each 5 wk period. It should be noted that initial slaughter animals and limit-fed castrates were used only for assessment of composition, and that tissue specific enzyme assays were not assessed on the pigs. In addition, limit fed castrates in the PREP study were not a part of the original design and were comprised of pigs from dissimilar litters.

All pigs were weighed and bled by venipuncture at weekly intervals. In addition, two pigs from each treatment were catheterized on d 17 in each study to obtain

TABLE I-1 EXPERIMENTAL DESIGN OF PREPUBERTAL AND PUBERTAL STUDIES[ab]

	Treatment or Group							
	Initial Slaughter Group	Castrates	Limit Fed Castrates	Boars	Dihydrotestosterone Implanted Castrates		Testosterone Implanted Castrates	
					Low	High	Low	High
Number of Pigs	4 to 6	4	4	4	4	4	4	4

[a]Prepubertal and Pubertal pigs weighed approximately 14 and 74 kg, respectively, at initiation of the respective study.

[b]Pigs were castrated and implanted at initiation of each 5 wk study.

serial blood samples over an 8 h period at 30 min intervals.

During each study, all pigs were housed in confinement at 25 C and three pigs from each treatment were individually penned to obtain feed consumption data. All pigs were fed ad libitum (except the limit-fed castrate group) an 18% crude protein corn-soybean meal diet with 1.08% lysine from weaning until termination of the studies. The added lysine provided an effective protein level equivalent to a 20% crude protein diet. Limit-fed castrates were fed twice daily the amount of feed which was the same as the average daily consumption of the boars. No antibiotics were fed or administered to pigs prior to initiation of the studies to enable usage of tetracycline marking procedures as outlined in Chapter VIII.

All pigs in a replicate (either littermates or pigs from pooled litters which were randomized into respective treatments) were slaughtered on a given day. In other words, one pig per treatment was slaughtered daily for 4 consecutive d. All pigs designated for a slaughter day were transported to the slaughter facility the night before slaughter (about 12 h) and given free access to feed and water until slaughter. All pigs were electrically stunned before exsanguination. After adipose tissue samples for lipoprotein lipase, hormone sensitive lipase, fatty acid synthesis assays and muscle strips from the left side

semitendinosus muscle for protein synthesis and degradation assays were obtained, the pigs were manually skinned. Prior to skinning, the dorsal and ventral midlines were marked to distinguish between right and left side skin. In addition, the fore and hind feet were removed. After skinning, the head was removed at the atlas-axis joint and the carcass eviscerated. Hot carcass weights were obtained to the nearest .05 kg) before splitting into right and left sides. After removal and weighing of the perirenal fat depot, the right side was measured for length, probed for fat depth at the 10th rib and then weighed. The semitendinosus (ST), pectineus (PC), longissimus dorsi (LD), triceps brachis (TB) and brachialis (BR) muscles as well as subcutaneous and perirenal adipose tissue samples for chemical analysis were removed from the left side, frozen in Dry Ice and isopentane and stored at -90 C until analyzed. The aforementioned muscles from the right side, plus the scapula, humerus, radius-ulna, femur and tibia-fibula were measured for length (to the nearest millimeter) and weighed (to the nearest .1 g). The right sides of each carcass were then separated into soft tissues and bone. Fat that remained on the skin during the skinning process was removed, weighed and added to the hot carcass weight and that which was specifically removed from the right side skin was added to the right side weight and included in the composite soft tissues. Soft tissues which

included muscle and fat was mixed and then ground twice through a .5 cm plate and subsampled. The soft tissue subsample was ground five times through a 3 mm plate and analyzed for moisture, ether extractable fat and Kjeldahl nitrogen (AOAC, 1980).

Procedures specifically addressing and pertaining to an enzyme assay will be discussed in subsequent chapters.

All data were analyzed by least squares analysis of variance using the method of unequal numbers solution to derive estimates of means and error (SAS Institute, 1977). Probability of differences between calculated means were estimated by general linear model procedures when protected by significant F tests ($P < .05$).

CHAPTER II

RELEASE RATES FROM TESTOSTERONE FILLED SILASTIC CAPSULES

Introduction

Silastic tubes filled with hormones have been used to provide a continuous release of hormone into the bloodstream (Stratton et al., 1973). Based upon in situ and in vitro experiments, estradiol, progesterone, androstenedione, testosterone and cortisol diffuse through the silastic tube membrane in a uniform and predictable manner (Dziuk and Cook, 1966; Kincl et al., 1968; Sommerville and Tarttelin, 1983). In addition, the rate of release of testosterone from silastic capsules is directly proportional to the total surface area of the silastic capsule (Dziuk and Cook, 1966; Gay and Kerlan, 1978) but not to the amount of steroid in the capsule (Dziuk and Cook, 1966; Schanbacher, 1980). Schanbacher (1980) implanted silastic capsules filled with testosterone into growing lambs and verified a continous release of physiological concentrations of testosterone.

The objectives of the present experiments were to ascertain the number of testosterone or dihydrotestosterone filled silastic capsules required to mimic physiologically low or high serum concentrations of testosterone in growing pigs.

Materials and Methods

Polydimethylsiloxane (Silastic; Dow Corning, Midland, MI) tubing (3.35 mm ID X 4.65 mm OD) was cut into various lengths (depending upon the study) and sealed at one end with Silastic adhesive (Type A) and cured for 24 h. Tubes were then packed with crystalline testosterone (17 β -hydroxy-3-0x0-4-androstene), dihydrotestosterone (5α - androstan-17 -ol-3-one) or testosterone propionate (preliminary studies) and then sealed with adhesive. Tubes were cured for 24 h before use. Prior to use, silastic capsules were soaked in absolute ethanol for 2 to 3 h and then in Nolvason surgical disinfectant for approximately 1 h.

After pigs were anesthetized with Ketamine and Rompun (general anesthesia) a local anesthetic (Lidocaine) was injected where the implant was to be inserted. The hair was clipped on the left side and the area scrubbed with Betadine solution. One centimeter incisions were made a few centimeters lateral to the left side teat line. Using a blunt-ended trocar as a needle, implants were placed subcutaneously parallel to the ribs by inserting the trocar into the ventral incision and it was exteriorized near the dorsal median plane.

From preliminary studies, the number of silastic capsules needed to maintain physiological prepubertal and postpubertal concentrations of testosterone or equivalent

concentrations of dihydrotestosterone was determined. Besides the preliminary studies, there was a pilot study involving boars, castrates and castrates implanted with three different doses of testosterone. In addition, there were two major studies (prepubertal and pubertal) and the design is described in Chapter I. In the pilot and the two major studies, weekly blood samples were obtained. For the prepubertal and pubertal studies, two pigs from each treatment were catheterized to enable serial blood sampling.

Implantation of Pilot Study Pigs. In a 3 wk long pilot study, pigs were implanted with enough testosterone filled silastic capsules to elevate serum testosterone to low, intermediate or high concentrations. At the start of the experiment pigs were castrated and implanted with three 10 cm tubes to provide a low serum concentration of testosterone (C_LTEST), ten 10 cm tubes to provide an intermediate serum concentration (C_ITEST) and eight 20 cm tubes to provide a high serum concentration of testosterone (C_HTEST). Pigs were bled immediately prior to implanting and castration and again at 1, 2 and 3 wk.

Implantation of Prepubertal and Pubertal Pigs. In the prepubertal (PREP) study, two 5.5 cm or five 10 cm capsules filled either with testosterone (TEST) or dihydrotestosterone (DHT) were implanted for the low and

high TEST or DHT groups, respectively. For the pubertal (POSTP) study, two 30 cm or nine 30 cm capsules filled with TEST or DHT were implanted for the low and high TEST or DHT groups, respectively. Boars and castrates, respectively, were implanted with empty silastic capsules. Pigs were bled immediately prior to implanting and castration, and at weekly intervals for the duration of the studies. In addition, two pigs from each treatment were catheterized about 10 d into the study and bled at 30 min intervals for 8 h.

Catheterization. Catheterization was performed in the MSU Veterinary Clinic. Pigs were given a intramuscular dose of Ketamine and Rompun and then anethesia was maintained with Helothane. Silastic tubing (2.1 mm OD X 1.9mm ID - was inserted into the anterior vena cava by directing the tubing through a 15 gauge thin wall needle used to cannulate the vein. Approximately 15 cm of the tubing was placed into the vein and the remaining tubing was directed subcutaneously along the neck with a trocar and exteriorized dorsally at the neck and then sutured to the skin. Catheters were flushed with heparinized saline twice daily. On the second day after catheterization, pigs were bled every 30 min for 8 consecutive h.

Blood Collection and Serum Preparation. Light weight pigs were restrained in a supine position and blood

collected in vacutainer tubes by vena cava puncture. In larger pigs, 20 ml syringes and 18 gauge needles were used after restraint by snaring. Separate syringes and needles were used for pigs differing in treatment, and syringes and needles were cleaned several times with heparinized saline.

Blood was allowed to clot in vacutainer tubes for approximately 1 h and then overnight in a cold room at 4 C. Clots were rimmed and serum was harvested by centrifugation at 2500 g for 30 min. Serum was stored in small culture tubes at -20 C until hormone analyses were performed.

Testosterone Determination. Serum testosterone was quantifed by radioimunoassay using MSU antitestosterone number 74 raised against testosterone-3-oxime-human serum albumin. The assay was validated by Kiser et al. (1978) and previously used for testosterone detection of boars (Kattesh et al., 1979). A detailed description of the steps involved is included in appendix A.

Results and Discussion

Preliminary Study Results. Sixteen castrated crossbred pigs were selected with eight weighing about 30 kg and eight weighing about 70 kg. Two pigs from the light weight group were implanted with one 7.5 or one 15 cm tube filled with testosterone or testosterone propionate. Two pigs from the heavy weight group were implanted with one 15 and

one 7.5 cm tube or three 15 cm tubes filled with testosterone or testosterone propionate. Pigs were weighed and bled by venapuncture for three consecutive d. Serum was prepared and assayed for testosterone. On the fifth day, pigs were reimplanted with additional tubes to increase dosage to 60, 90, 150 or 210 total centimeters of tubing. Serum was collected for three more consecutive d and assessed for testosterone concentrations. The results are presented in figure II-1 as the $cm \cdot kg^{-1}$ required to elevate testosterone to specific concentrations.

Two additional pigs each weighing about 38 kg and two pigs weighing 58 kg were used to assess release of dihydrotestosterone from silastic tubes. The 38 kg pigs were implanted with one 10 cm tube and the 58 kg pigs implanted with two 10 cm tubes filled with dihydrotestosterone. After 3 successive d of bleeding and weighing, pigs were additionally implanted with four 15 cm long tubes or with eight more 15 cm long tubes for the light weight and heavy pigs, respectively. Pigs were bled for 3 consecutive d, serum prepared and assayed for testosterone. The surface area of tubes to weight of pigs ($cm \cdot kg^{-1}$) ratio for dihydrotestosterone filled tubes relative to testosterone concentrations are shown in figure II-1.

Pilot, Pre-Pubertal and Pubertal Study Results. The least square means of testosterone concentration of initial

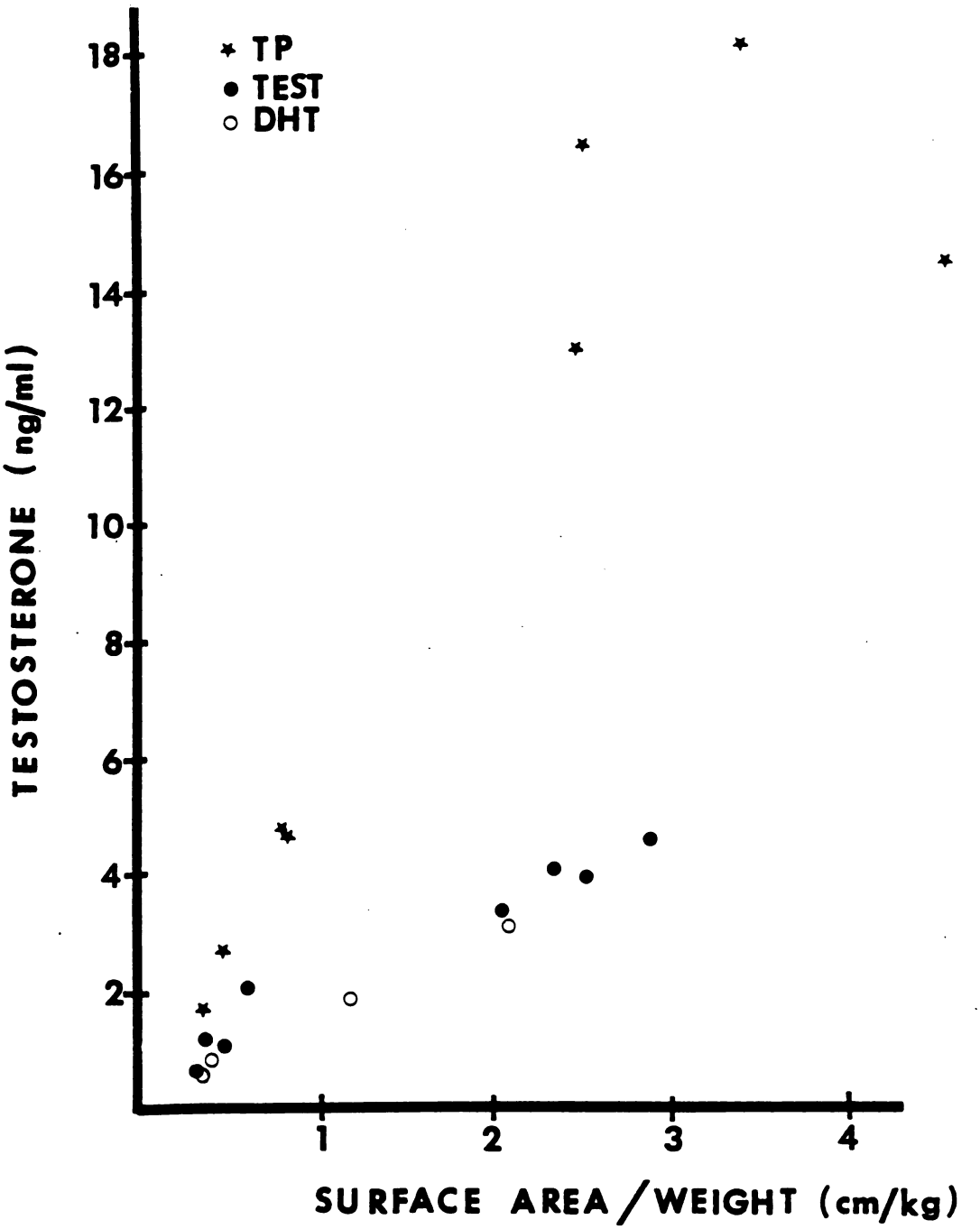


FIGURE II-1. Silastic implant surface area per kilogram body weight required to elevate serum testosterone for tubes filled with testosterone, dihydrotestosterone or testosterone propionate

TABLE II-1 SERUM TESTOSTERONE (TEST) CONCENTRATIONS IN BOARS, CASTRATES AND TESTOSTERONE IMPLANTED CASTRATES IN THE PILOT STUDY[a]

Treatment Group[b]	Sample Period			
	Initial[c]	Week 1	Week 2	Week 3
	ng/ml			
B	1.08	1.51[e]	1.67[ef]	1.40[e]
C[d]	2.10	ND	ND	ND
C_LTEST	1.53	1.37[e]	1.05[e]	.82[e]
C_ITEST	.85	4.05[f]	2.22[f]	1.94[e]
C_HTEST	.97	8.15[g]	4.48[g]	3.36[f]
SEM[e]	1.02	.86	.46	.48

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrate, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[c]Initial testosterone concentrations determined on pigs prior to castration and implantation.

[d]ND=not detectable.

[e]SEM=standard error of least squares means.

blood samples and single samplings at initiation of the study, and at 1, 2 and 3 wk for the pigs in the pilot study are presented in table II-1. There was considerable variation in the initial serum sample for testosterone concentrations as well as in serum prepared from boars at weekly intervals. Based upon the data in table II-1, differential release rates were obtained. Testosterone concentrations declined by 40, 52 and 59% from the first to the third week for the low C_LTEST, C_ITEST and C_HTEST groups, respectively. Since release rates from the tubes did not change, the increased body weight over time apparently influenced testosterone concentrations. A similar decrease in testosterone concentration was observed by Schanbacher (1980) for lambs implanted with testosterone filled silastic tubes.

For the PREP and POSTP studies, the number and length of silastic tubes were adjusted to provide a low concentration (about .6 ng/ml) or a high concentration (about 4 ng/ml) of testosterone in the pigs during wk 3 of the 5 wk periods. Potential live weight gains were approximated, and the number and length of tubes needed to achieve the targeted low and high testosterone concentrations were calculated.

Table II-2 contains the least square mean testosterone concentration for the PREP study obtained by single sampling. There were no differences among the 15 kg pigs

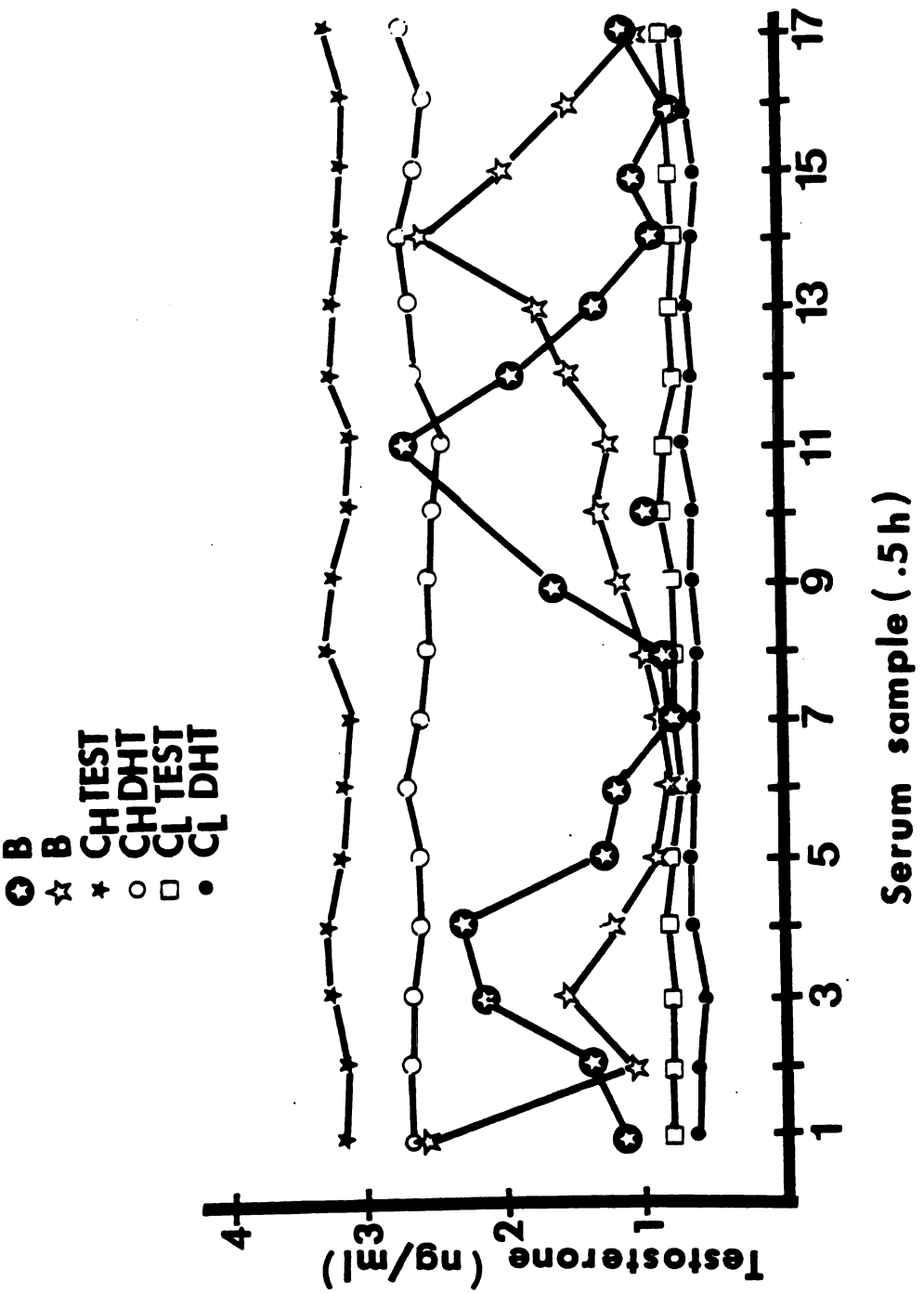
TABLE II-2 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SERUM TESTOSTERONE CONCENTRATIONS[a,b]

Treatment Group[c]	Sample Period					
	Initial[d]	Week 1	Week 2	Week 3	Week 4	Week 5
	ng/ml					
B	1.31	.90[f]	.89[f]	.83[f]	.86[f]	1.29[f]
C	1.36	.24[e]	.26[e]	.25[e]	.15[e]	.31[e]
C_LDHT	1.37	1.22[fg]	.93[f]	.70[f]	.56[g]	.52[e]
C_HDHT	1.34	4.90[h]	3.48[g]	2.59[g]	1.22[h]	.95[g]
C_LTEST	1.12	1.38[g]	.98	.73[f]	.66[fg]	.55[e]
C_HTEST	1.19	5.02[h]	4.02[h]	3.05[h]	1.31[h]	.97[g]
C_LFED	.85	.36[e]	.37[e]	.47[e]	.31[e]	.33[e]
SEM[e]	.26	.14	.09	.10	.08	.11

[a]Serum obtained from single samples.
[b]Treatment means within columns with different superscripts differ ($P < .05$).
[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[d]Initial testosterone concentration determined on pigs prior to castration and implantation.
[e]SEM=standard error of least square means.

for prepubertal testosterone. These values are consistent with those reported by Colenbrander et al. (1978) and Allrich et al. (1982). Prepubertal boars had higher testosterone concentrations than castrates and tended to be similar to low implant pigs during wk 3 of the study. Testosterone concentrations in implanted pigs were higher than expected. Testosterone concentrations declined fairly consistently for all implant groups as there was a 57, 81, 60 and 81% decrease in serum testosterone over the study for C_LDHT, C_HDHT, C_LTEST and C_HTEST groups, respectively. This decreased serum testosterone over the study was not due to complete silastic capsule depletion. However, it may be due to a combination of increased body weight and changes in metabolism and clearance of testosterone. Some pigs had indications of walling-off around the subcutaneous site of implantation but this was only observed on a few pigs. Nevertheless, high concentrations were maintained in the high implanted groups and relatively low concentrations in the low implanted groups. The testosterone concentratons of 1 boar and the mean of 2 pigs per PREP study treatment over the 8 h serial bleeding period are presented in figure II-2. Testosterone concentrations in castrates were essentially nondetectable. The important point of the data in figure II-2 is that release was constant for all implanted groups. The boar appeared to have had a peak testosterone

FIGURE II-2. Serum testosterone concentrations in high (C_H) and low (C_L) testosterone (TEST) and dihydrotestosterone (DHT) implanted pigs and two boars from the prepubertal study.


B
B
CH TEST
CH DHT
CL TEST
CL DHT
Testosterone (ng/ml)
4
3
2
1
1
3
5
7
9
11
13
15
17
Serum sample (.5h)

concentration before the start of bleeding and an apparent peak was observed (not tested statistically) by the end of the bleeding period. Base line testosterone values for these boars was about 1.5 $ng.ml^{-1}$.

The initial testosterone concentrations for boars in the POSTP study were not different among all pigs (table II-3). The overall mean of 5.2 $ng.ml^{-1}$ are similar to values of comparable age boars reported by Kattesh et al. (1979) but slightly lower than those of Allrich et al. (1982). Castration decreased testosterone concentrations to low or nondetectable concentrations. Based upon the single bleedings, pigs implanted with high levels of androgen had testosterone concentrations similar to boars until the third week bleeding at which time values were about one-half those found in boars. Testosterone concentrations decreased by 53, 82, 56 and 86% from the first week to the last week for C_LDHT, C_HDHT, C_LTEST and C_HTEST groups, respectively. These decreases are similar to those found for PREP implanted pigs. However, the PREP pigs doubled in weight over the 5 wk study but the POSTP pigs only increased in weight by about 40%. Figure II-3 shows the release of testosterone from 2 implanted pigs per treatment and 1 boar from the POSTP study over an 8 h period. This figure illustrates the episodic release of testosterone from boars but a constant release from silastic capsules.

TABLE II-3 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SERUM TESTOSTERONE CONCENTRATIONS[a,b]

Treatment Group[c]	Sample Period					
	Initial[d]	Week 1	Week 2	Week 3	Week 4	Week 5
	ng/ml					
B	4.10	5.41^{g}	3.83^{f}	4.86^{g}	4.37^{g}	2.84^{f}
C	5.21	.42^{e}	.37^{e}	.27^{e}	.23^{e}	.30^{e}
C_LDHT	5.21	1.18^{ef}	1.01^{e}	.79^{e}	.81^{e}	.55^{e}
C_HDHT	7.20	4.98^{g}	3.29^{f}	2.19^{f}	1.68^{f}	.89^{e}
C_LTEST	6.04	1.36^{f}	1.04^{e}	.85^{e}	.97^{e}	.60^{e}
C_HTEST	3.79	5.07^{g}	3.77^{f}	2.30^{f}	1.45^{f}	.73^{e}
C_LFED	4.84	.25^{e}	.33^{e}	.43^{e}	.47^{ef}	.44^{e}
SEM[e]	1.27	.30	.43	.26	.47	.26

[a] Serum obtained from single samples.
[b] Treatment means within columns with different superscripts differ ($P < .05$).
[c] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[d] Initial testosterone concentrations determined on pigs prior to castration and implantation.
[e] SEM=standard error of least square means.

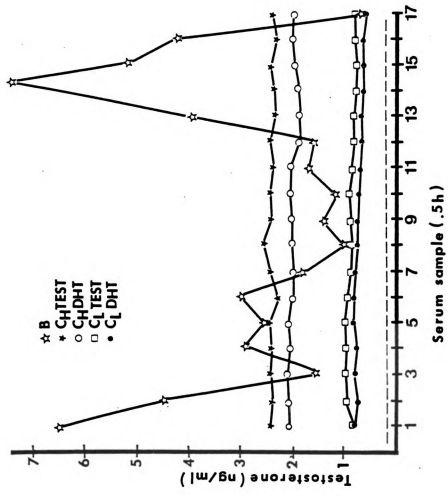


FIGURE II-3. Serum testosterone concentrations in high (C_H) and low (C_L) testosterone (TEST) and dihydrotestosterone (DHT) implanted pigs and one boar from the pubertal study.

Silastic capsules provided a suitable mode of testosterone delivery in the pigs of this study. However, the constant release of testosterone by this method relative to the natural episodic release must be kept in mind.

CHAPTER III

EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGENS ON BODY GROWTH AND CARCASS COMPOSITION

Introduction

The composition advantages of boars relative to male castrates are well documented (Walstra and Kroeske, 1968; Wismer-Pederson, 1968; Field, 1971; Seidemann et al., 1982). In general the effects of castration upon growth rate in swine are not as dramatic as observed in cattle, sheep (Seideman et al., 1982) or rodents (Kochakian, 1976). Administration of testosterone to rats and guinea pigs enhances nitrogen balance (Kochakian, 1976), and in rats definite changes in lipid metabolism were observed (Laron and Kowaldo-Silbergeld, 1964; Aloia and Field, 1976; Wilson et al., 1976; Hansen et al., 1980). Attempts to alter swine composition via exogenous androgens have had limited success (Galbraith and Topps, 1981).

In order to study the effects of testosterone on either muscle or adipose tissue metabolism these effects should be correlated to detectable changes in overall growth and composition. Because the responses to testosterone are subtle in swine, this study was designed to examine changes in adipose tissue growth and composition. Since it was

hypothesized (Wade and Gray, 1979) that the effects of testosterone upon adipose tissue are mediated through its aromatized metabolites, estrone and estradiol 17β , we chose to administer a nonaromatizable form of testosterone (dihydrotestosterone) as well as testosterone to prepubertal and pubertally castrated male pigs.

Materials and Methods

Soft tissue subsamples from the right side of carcasses were analyzed for moisture, ether extractable lipid and Kjeldahl nitrogen (AOAC, 1980). Protein was determined by multiplying nitrogen by 6.25. After subtracting the soft tissue weight (STW) and total bone weight (TBW) from the right side weight (RSW) it was apparent moisture losses (approximately .5 kg) had occurred. It was decided to attribute these moisture losses totally to either muscle or fat. Based upon the relative moisture content of subcutaneous (SQ) and composite muscle (CM) samples as determined by:

Equation 1.
$$\frac{\text{SQ Moisture}}{\text{SQ moisture + CM moisture}}$$

Equation 2.
$$\frac{\text{CM Moisture}}{\text{SQ moisture + CM moisture}}$$

approximately 20% of the moisture loss was added to a calculated fat weight and the remaining moisture loss added to a calculated fat free muscle weight. Total soft tissue fat was calculated by multiplying the analyzed percentage ether extractable fat by the soft tissue weight. The calculated fat was then subtracted from the STW to arrive at fat free muscle after correcting for the moisture losses. The corrected carcass fat (CF) and fat-free muscle (FFM) weights were then divided by the right side weight to determine relative percentages of carcass FFM and fat. These percentages were then multiplied by the hot carcass weight to obtain total carcass fat or total carcass fat-free muscle. These totals were then divided by live weights to derive fat and FFM percentages of live body weight.

Results and Discussion

Growth and Feed Efficiency. The initial body weights (IBW), final body weights (FBW), average daily gain (ADC), feed intake (TFI) and feed to gain ratio (F/G) of pigs in the pilot study are shown in table III-1. While there were no differences in IBW, FBW or ADG, there was a trend for testosterone (TEST) implanted pigs to have depressed growth rates. Even over this short 3 wk period, boars consumed 15% less feed than castrates and converted feed into body gain with over 15% greater efficiency. Feed intake was

TABLE III-1 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON BODY GAINS, FEED INTAKE AND FEED EFFICIENCY[a,b]

Treatment Group[c]	Initial Body Weight (kg)	Final Body Weight (kg)	Average Daily Gain (g/d)	Feed/Gain	TFI (kg)
B	40.3	59.8	930.3	2.41[e]	47.1[e]
C	36.5	55.9	821.2	2.85[g]	55.2[f]
C_LTEST	37.0	55.0	857.8	2.70[fg]	48.6[e]
C_ITEST	38.7	56.4	842.3	2.74[fg]	48.4[e]
C_HTEST	38.2	55.9	840.9	2.55[ef]	45.0[e]
SEM[d]	1.9	1.4	70.0	.10	1.6

[a]Gain and feed intake over 3 wk period of pilot study.

[b]Treatment means within column with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[d]SEM=standard error of least squares means.

also depressed by 12 to 18% in the TEST implanted castrates. There were no differences in feed conversion among TEST implanted castrates but the high implanted (C_HTEST) group was 10.5% more efficient than castrates. Corresponding data from the prepubertal (PREP) and pubertal (POSTP) studies are presented in tables III-2 and 3, respectively. In neither of these two studies did IBW differ. Pigs implanted with high dihydrotestosterone (C_HDHT), low testosterone (C_LTEST) and high testosterone (C_HTEST) in the PREP study grew more slowly than boars, castrates, castrates implanted with low dihydrotestosterone (C_LDHT) or limit-fed castrates (C_LFED). Over the 5 wk duration of the PREP study, boars consumed 23% less feed and were 23% more efficient in conversion of feed to gain than castrates. Testosterone but not DHT caused a feed intake depression. In fact, C_HTEST pigs voluntarily consumed 38% less feed than castrates but were not different from boars in feed efficiency.

In the POSTP study (table III-3), there were no differences in IBW or FBW between treatments. However, boars tended to be faster growing (10 to 19%) than castrates or C_LFED pigs. Dihydrotestosterone implanted pigs grew more slowly than boars or C_LTEST pigs ($P < .06$) and tended to be slower growing than all other treatments. Boars and testosterone implanted pigs consumed

TABLE III-2 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON BODY GAIN FEED INTAKE, AND FEED EFFICIENCY[a,b]

Treatment Group[c]	Initial Body Weight (kg)	Final Body Weight (kg)	Average Daily Gain (g/d)	Feed/Gain	TFI (kg)
B	14.99	39.63^{f}	703.6^{f}	1.94^{e}	47.41^{f}
C	14.98	40.13^{f}	715.9^{f}	2.51^{fg}	61.88^{h}
C_LDHT	15.21	39.65^{f}	695.9^{f}	2.45^{fg}	59.01^{h}
C_HDHT	14.64	35.71^{e}	600.8^{e}	2.68^{g}	56.54^{bg}
C_LTEST	14.88	36.40^{e}	613.7^{e}	2.35^{f}	51.55^{fg}
C_HTEST	15.05	37.42^{e}	636.2^{e}	1.86^{e}	38.46^{e}
C_LFED	15.59	40.61^{f}	714.5^{f}	1.93^{e}	47.47^{f}
SEM[d]	.58	1.10	24.5	.09	2.28

[a]Gain and feed intake over 5 wk period.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrate, C_L=low implants, C_I,intermediate implants, C_H=high implants.

[d]SEM=standard error of least squares means.

TABLE III-3 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON BODY GAINS, FEED INTAKE AND FEED EFFICIENCY[a,b]

Treatment Group[c]	Initial Body Weight (kg)	Final Body Weight (kg)	Average Daily Gain (g/d)	TFI (kg)	Feed/Gain
B	74.85	105.78	881.1^{f}	78.7^{e}	$3.42^{e,f}$
C	73.56	101.74	$794.9^{e,f}$	$91.15^{g,h}$	4.30^{h}
C_LDHT	75.11	99.61	700.7^{e}	$81.08^{f,g}$	3.62^{f}
C_HDHT	75.67	100.67	715.7^{e}	94.95^{h}	3.93^{g}
C_LTEST	74.54	104.30	861.1^{f}	$81.75^{e,f}$	3.40^{e}
C_HTEST	72.74	100.35	$775.0^{e,f}$	$75.4^{e,f}$	3.39^{e}
C_LFED	75.17	100.31	715.0^{e}	$79.3^{e,f}$	3.41^{e}
SEM[d]	2.08	2.50	46.9	2.32	.06

[a]Gain and feed intake over 5 wk period.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[d]SEM=standard error of least squares means.

approximately 14% less feed than castrates and about 3 to 16% less feed than the C_LDHT and C_HDHT groups, respectively. Boars and testosterone implanted pigs had 20% lower feed to gain ratios indicative of more efficient feed conversion. Dihydrotestosterone implanted pigs in the POSTP study were intermediate to boars and castrates for feed to gain ratios but were less efficient than testosterone implanted pigs. In a longitudinal growth study of pigs reported by Walstra (1980) castrates tended to consume more feed per day than either boars or gilts up to 6 mo of age. Maximum growth rates for boars were reached between 4.5 and 6 mo while castrates attained maximum growth rates between 3 and 4.5 mo of age. In addition, Newell and Bowland (1972) and Walstra (1980) found that boars required approximately 12% less feed per kg gain than castrates when fed ad libitum.

Boars voluntarily consumed less feed than castrated male pigs in other reported work (Pay and Davies, 1973; Campbell and King, 1982) but boars normally gain at rates equal to or faster than castrates (Wood and Riley, 1982). Bratzler et al. (1954) observed an 8% higher ADG for testosterone implanted castrates but no difference in feed efficiency relative to castrates. The data in the present study confirm data of Walstra (1980) and Wood and Riley (1982) in that boars grew equally as well as castrates and had enhanced feed efficiencies. The observed decreased

feed intake of boars and testosterone implanted pigs but not in dihydrotestosterone implanted pigs relative to castrates suggests a role of testosterone or its aromatized metabolite in regulation of feed intake in pigs. This is supported by tendencies of female pigs to consume less feed than castrates and increase feed intake when they are castrated (Rerat, 1976).

The growth data for the C_LFED pigs of this study are not consistent with the data of Campbell and King (1982) in which castrates were restricted fed to 82% of ad libitum amounts of castrates. In that study, restriction caused a 10% depression in daily gain. The C_LFED pigs of the present study indicated no large restrictions in daily gain relative to ad libitum fed castrates. One possible explanation is that the C_LFED pigs in this study were restricted to the voluntary intake of boars but were still consuming adequate amounts of nutrients. A more plausible explanation for the lack of any growth depression is that some of the pigs used in PREP C_LFED group were from litters not represented elsewhere in the study and since litter and treatment were used to calculate least squares means the genetic difference in growth potential of these pigs was detected.

Carcass Length, Fat Depth and Longissimus Muscle Area.

Carcass length (CL), fat depth at the tenth rib (FD) and longissimus muscle area (LMA) are given in tables III-4, 5

and 6 for the pilot, PREP and POSTP studies respectively. In the pilot study, boars and C_LTEST treated pigs had slightly longer (5%) carcasses than castrates. However, neither PREP nor POSTP studies showed any statistical differences between boars and castrates but PREP castrates tended to be longer (nonsignificant) than boars. The trend for boars to be longer than castrates found in the pilot and POSTP studies are consistent with data of Knudson (1983) in which boars were about 3% longer than castrates at 105 kg live weight. Walstra (1980) and Wood and Riley (1982) found no differences in carcass length between boars and castrates. As indicated by Brannang (1971) the age of castration can influence differences observed in skeletal growth and that vertebrae lengths were reduced in steers compared to bulls.

Based upon FD measurements alone in the 3 wk pilot study, castration was without effect. However, there was a trend for low and intermediate testosterone treated pigs to have 8 to 10% lower (nonsignificant) FD than castrates and the C_HTEST pigs had 25% lower FD than castrates. In the PREP and POSTP studies, boars had 35 to 43% less subcutaneous fat at the tenth rib. This is consistent with the 45% reduction in backfat at the tenth rib recently reported for boars compared to castrates (Knudson, 1983). Walstra (1980) also reported that boars had over 20% less backfat than castrates. Prepubertal and pubertal

TABLE III-4 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON CARCASS LENGTH, FAT DEPTH AT TENTH RIB AND LONGISSIMUS MUSCLE AREA[a,b]

Treatment Group[c]	Carcass Length (cm)	Fat Depth (cm)	LMA[e] (cm^2)
B	73.50^{h}	$.92^{h}$	21.68
C	69.45^{g}	$.94^{h}$	19.97
C_LTEST	73.01^{h}	$.84^{h}$	21.59
C_ITEST	$71.16^{g,h}$	$.86^{h}$	21.52
C_HTEST	$72.60^{g,h}$	$.70^{g}$	20.43
SEM[f]	1.20	.08	.76
Initial Slaughter Group	63.15	.69	13.23

[a]Pilot study data.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[d]Fat depth excludes skin.

[e]LMA-longissimus muscle area.

[f]SEM=standard error of least squares means.

TABLE III-5 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON CARCASS LENGTH, FAT DEPTH AT THE TENTH RIB AND LONGISSIMUS MUSCLE AREA[a]

Treatment Group[b]	Carcass Length (cm)	Fat Depth (cm)	LMA[d] (cm^2)
B	$62.8^{f,g}$	$.51^{f}$	$17.4^{f,g}$
C	63.8^{g}	$.90^{g}$	$17.3^{f,g}$
C_LDHT	$62.1^{f,g}$	$.90^{g}$	18.5^{g}
C_HDHT	$62.0^{f,g}$	$.71^{f,g}$	15.4^{f}
C_LTEST	61.2^{f}	$.67^{f}$	16.3^{g}
C_HTEST	$62.4^{f,g}$	$.64^{f}$	18.5^{g}
C_LFED	63.7^{g}	$.60^{f}$	20.5^{g}
SEM[e]	.778	.076	1.002
Initial Slaughter Group	45.9	.34	7.08

[a]Least square means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Fat depth excludes skin.

[d]LMA=longissimus muscle area.

[e]SEM=standard error of least square means.

TABLE III-6 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON CARCASS LENGTH, FAT DEPTH AT TENTH RIB AND LONGISSIMUS MUSCLE AREA[a]

Treatment Group[b]	Carcass Length (cm)	Fat Depth (cm)	LMA[d] (cm^2)
B	86.51	1.66^{f}	$43.76^{g,h}$
C	85.33	$2.57^{g,h}$	33.51^{f}
C_LDHT	84.17	$1.94^{f,g,h}$	$38.76^{f,g,h}$
C_HDHT	84.43	$1.76^{f,g,h}$	$36.11^{f,g}$
C_LTEST	85.34	1.63^{f}	$37.79^{f,g,h}$
C_HTEST	85.89	1.41^{f}	41.18^{g}
C_LFED	85.69	2.16^{h}	33.47^{f}
SEM[e]	.90	.26	2.13
Initial Slaughter Group	76.95	1.24	24.57

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implant, C_H=high implant, C_LFED=limit-fed castrates.

[c]Fat depth does not include skin.

[d]LMA=longissimus muscle area.

[e]SEM=standard error of least squares means.

testosterone implanted castrates in the present study also had 25 to 29% and 36 to 45% less fat at the tenth rib relative to castrates for C_LTEST and C_HTEST pigs of each study, respectively. Even though C_HTEST pigs of each study tended to have less FD than the castrates or C_LDHT, there was a trend for dihydrotestosterone implanted pigs to have greater FD than boars or testosterone treated pigs. Limit-feeding castrates to the ad libitum consumption rates of boars reduced FD by 33% in PREP pigs ($P < .05$) and by 16% ($P < .05$) POSTP pigs relative to castrate values. It appears that restriction of feed intake alone was inadequate in reducing fat depth to the same magnitude as observed in boars or testosterone implanted pigs.

Longissimus muscle area is often used as an indicator of muscling. Many studies have shown larger LMA for boars compared to castrates (Blair and English, 1965; Pay and Davies, 1973; Siers, 1975). There were no differences in LMA of pigs in the pilot study or in the study with the PREP pigs. However, POSTP boars and C_HTEST pigs had 31 and 23% larger LMA, respectively, than castrates. Limit-feeding castrates appeared to inhibit LMA in POSTP but not in PREP pigs. Based upon LMA, no definitive statements can be made about the effects of castration or exogenous testosterone upon muscling. This may relate to the relative insensitivity of LMA mesurements for detecting small muscle area differences.

Carcass Weights and Soft Tissue Composition. Hot carcass weights (HCW), right side weights (RSW), total soft tissues dissected from the skinless right side (TSTW) and proximate analysis of composite soft tissues are given in tables III-7, 8 and 9 for the pilot, PREP and POSTP studies, respectively. Walstra (1980) indicated that boars tended to have less combined muscle and fat than castrates. No consistent trends in HCW, RSW or TSTW were observed in this study except that PREP C_HDHT pigs had reduced RSW and TSTW relative to other treatments and that PREP C_LFED pigs had heavier carcasses and more TSTW than other treatments. This observation indicates that limit-feeding of high protein diets as used here may not be growth limiting over the 15 to 40 kg growth period. Over a 20 to 45 kg growth period in which intake of a 17% crude protein diet was limited to 75% of ad libitum castrate values, carcass weights were not different (Campbell and King, 1982).

In soft tissues of pigs from the pilot study, boars had 7% higher (1.14% absolute difference) concentrations of protein than castrates, but no differences in percentage moisture or ether extractable lipid between treatments were detected. In the PREP and POSTP studies, boars had higher percentages of moisture (4 and 10%, respectively) and lower percentages (12 and 26%, respectively) of ether extractable lipid than castrates. Prepubertal boars did not differ but

TABLE III-7 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON HOT CARCASS, RIGHT SIDE AND SOFT TISSUE WEIGHTS AND COMPOSITION OF THE SOFT TISSUES[a,b]

Treatment Group[c]	HCW[d,e] (kg)	RSW[d,e] (kg)	TSTW[d] (kg)	Soft Tissue Composition (%)		
				Moisture	Protein	Fat
B	35.63	18.17	15.24[h]	63.82	18.28[h]	17.22
C	35.18	16.11	13.14[g]	64.05	17.14[g]	18.15
C_LTEST	35.94	17.12	14.15[g,h]	63.98	17.22[g]	18.18
C_ITEST	34.27	17.20	13.87[g,h]	63.05	16.82[g]	19.38
C_HTEST	33.60	17.01	13.77[g,h]	64.92	17.08[g]	17.25
SEM[f]	1.13	.99	.91	.74	.31	.84
Initial Slaughter Group	20.42	10.00	8.13	66.52	17.40	15.13

[a] Pilot study.
[b] Treatment means within columns with different superscripts differ ($P < .05$).
[c] B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.
[d] HCW=hot carcass weight, RSW=right side weight, TSTW=total soft tissue weight.
[e] Carcass weights exclude head, skin and feet.
[f] SEM=standard error of least squares means.

TABLE III-8 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON HOT CARCASS, RIGHT SIDE AND SOFT TISSUE WEIGHTS AND COMPOSITION OF THE SOFT TISSUES[a]

Treatment Group[b]	HCW[c,d] (kg)	RSW[c,d] (kg)	TSTW[c] (kg)	Soft Tissue Composition (%)		
				Moisture	Protein	Fat
B	$20.90^{f,g}$	$10.45^{g,h}$	8.36^{h}	64.77^{h}	16.01^{f}	17.91^{f}
C	21.65^{g}	10.18^{fgh}	$7.80^{f,g,h}$	$62.34^{f,g}$	16.04^{f}	$20.43^{g,h}$
C_LDHT	$22.11^{g,h}$	$10.79^{h,i}$	8.70^{h}	61.37^{f}	15.98^{f}	21.34^{h}
C_HDHT	18.93^{f}	$9.13^{f,g}$	7.10^{f}	$63.68^{g,h}$	16.11^{f}	$19.05^{f,g}$
C_LTEST	$20.35^{f,g}$	9.93^{fgh}	$7.73^{f,g,h}$	$62.42^{f,g}$	16.62^{f}	$20.66^{g,h}$
C_HTEST	$20.11^{f,g}$	10.52^{g}	$8.08^{f,g,h}$	$63.91^{f,g}$	16.60^{f}	$18.81^{f,g}$
C_LFED	24.45^{h}	12.02	10.09^{i}	$62.64^{f,g}$	17.70^{g}	$19.09^{f,g}$
SEM[e]	.81	.38	.34	.56	.36	.70
Initial Slaughter Group	7.39	3.50	2.60	13.47	68.82	16.71

[a]
[b]Treatment means within columns with different superscripts differ ($P < .05$).
[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[d]HCW=hot carcass weight, RSW=right side weight, TSTW=total soft tissue weight.
[e]Carcass weights exclude head, skin and feet.
SEM=standard error of least squares means.

TABLE III-9 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON HOT CARCASS, RIGHT SIDE AND SOFT TISSUE WEIGHTS AND COMPOSITION OF THE SOFT TISSUES[a]

Treatment Group[b]	HCW[cd] (kg)	RSW[cd] (kg)	TSTW[c] (kg)	Soft Tissue Composition (%)		
				Moisture	Protein	Fat
B	68.94	34.31	28.59	61.63^{ac}	17.23^{ac}	20.16^{a}
C	64.82	32.36	27.07	55.95^{b}	15.40^{b}	27.07^{bc}
C_LDHT	64.06	32.34	26.82	58.39^{b}	16.23^{bc}	24.14^{b}
C_HDHT	67.15	33.33	27.59	57.92^{b}	16.28^{abc}	24.57^{bc}
C_LTEST	68.74	34.70	28.63	58.55^{ab}	16.11^{bc}	24.16^{bc}
C_HTEST	60.85	32.25	27.37	63.24^{c}	16.91^{cd}	18.35^{a}
C_LFED	64.87	32.20	26.79	55.57^{b}	16.10^{bd}	27.73^{c}
SEM[e]	2.76	1.44	1.20	1.03	.31	1.28
Initial Slaughter Group	42.63	21.27	15.83	62.90	16.84	19.96

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]HCW=hot carcass weight, RSW=right side weight, TSTW=total soft tissue weight.

[d]Carcass weights exclude head, skin and feet.

[e]SEM=standard error of least squares means.

POSTP boars had 12% higher ($P < .05$) protein in soft tissue relative to castrates. Pubertal C_HTEST pigs also tended to have higher percentages of moisture, protein and less lipid than castrates. Soft tissue composition of POSTP pigs presented in table III-9 are higher in fat and lower in moisture than data presented by Fortin et al. (1983) but the trend of higher moisture and less fat in boars compared to castrates coincides between that study and data presented here. The relatively constant percentage protein, increased percentage lipid and decreased moisture observed in a comparison of PREP pigs to POSTP pigs is consistent with the changes in body composition with increasing body weight reported by McMeekan (1940).

Carcass Fat-Free Muscle, Fat and Bone. The method of calculating fat free muscle (FFM) and carcass fat (CF) were described in the methods section of this chapter. Tables III-10, 11 and 12 summarize the FFM, CF and bone totals in the carcasses of the pilot, PREP and POSTP studies, respectively. No difference in FFM, fat or bone were observed in the 3 wk pilot study. However, boars at this early stage (55 to 60 kg) tended to have slightly greater FFM and bone and less CF than castrates. All three testosterone groups tended to have less CF than castrates.

Slightly different results were obtained for the PREP and POSTP pigs (tables III-11 and 12). PREP boars were not different from castrates in FFM or bone but they had 15%

TABLE III-10 EFFECTS OF CASTRATION AND ADMININISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON TOTAL CARCASS FAT-FREE MUSCLE, FAT AND BONE[a]

Treatment Group[b]	Fat Free Muscle[c] (kg)	Fat[c] (kg)	Bone[c] (kg)
B	25.03	5.21	5.39
C	24.56	5.51	5.11
C_LTEST	25.31	5.29	5.35
C_ITEST	24.10	4.91	5.26
C_HTEST	23.71	4.92	4.97
SEM[d]	1.56	.54	.10
Initial Slaughter Group	14.21	2.54	3.66

[a]Pilot study pigs.

[b]B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[c]Fat-free muscle (FFM) and fat derived from soft tissue of right side and percentages of side multiplied by hot carcass weight.

[d]SEM=standard error of least squares means.

TABLE III-11 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL CARCASS FAT-FREE MUSCLE, FAT AND BONE[a]

Treatment Group[b]	Fat-Free Muscle[c] (kg)	Fat[c] (kg)	Bone[c] (kg)
B	13.93^{ef}	3.05e	3.91f
C	14.14^{ef}	3.61^{fg}	3.89^{f}
C_LDHT	14.56^{f}	3.94^{g}	3.61^{ef}
C_HDHT	12.74^{e}	3.04^{e}	3.22^{e}
C_LTEST	13.26^{ef}	3.46^{eg}	3.64^{ef}
C_HTEST	13.31^{e}	3.11^{ef}	3.73^{f}
C_LFED	16.68^{g}	3.98^{g}	3.40^{ef}
SEM[d]	.67	.18	.16
Initial Slaughter Group	4.91	.79	1.69

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrate, C_L=low implant, C_H=high implant, C_LFED=limit-fed castrates.

[c]Fat-free muscle (FFM) and fat derived from soft tissue of right side and percentage of right side multiplied by hot carcass weight.

[d]SEM=standard error of least square means.

TABLE III-12 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL CARCASS FAT-FREE MUSCLE, FAT AND BONE[a]

Treatment Group[b]	Fat-Free Muscle[c] (kg)	Fat[c] (kg)	Bone[c] (kg)
B	47.56^{g}	11.94^{ef}	9.43^{f}
C	41.68^{e}	15.03^{g}	8.11^{e}
C_LDHT	42.73^{ef}	13.28^{fe}	8.05^{e}
C_HDHT	44.69^{efg}	14.18^{fg}	8.28^{e}
C_LTEST	46.21^{fg}	14.30^{fg}	8.23^{e}
C_HTEST	42.73^{ef}	9.58^{e}	8.54^{e}
C_LFED	41.25^{ef}	15.39^{g}	8.23^{e}
SEM[d]	1.65	1.04	.11
Initial Slaughter Group	29.26	7.15	6.22

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implant, C_H=high implant, C_LFED=limit-fed castrates.

[c]Fat-free muscle (FFM) and fat derived from soft tissue of right side and percentage of right side multiplied by hot carcass weight.

[d]SEM=standard error of least squares means.

less CF. Pubertal boars had 14% more FFM, 21% less CF and 16% more bone than castrates. Others have shown that boars have greater muscle and bone but less fat than castrates at typical market weights (Prescott and Lamming, 1967; Walstra, 1980; Wood and Riley, 1982). With the exception of POSTP C_LTEST pigs which had 11% more FFM, no definite effects of testosterone or dihydrotestosterone administration were detected in either the PREP or POSTP study reported here. Limit-feeding PREP castrates had a positive influence upon FFM accretion as these pigs had almost 20% more FFM than boars. Limit-feeding of POSTP castrates did not reduce FFM accretion relative to ad libitum fed castrates but did result in 13% less FFM relative to boars. Walstra (1980) reported that the amounts of muscle in carcasses from both boars and castrates were increased when fed 70% of ad libitum. However, part of these observed increases may be attributable to the fact that pigs were older when slaughter weights were attained.

Aside from boars in the PREP study, only C_HDHT and C_HTEST pigs had less CF than castrates and they were similar to boars. In the POSTP study, C_HTEST depressed fat accretion by 36% relative to castrates but the other implant groups were intermediate to boars and castrates. Limit-feeding was ineffective in depressing carcass fat accretion relative to ad libitum fed castrates. This

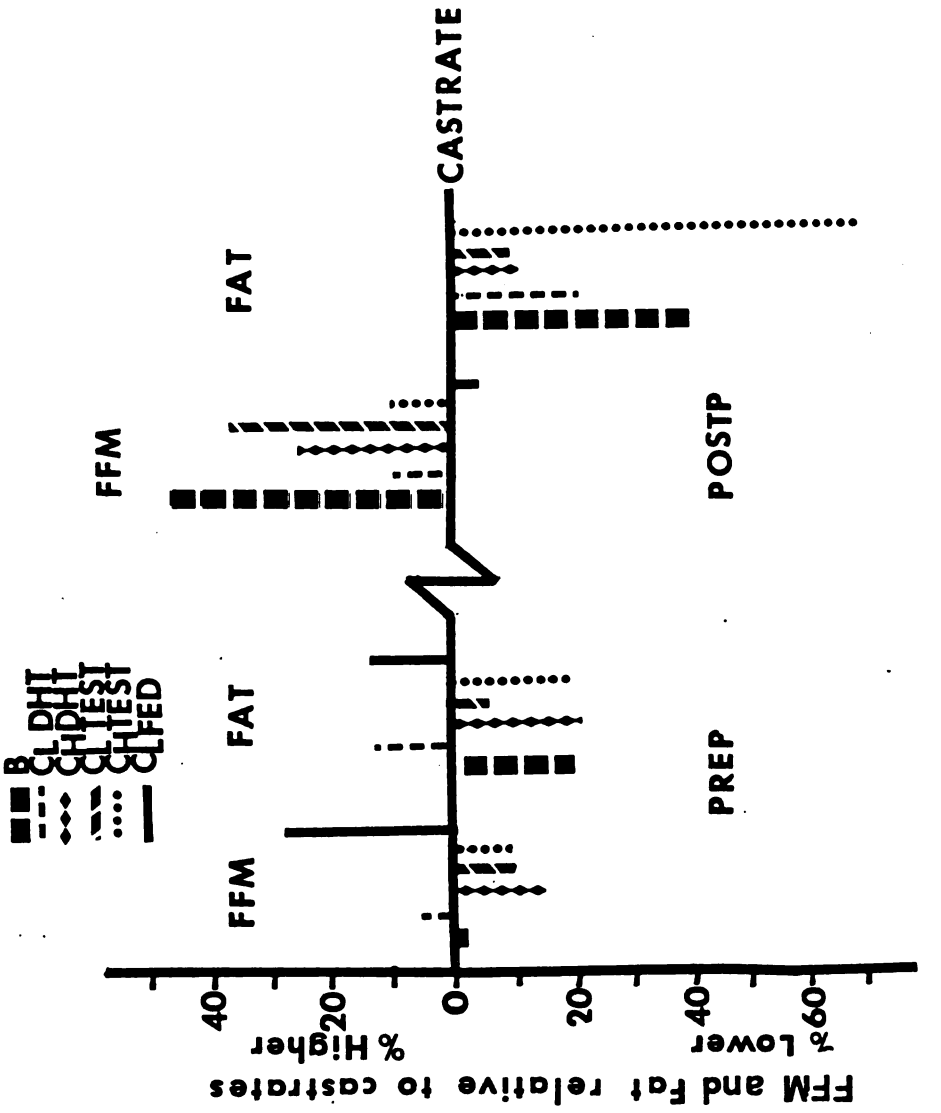


FIGURE III-1. Fat-free muscle and fat in carcasses of boars and androgen implanted castrates as a percentage of castrates.

observation substantiates the hypothesis that testosterone alters fat deposition by mechanisms other than feed intake depression (Wade and Gray, 1979). Bone accretion was relatively unaffected by either form of androgen treatment or limit-feeding (further data on bone in Chapter VIII). Tables III-10, 11 and 12 also present the data derived from the initial slaughter pigs. Accretion of FFM, CF or bone can be derived by subtracting the initial values from the treatment means. However, the trends remain the same since this was a subtraction of a constant from all treatments.

When the FFM, CF and bone data in tables III-10, 11 and 12 are divided by HCW or live weight, the percentages of FFM, CF or bone are derived. These percentages for the pilot, PREP and POSTP studies are presented in tables III-13, 14 and 15, respectively. In the pilot study no consistent trends in percentage FFM or bone were detected but castration depressed percentage fat by 6 and 12% on a carcass and live weight basis, respectively. Testosterone also tended to result in lower fat percentages on a carcass and live weight basis. In the PREP and POSTP studies no new trends between treatments were apparent when data were expressed as a percentage of carcass or live weight.

Subcutaneous and Perirenal Adipose Tissue Composition. Subcutaneous (SQ) and perirenal (PR) adipose tissue were analyzed for moisture, ether extractable lipid and protein

TABLE III-13 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON THE PERCENTAGE OF FAT-FREE MUSCLE, FAT OR BONE OF THE CARCASS OR LIVE WEIGHT[ab]

Treatment Group[c]	% of Carcass[d]			% of Live Weight		
	FFM[e]	Fat[f]	Bone	FFM[e]	Fat[f]	Bone
B	70.24	14.63[h]	15.03[i]	41.85[h]	8.72[hi]	9.14
C	69.80	15.57[i]	14.53[h]	43.92[hi]	9.87[j]	9.01
C_LTEST	70.41	14.71[hi]	14.88[hi]	46.01[i]	9.61[hij]	9.72
C_ITEST	70.31	14.33[h]	15.36[i]	42.72[h]	8.71[hi]	9.33
C_HTEST	70.57	14.65[hi]	14.78[hi]	42.42[h]	8.80[h]	8.82
SEM[g]	.45	.43	.10	1.15	.42	.50
Initial Slaughter Group	69.61	12.45	17.94	38.26	6.84	9.86

[a]Pilot study.

[b]Treatment means within columns with different superscripts differ ($P < .06$).

[c]B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[d]Carcass defined as headless, skinless and feet removed.

[e]FFM=fat-free muscle.

[f]Fat includes subcutaneous, intra- and intermuscular depots.

[g]SEM=standard error of least squares means.

TABLE III-14 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON THE PERCENTAGE OF FAT-FREE MUSCLE, FAT OR BONE OF THE CARCASS OR LIVE WEIGHT[a]

Treatment Group[b]	% of Carcass[c]			% of Live Weight		
	FFM[d]	Fat[e]	Bone	FFM[d]	Fat[e]	Bone
B	66.66^{g}	14.61^{g}	18.73^{h}	31.15^{g}	7.71^{g}	9.88^{g}
C	65.35^{g}	16.70^{hj}	17.95^{h}	35.25^{g}	9.01^{hij}	9.68^{h}
C_LDHT	65.85	17.84^{j}	16.37^{gh}	36.72^{g}	9.95^{k}	9.10^{gh}
C_HDHT	67.06^{g}	16.02^{gh}	16.92^{h}	35.69^{g}	8.52^{gh}	9.00^{gh}
C_LTEST	65.14^{g}	16.98^{hj}	17.88^{h}	36.43^{g}	9.49^{ik}	9.99^{h}
C_HTEST	66.17^{g}	15.44^{gi}	17.88^{h}	35.56^{g}	8.30^{ghi}	9.88^{h}
C_LFED	69.35^{h}	16.53^{hij}	14.12^{g}	41.07^{h}	9.79^{ik}	8.36^{g}
SEM[f]	.65	.64	.81	1.28	.34	.48
Initial Slaughter Group	66.49	10.64	22.87	33.58	5.37	11.55

[a] Treatment means within columns with different superscripts differ ($P < .05$).
[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[c] Carcass defined as headless, skinless and with front and hind feet removed.
[d] FFM=fat-free muscle.
[e] FAT includes subcutaneous, intramuscular and intermuscular depots.
[f] SEM=standard error of least squares means.

TABLE III-15 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON THE PERCENTAGE OF FAT-FREE MUSCLE, FAT OR BONE OF THE CARCASS OR LIVE WEIGHT[a]

Treatment Group[b]	% of Carcass[c]			% of Live Weight		
	FFM[d]	Fat[e]	Bone	FFM[d]	Fat[e]	Bone
B	68.99^{h}	17.33^{g}	13.68^{hi}	44.96^{i}	11.29^{gh}	8.92^{h}
C	64.30^{g}	23.18^{h}	12.51^{gh}	41.10^{g}	14.82^{i}	8.00^{g}
C_LDHT	66.70^{h}	20173^{h}	12.57^{gh}	42.90^{gij}	13.33^{hi}	8.08^{g}
C_HDHT	66.56^{g}	21.12^{h}	12.32^{gh}	44.40^{ij}	14.09^{hi}	8.22^{g}
C_LTEST	67.22^{h}	20.80^{h}	11.98^{g}	44.30^{ij}	13.71^{hi}	7.89^{g}
C_HTEST	70.22^{h}	15.74^{g}	14.04^{i}	42.58^{hi}	9.55^{g}	8.51^{h}
C_LFED	63.60^{g}	23.72^{h}	12.68^{gh}	41.12^{gh}	15.34^{i}	8.20^{g}
SEM[f]	.91	1.10	.55	1.09	.89	.22
Initial Slaughter Group	68.63	16.78	14.59	40.50	9.89	8.61

[a] Treatment means within columns with different superscripts differ ($P < .05$).
[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[c] Carcass defined as headless, skinless and with front and hind feet removed.
[d] FFM=fat-free muscle.
[e] FAT includes subcutaneous, intramuscular and intermuscular depots.
[f] SEM=standard error of least squares means.

in the PREP and POSTP studies. The proximate analysis results are presented in tables III-16 and 17 for PREP and POSTP studies, respectively. Subcutaneous adipose tissue from the dorsal neck region of boars had 37 and 35% higher moisture, 10 and 6% lower lipid and 37 and 33% higher protein than PREP and POSTP castrates, respectively. While DHT implanted pigs did not differ from castrates, C_LTEST and C_HTEST pigs had higher percentages of moisture and protein but lower percentages of lipid relative to castrates in both the PREP and POSTP studies. This observation indicates that lipid filling was being depressed by testosterone treatment but not by dihydrotestosterone. For the earlier developing PR depot compared to the SQ depot, similar trends were observed. Since lipid filling would have its greatest effect on decreasing percentage moisture the effects of castration and C_HTEST are most evident on this aspect in the PR depot. Lipid filling also would decrease the concentration of protein in the depot but some of this decrease would be offset by increased synthesis of membrane proteins due to cell enlargement. In comparing PREP with POSTP pigs it is apparent that PREP pigs had much less lipid but higher protein and moisture concentrations. These developmental changes are in agreement with those reported by Topel (1971) and indicate a potential method of predicting composition in pigs (Aberle et al., 1977).

TABLE III-16 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON CHEMICAL COMPOSITION OF SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE[a]

Treatment Group[b]	Fat Depot					
	Subcutaneous[c]			Perirenal		
	%Mo[d]	%EEL[d]	%P[d]	%Mo[d]	%EEL[d]	%P[d]
B	21.89^{i}	72.03^{f}	5.87^{i}	16.75^{g}	79.97^{f}	3.47
C	16.00^{f}	79.97^{h}	4.29^{f}	11.81^{f}	85.00^{g}	3.36
C_LDHT	16.34^{f}	79.34^{h}	4.40^{fg}	12.29^{f}	84.13^{g}	3.48
C_HDHT	17.00^{fg}	78.53^{h}	4.70^{fgh}	12.91^{f}	83.17^{g}	3.96
C_LTEST	20.55^{hi}	73.87^{fg}	5.37^{g}	16.26^{g}	79.84^{f}	3.62
C_HTEST	19.03^{hi}	75.14^{g}	5.76^{i}	16.44^{g}	79.75^{f}	3.97
C_LFED	18.86^{gh}	74.86^{g}	5.62^{hi}	13.00^{f}	83.59^{g}	3.36
SEM[e]	.67	.86	.33	.95	1.04	.25
Initial Slaughter Group	30.84	62.84	5.92	26.20	69.35	4.28

[a] Treatment means within columns with different superscripts differ ($P < .05$).
[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[c] Subcutaneous sample from the dorsal neck region.
[d] Percentages of moisture (Mo), ether extractable lipid (EEL) and Kjeldahl N X6.25=(P).
[e] SEM=standard error of least squares means.

TABLE III-17 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON CHEMICAL COMPOSITION OF SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE[a]

Treatment Group[b]	Fat Depot: Subcutaneous[c] %Mo[d]	Subcutaneous %EEL[d]	Subcutaneous %P[d]	Perirenal %Mo[d]	Perirenal %EEL[d]	Perirenal %P[d]
B	14.77^{gh}	79.90^{fg}	5.14^{gh}	14.22^{h}	81.88^{f}	3.44^{i}
C	10.94^{f}	85.19^{h}	3.86^{f}	9.00^{fg}	88.21^{gh}	2.35^{f}
C_LDHT	10.48^{f}	85.40^{h}	3.97^{f}	9.23^{fg}	88.47^{gh}	2.24^{f}
C_HDHT	10.49^{f}	85.67^{h}	3.66^{f}	11.35^{g}	85.67^{g}	2.68^{fhi}
C_LTEST	14.22^{g}	80.98^{g}	4.42^{f}	7.59^{f}	90.46^{h}	2.43^{fg}
C_HTEST	16.00^{h}	77.41^{f}	6.07^{h}	14.41^{h}	81.79^{f}	3.24^{ghi}
C_LFED	10.43^{f}	85.20^{h}	3.67^{f}	9.18^{fg}	88.57^{gh}	2.00^{f}
SEM[e]	.49	.82	.33	.66	.90	.26
Initial Slaughter Group	16.00	78.18	5.41	14.25	81.50	3.51

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Subcutaneous sample from the dorsal neck region.

[d]Percentages of moisture (Mo), ether extractable lipid (EEL), and Kjeldahl N X 6.25=(P).

[e]SEM=standard error of least squares means.

When the percentages presented in tables III-16 and 17 are multiplied by the total CF or PR weight, the total amount of moisture, ether extractable lipid or protein result. These data are shown in tables III-18 and 19 for the PREP and POSTP studies, respectively. Based upon these data it appears that boars and C_HTEST were the two treatments with the most obvious trends for reduced lipid filling in both studies.

As mentioned in the introduction, only minor alterations in pork carcass composition have been observed after exogenous androgen treatment. Bratzler et al. (1954) intramuscularly implanted castrated male pigs with 193 mg pellets of testosterone propionate. No significant differences in performance or composition were detected. These data are consistent with results of Sleeth et al. (1953) in which 1 mg of testosterone propionate was injected per kilogram of body weight weekly and caused no differences relative to untreated castrates.

Beeson et al. (1955) fed methyltestosterone (MT) at 20 mg/d to pigs from weaning to 100 kg live weight and found variable effects upon growth but MT reduced the percentage of fat (based on lean cut percentages) was by 9%. After determining proximate analysis of the tissue from the carcasses, MT treated pigs had 5% less fat than controls. Bidner et al. (1972) added 2.2 mg of diethylstilbestrol (DES) and MT per kg feed fed to barrows and gilts. The

TABLE III-18 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL MOISTURE, ETHER EXTRACTABLE LIPID AND PROTEIN IN COMPOSITE CARCASS AND PERIRENAL ADIPOSE TISSUE[a]

Treatment Group[b]	Carcass Fat[c]			Perirenal Fat[c]		
	Mo[d] (kg)	EEL[d] (kg)	P[d] (kg)	Mo[d] (g)	EEL[d] (g)	P[d] (g)
B	.66^{fh}	2.16^{e}	.17^{f}	14.5	67.5^{ef}	3.0^{ef}
C	.56^{eg}	2.87^{fh}	.15^{ef}	14.7	107.1^{g}	4.0^{g}
C_LDHT	.64^{fgh}	3.11^{h}	.17^{f}	11.7	81.6^{f}	3.3^{ef}
C_HDHT	.49^{e}	2.27^{ef}	.13^{e}	11.4	75.1^{f}	3.6^{fg}
C_LTEST	.71^{h}	2.51^{fg}	.18^{fg}	12.1	58.5^{e}	2.7^{e}
C_HTEST	.61^{f}	2.38^{f}	.18^{fg}	11.4	54.2^{e}	2.8^{e}
C_LFED	.74^{h}	2.96^{gh}	.22^{g}	11.8	74.8^{f}	3.0^{ef}
SEM[d]	.04	.18	.01	1.3	5.7	.22

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Total calculated carcass fat or perirenal weight times respective percentage moisture, ether extractable lipid or protein.

[d]Percentages of moisture (Mo), ether extractable lipid (EEL), and Kjeldahl NX6.25=(P).

[e]SEM=standard error of least squares means.

TABLE III-19 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL MOISTURE, ETHER EXTRACTABLE LIPID AND PROTEIN IN COMPOSITE CARCASS AND PERIRENAL ADIPOSE TISSUE[a]

Treatment Group[b]	Carcass Fat[c] Mo[d] (kg)	Carcass Fat[c] Fat[d] (kg)	Carcass Fat[c] p[d] (kg)	Perirenal Fat[c] Mo[d] (g)	Perirenal Fat[c] Fat[d] (g)	Perirenal Fat[c] p[d] (g)
B	1.74^{fg}	9.44^{ef}	.60	60.4^{g}	349.7^{ef}	14.7^{f}
C	1.63^{ef}	12.90^{g}	.57	62.1^{fg}	511.0^{gh}	13.6^{ef}
C_LDHT	1.39^{e}	11.17^{fg}	.52	55.2^{fg}	527.4^{h}	13.4^{ef}
C_HDHT	1.48^{ef}	11.98^{fg}	.52	61.0^{fg}	457.7^{gh}	14.5^{ef}
C_LTEST	2.01^{g}	11.44^{fg}	.64	36.0^{ef}	420.1^{fg}	11.4^{ef}
C_HTEST	1.50^{ef}	7.33^{e}	.57	48.6^{ef}	273.1^{e}	11.0^{ef}
C_LFED	1.59^{ef}	12.90^{g}	.56	47.5^{f}	454.4^{gh}	10.3^{e}
SEM[e]	.11	1.10	.07	4.3	33.8	1.5

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Total calculated carcass fat or perirenal weight times respective percentage moisture, ether extractable lipid or protein.

[d]Percentages of moisture (Mo), ether extractable lipid (EEL), and Kjeldahl NX6.25=(P).

[e]SEM=standard error of least squares means.

main effect of the feeding of DES-MT was an 11% decrease in backfat thickness. Numerous other investigations have indicated oral administration of MT to pigs increased lean content and reduced fatness (Noland and Burris, 1956; Johnston et al., 1957; Thrasher et al., 1959; Whitaker et al., 1959; Elliot and Fowler, 1974; Fowler et al., 1978). Elliot and Fowler (1973) observed that the addition of DES (2.0 mg/kg feed) to diets of castrated male pigs receiving testosterone caused greater gains and feed efficiencies than testosterone alone.

Oral administration of trenbolone acetate (TBA) to castrated males resulted in less backfat relative to controls (Fowler et al., 1978). Similar results were reported by VanWeerden and Grandadam (1976) when TBA was combined with ethanylestradiol. These latter workers also observed a 56% increase in protein deposition (derived from nitrogen balance) and a 15% decrease in fat deposition (measured from energy balance) in pigs implanted with 20 mg estradiol and 140 mg TBA.

The results of the composition data in the present study indicate that prepubertal and pubertal boars have less fat than castrates. However, advantages in FFM accretion of boars were not observed prior to puberty. Based upon soft tissues, SQ and PR composition, high testosterone implants (and possibly high dihydro-testosterone implants prepubertally) were effective in

decreasing lipid deposition. Over the time periods of these studies, neither testosterone nor dihydrotestosterone implants were effective in stimulating total FFM accretion. In these studies, limit-feeding castrates to the consumption levels of boars appeared to depress fattening but not to the same degree as boars. Muscle deposition was enhanced by limit feeding prepubertally but was reduced after puberty.

The inability to show positive effects of testosterone upon FFM accretion is consistent with previous data in the literature. It would appear that other testicular secretions are involved in coordinating the maximal anabolic differences observed between boars and castrates. Testosterone but not DHT appears to be involved in appetite control of boars and in lipid deposition.

Because of the variation in feed intake of the pigs in these studies, it is apparent that future endeavors for investigating testosterone effects upon growth and composition must be carried out on pigs fed similar intakes of isocaloric and isonitrogenous diets.

SUMMARY

Body Growth. Generally speaking boars grew at rates equal to castrates. Androgen administration tended to reduce average daily gain relative to castrates but generally this trend was only significant in prepubertal

pigs implanted with low and high testosterone and high dihydrotestosterone.

Feed Intake. In the 3 wk growth study of pigs from 38 to 57 kg, boars consumed 15% less feed and had 15% lower feed to gain ratios than castrated males. In two subsequent studies for 5 wk growth periods from 15 to 38 kg and from 74 to 102 kg live body weight, boars consumed 23 and 14% less feed, respectively, than castrates and had 23 and 20% lower feed to gain ratios, respectively. In all studies, testosterone implanted but not dihydrotestosterone implanted castrates, reduced feed intake by 12 to 38% when compared to castrates. These data suggest a definite role of testosterone in feed intake suppression. The inability of DHT administration to suppress feed consumption suggests that 1) reduction of testosterone to dihydrotestosterone is not required and 2) testosterone itself or a testosterone metabolite such as aromatized products may be important in mediating the appetite depression. The use of aromatization inhibitors may answer this question.

Bone. The 3 wk study showed that bone growth may be increased in young boars and low testosterone implanted castrates relative to castrates as reflected by carcass length. Subsequent studies showed no differences in carcass length between treatments.

Fattening. Based upon fat depth at the tenth rib alone, testosterone had a definite effect on reducing fat compared to castrates. Dihydrotestosterone implanted pigs

also tended to have reduced fat compared to castrates but only the high treatment of pubertal pigs was significantly lower. Except for the 3 wk study, boars had 35 to 43% less fat at the tenth rib than castrates.

Total carcass fat data followed similar trends as the fat depth data. Except for high dihydrotestosterone implanted prepubertal pigs, neither dihydrotestosterone nor low testosterone implanted pigs had less fat than castrates. However, boars and high testosterone implanted pigs had reduced fat deposition compared to castrates. Composition of subcutaneous and perirennal fat clearly demonstrate that castration increased lipid filling and high testosterone implanted pigs but not dihydrotestosterone prevented this lipid deposition.

Muscle. Based upon longissimus muscle areas, castration and administration of androgen to castrates had no influence except for pubertal pigs. Pubertal boars had 1.3-fold larger and pubertal pigs administered high testosterone had 1.2-fold larger longissimus muscle areas compared to castrates. Total fat-free muscle in carcasses followed similar trends in that only pubertal boars had 1.1-fold greater and low testosterone implanted pubertal pigs had 1.1-fold greater fat-free muscle compared to castrates.

CHAPTER IV

EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGENS UPON SKELETAL MUSCLE ACCRETION

Introduction

Castration of male rodents (Kochakian, 1976), sheep (Lohse, 1973) and cattle (Brannang, 1971) suppresses skeletal muscle growth. The effects of castration of male pigs are minimal for growth rate and total muscle mass but does increase fat deposition (Field, 1971). Intact male pigs have been shown to have 12% more muscle than castrated males (Walstra, 1980). Exogenous testosterone administered to guinea pigs (Kochakian, 1976) or rabbits (Grigsby et al., 1976) resulted in trends for increased muscle growth which opposed the castration associated decrease in muscle. Individual guinea pig muscles varied in their growth response to exogenosus testosterone (Kochakian, 1976). Recent studies involving myogenic cell culture failed to demonstrate any stimulatory effect on α-actin synthesis (Allen et al., 1983) and other proteins (Ballard and Francis, 1983). Powers and Florini (1975) observed increased incorporation of labeled thymidine into myogenic cells indicating increased cell proliferation. This latter observation provides a basis for suggesting that

testosterone may act on muscle growth by stimulating satellite cell proliferation. Florini (1970) reported that castration of male rats decreased body growth and muscle ribosomal activity. After 8 d of administering .5 mg of testosterone propionate to castrated rats, body weight and ribosomal activity were restored. In response to androgens and synthetic anabolic steroids muscle has been shown to have increased amino acid uptake and incorporation into protein (Breuer and Florini, 1965; Arvill, 1967; Grigsby et al., 1976; Rogozkin, 1979), increased m- and rRNA synthesis (Kochakian et al., 1964; Rogozkin, 1979), and decreased protein catabolism (Bullock et al., 1968, 1969; White, 1967; Young, 1970, Santidrian et al., 1982; Sinnett-Smith et al., 1983).

Dihydrotestosterone appears to be the primary active form in skin and sexual tissues (Williams-Ashman, 1975). Since the reductase enzymes required for conversion of testosterone to dihydrotestosterone are virtually absent in skeletal muscle (Krieg and Voight, 1977; Michel and Baulieu, 1980), it is uncertain what form of androgen is active for skeletal muscles. The effects of castration and administration of testosterone to pigs upon growth of specific muscles have not been investigated extensively. Telegdy (1980) reviewed numerous studies which indicated that animals responsiveness to hormones and drugs change with age and reach functional capacity after puberty. This

study was designed to investigate the effects of prepubertal and pubertal castration and the administration of testosterone or dihydrotestosterone to castrated males upon skeletal muscle growth.

Materials and Methods

The overall design of the study and other pertinent methods are described in Chapters I and II.

Results and Discussion

Muscle Weights. In addition to determining total fat-free muscle (FFM) described in Chapter III, several muscles were removed from pigs in the pilot study. Table IV-1 lists the least squares treatment means for the combined right and left side deltoid (D), brachialis (BR), longissimus muscle (LD), pectineus (PC), satorius (SA) and semitendinosus (ST) as well as, the right side infraspinatus (IF), triceps brachii (TB), semimembranosus (SM) and adductor (AD) muscles. Weights of these muscles from the initial slaughter group of pigs are also listed in table IV-1. There was a trend for boars to have slightly heavier muscles than castrates except for the SA and IF. Significant differences existed between boars and castrates for the combined right and left side PC (17%) and the right side TB (14%) and SM (19%) muscles. There appeared to be a trend for the PC, ST, TB and possibly the BR and LD muscles

TABLE IV-1 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON SELECTED MUSCLE WEIGHTS[abc]

Muscle	Boars	Castrates	TEST Implant Dose			SEM[d]	Initial Group
			Low	Inter	High		
Right & Left Side							
Deltoid	124.5	114.9	114.6	133.3	117.1	3.8	70.4
Brachialis	125.5	118.9	120.6	123.5	123.2	7.2	75.4
Longissimus	2326.6	2069.2	2205.0	2020.8	2118.8	119.1	1267.8
Pectineus	110.5[f]	94.1[e]	99.3[ef]	101.1[ef]	102.1[ef]	5.4	57.1
Rectus femoris	24.4	25.2	25.1	26.0	25.2	2.1	15.1
Semitendinosus	487.5	410.0	437.9	451.4	450.4	33.6	266.7
Right Side							
Infraspinatus	173.7	174.8	176.1	175.5	183.5	13.75	89.5
Triceps Brachii	472.1[f]	413.9[e]	418.3[ef]	431.[ef]	455.3[ef]	23.3	246.2
Semimembranosus	561.6[f]	471.8[e]	469.2[e]	516.0[ef]	504.8[ef]	32.8	287.0
Adductor	199.4	180.5	180.5	167.6	181.4	14.9	116.0

[a]Pilot study.

[b]Weight in grams.

[c]Mean within a live with different superscripts differ ($P < .05$).

[d]SEM=standard error of least squares means.

to respond to testosterone implants. Thus it was decided that these muscles could be used as indicator muscles in the subsequent trials of this study.

Gross muscle weights of pigs in the prepubertal (PREP) and pubertal (POSTP) studies, respectively, are listed in Tables IV-2 and 3. Boars from the PREP and POSTP studies, respectively, had the following heavier muscles relative to castrates: TB (by 17 and 30%), BR (by 24 and 31%, LD (by 19 and 10%, PC (by 20 and 14%), teres major (TM) (by 35 and 22%) and ST (by 19 and 21%). As indicated in Chapter III, neither castration nor supplementation with TEST or DHT affected total carcass FFM accretion in PREP pigs. However, in POSTP implanted pigs, total carcass FFM accretion rates were elevated over castrates by 8.5 to 36%. An initial interpretation is that muscle in PREP pigs were not affected by testosterone. However, both androgens had positive effects upon individual muscle weights in the PREP study. If the muscle weight means of the implanted groups in the PREP study are averaged, implanted pigs had 12, 16, 22 and 11% heavier TB, BR, LD and ST muscles, respectively, than castrates. PC, SA and TM muscles of the implanted pigs in the PREP study did not differ from castrates. In light of the total FFM accretion data (Chapter III), these responses appear surprising but indicate that some muscles of the PREP pigs were responsive to both forms of androgen. The data in figure IV-1

TABLE IV-2 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SELECTED MUSCLE WEIGHTS[a]

	Muscle[b]						
Treatment Group[c]	Triceps Brachii	Brachialis	Longissimus	Pectineus	Satorius	Teres Major	Semitendinosus
B	535.9^{fg}	80.5^{hi}	1311.1^{fg}	71.3^{f}	13.8	31.1^{g}	285.0^{f}
C	456.7^{e}	65.1^{e}	1099.9^{e}	59.5^{e}	16.2	23.1^{ef}	239.5^{e}
C_LDHT	523.2^{fg}	76.1^{gh}	1359.8^{gh}	65.5^{ef}	16.2	25.1^{efg}	266.7^{f}
C_HDHT	505.7^{f}	78.7^{ghi}	1335.3^{fgh}	57.2^{e}	12.4	25.9^{fg}	259.4^{ef}
C_LTEST	492.5^{e}	71.4^{fg}	1230.5^{f}	60.8^{e}	15.7	22.4^{f}	262.7^{ef}
C_HTEST	560.0^{g}	82.4^{j}	$1456.^{eh}$	62.7^{ef}	13.4	19.1^{e}	278.2^{f}
C_LFED	492.9^{ef}	68.9^{ef}	1262.8^{fg}	66.0^{ef}	16.9	28.8^{fg}	245.8^{e}
SEM[d]	18.11	1.69	42.04	3.10	1.53	2.06	8.86
Initial Slaughter Group	170.9	25.9	352.1	19.3	5.0	10.0	79.2

[a]Least square treatment means within columns with different superscripts differ ($P<.05$).

[b]Muscle weight in grams.

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

TABLE IV-3 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SELECTED MUSCLE WEIGHTS[d]

Treatment Group[c]	Muscle[b]						
	Triceps Brachii	Brachialis	Longissimus	Pectineus	Satorius	Teres Major	Semitendinosus
B	1727.6^{f}	264.1^{g}	4036.9	190.0^{e}	30.8^{f}	47.5^{f}	867.7^{g}
C	1328.6^{e}	201.3^{ef}	3670.6	166.5^{ef}	21.7^{e}	39.0^{e}	715.8^{ef}
C_LDHT	1456.3^{f}	210.1^{ef}	4058.1	162.4^{e}	21.9^{e}	38.6^{e}	776.4^{efg}
C_HDHT	1515.3^{ef}	222.9^{f}	4256.4	177.0^{e}	25.6^{ef}	49.1^{f}	821.1^{fg}
C_LTEST	1515.7^{ef}	220.0^{f}	4114.5	175.6^{e}	24.6^{ef}	35.6^{e}	816.2^{fg}
C_HTEST	1476.6^{e}	200.8^{ef}	4039.3	185.7^{e}	23.4^{e}	38.8^{ef}	829.5^{g}
C_LFED	1382.3^{e}	194.2^{e}	3706.1	177.0^{e}	21.8^{e}	38.8^{e}	694.7^{e}
SEM[d]	72.85	7.68	170.78	8.83	2.44	2.79	30.75
Initial Slaughter Group	936.0	138.8	2325.6	108.6	16.2	23.8	482.7

[a]Leas square treatment means within columns with different superscripts differ ($P<05$).

[b]Muscle weight in grams.

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_IFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

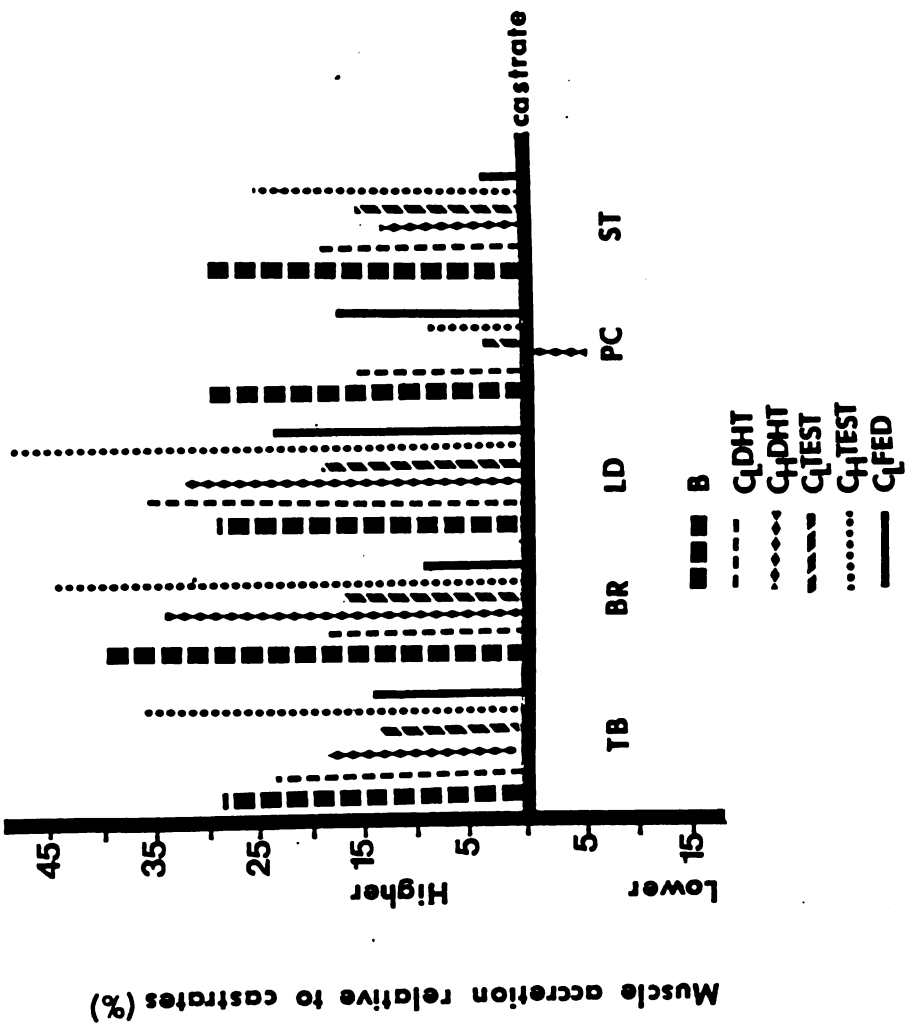


FIGURE IV-1. Effects of prepubertal castration and administration of androgens to castrated male pigs upon triceps brachii, brachialis, longissimus, pectineus and semitendinosus muscle accretion.

represent the difference in respective muscle weights between the initial slaughter group and the implant groups expressed as a percentage of the nonimplanted castrates for comparison among treatments. The magnitude of response varied with muscle but from the figure it appears that C_HTEST was more effective in stimulating growth in these selected muscles than the other implants.

In the POSTP study (table IV-3), there were fewer significant differences for specific muscles between implant groups and castrates but muscles tended to be heavier in the implanted groups. If the implanted group treatment means are averaged for the TB, BR, LD and ST, it appears that implanted androgens stimulated these muscles by 12, 6, 12 and 13%, respectively, relative to castrates. Individual muscle accretion rates were determined relative to castrates and are illustrated for the POSTP study in figure IV-2. It appeared that high DHT had a greater effect on muscle growth than the other implants. However, in the POSTP study no treatment was as effective for muscle growth as intact boars. The inability to detect differences between TEST or DHT upon muscle growth contrasts with recent reports which suggest that some androgen effects on skeletal muscle may be mediated by aromatization of testosterone to estradiol (Dionne et al., 1979; Knudsen and Max, 1980; Max, 1981).

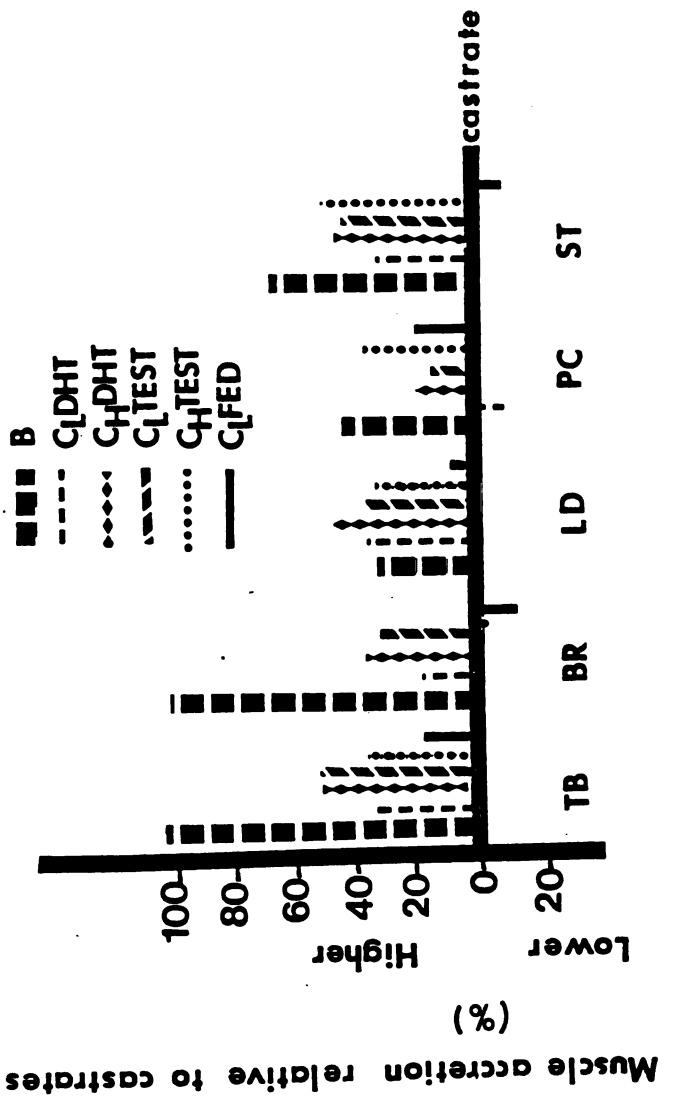


FIGURE IV-2. Effects of pubertal castration and administration of androgens to castrated male pigs upon triceps brachii, brachialis, longissimus, pectineus and semitendinosus muscle accretion.

It is puzzling to explain why individual muscles were stimulated by androgen but no differences in total carcass fat-free muscle mass were evident in the PREP pigs, except that certain muscles (some of those measured) are responsive to androgens, whereas most muscles are not. On the other hand, POSTP pigs showed a response to androgen in individual and total carcass total fat-free muscle accretion rates. Brannang (1971) used monozygous twin cattle to show that the presence or absence of the testes selectively affected certain muscles more than others. Castration decreased shoulder muscle weights over 15 to 28% but hind leg muscles by less than 15% on the average. Walstra (1980) made similar observations for groups of muscles in pigs. Kochakian and Tillotson (1957) observed similar trends in castrated guinea pigs given exogenous testosterone. Explanation of the diverse responses to androgen observed between muscles could be related to receptor presence, number and function. Androgen receptors have been identified in porcine rectus femoris muscle (Snochowski et al., 1981; Lundstrom et al., 1983). In rat skeletal muscle, androgen and glucocorticoid receptors both interact with DNA (Snochowski et al., 1980). It also is uncertain whether TEST or DHT is the active ligand which binds to muscle receptors to elicit responses (Gustafsson and Pousette, 1975; Krieg and Voight, 1976; Krieg et al., 1974; Tremblay et al., 1977; Michel and Baulieu, 1980).

Strong evidence indicates that 5 α-reductase activity is nonexistent in skeletal muscle (Krieg and Voight, 1976; Liao et al., 1976; Michel and Baulieu, 1980). Warrenski and Almon (1983) reported that skeletal muscle has considerable 3 α -hydroxysteroid oxidoreductase activity which converts DHT to androstanediol.

Chin and Almon (1980) reported that the number of cholinergic receptor sites in some muscles (extensor digitorum longus) are increased but in others (soleus) no change is observed after castration. The demonstration of variation in response to castration indicates that muscles may vary in the species of receptor proteins made as well as responsiveness to other hormones such as glucocorticoids.

The data in the present study shows that selected skeletal muscles of pigs were responsive to both TEST and DHT both prepubertally and pubertally. The mechanisms underlying this similarity of action cannot be ascertained from these data. Considering the boar taint problem associated with producing boars for meat, it appears that administration of androgens after castration may be a practical approach worthy of further investigation.

Muscle Lengths. Muscle lengths taken before removal of muscles from the right sides of the carcasses from the PREP and POSTP studies are presented in tables IV-4 and 5, respectively. Very few differences existed between

TABLE IV-4 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON LENGTH OF SELECTED MUSCLES[a]

Treatment Group[c]	Muscle[b]					
	Teres Major	Triceps Brachii	Brachialis	Satorius	Pectineus	Semitendinosus
B	11.38^{f}	11.17^{fg}	10.30^{e}	10.18	9.51^{g}	18.73^{f}
C	10.26^{e}	10.71^{ef}	10.71^{e}	10.16	8.46^{e}	16.98^{e}
C_LDHT	10.79^{ef}	11.58^{g}	11.38^{f}	10.36	9.46^{g}	18.98^{f}
C_HDHT	10.07^{e}	11.11^{fg}	10.83^{ef}	9.81	8.88^{ef}	18.43^{f}
C_LTEST	10.11^{e}	11.44^{g}	10.80^{ef}	9.75	9.18^{fg}	19.26^{f}
C_HTEST	9.48^{e}	11.12^{fg}	10.78^{e}	9.63	9.03^{fg}	19.26^{f}
C_LFED	9.43^{e}	10.47^{e}	10.71^{e}	9.71	9.21^{fg}	17.24^{e}
SEM[d]	.33	.18	.18	.36	.18	.36
Initial Slaughter Group	7.44	8.32	7.44	7.58	6.66	12.76

[a]Least square treatment means within columns with different superscripts differ ($P < .05$).

[b]Muscle weight in grams.

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

TABLE IV-5 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON LENGTH OF SELECTED MUSCLES[a]

Treatment Group[c]	Muscle[b]					
	Teres Major	Triceps Brachii	Brachialis	Satoruis	Pectineus	Semitendinosus
B	15.39	15.08	14.75^{f}	14.16	13.32^{f}	24.99^{f}
C	13.31	14.22	14.23^{e}	13.70	11.99^{e}	21.56^{e}
C_LDHT	13.83	15.05	14.04^{e}	13.63	12.53^{ef}	24.35^{f}
C_HDHT	14.65	14.87	13.70^{e}	13.54	12.37^{e}	26.09^{fg}
C_LTEST	14.48	14.56	13.91^{e}	13.20	12.54^{ef}	25.24^{fg}
C_HTEST	13.51	14.30	14.27^{ef}	13.54	11.75^{e}	27.41^{g}
C_LFED	13.75	15.40	13.78^{e}	13.79	11.96^{e}	22.18^{e}
SEM[d]	.46	.36	.20	.40	.31	.69
Initial Slaughter Group	12.45	12.32	12.58	11.65	10.22	20.58

[a]Least square treatment means within columns with different superscripts differ ($P < .05$).

[b]Muscle weight in grams.

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

treatments. However, the TB and ST muscles tended to be longer in boars and androgen treated pigs relative to castrates.

Muscle Composition. Since wet muscle weights tended to be heavier in boars and androgen treated pigs relative to castrates, composition of TB, BR, PC, LD and ST muscles was determined. Tables IV-6 and 7 show the percentage moisture, ether extractable fat and protein (Kjeldahl nitrogen X 6.25) determined on muscles from the PREP pigs and tables IV-8 and 9 present the data for the POSTP pigs.

The most common feature of castration was to increase percentage fat in muscle by 40% or more, decrease percentage protein by about 3% and percentage moisture by 1 to 2% in both studies. High testosterone implanted pigs tended to have lower percentages of lipid relative to castrates, and in some cases similar or lower percentages of lipid than the initial slaughter group of pigs which in itself indicates an effect on lipid metabolism of the intramuscular depots. Initial group data of the POSTP study which weighed about 75 kg were similar in muscle composition to the boars in the PREP study which weighed about 40 kg. Muscle composition data comparing POSTP boars to castrates are in agreement with data of Newell and Bowland (1972) where muscles from boars had 1 to 2% less fat than castrates.

TABLE IV-6 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE OR DIHYDROTESTOSTERONE TO CASTRATED MALE PIGS UPON CHEMICAL COMPOSITION OF TRICEPS BRACHII, BRACHIALIS AND PECTINEUS MUSCLES

Treatment Group[c]	Muscle[ab]								
	Triceps Brachii			Brachialis			Pectineus		
	Mo	P	F	Mo	P	F	Mo	P	F
B	77.62	20.50^{f}	$.87^{ef}$	77.70^{g}	18.87	2.50^{e}	77.52^{gh}	19.81^{f}	1.67^{e}
C	77.64	19.41^{e}	1.80^{gh}	76.84^{ef}	18.76	3.54^{f}	76.77^{ef}	19.73^{e}	2.35^{fg}
C_LDHT	77.17	20.03^{ef}	1.70^{gh}	77.46^{fg}	18.72	3.27^{f}	76.53^{ef}	20.52^{g}	1.66^{e}
C_HDHT	77.40	19.55^{ef}	1.96^{g}	77.33^{fg}	18.90	3.50^{f}	76.24^{e}	19.76^{ef}	2.69^{g}
C_LTEST	77.61	19.92^{ef}	1.43^{eg}	77.06^{efg}	18.75	3.55^{f}	76.74^{eg}	20.05^{efg}	1.72^{e}
C_HTEST	78.12	19.64^{ef}	1.15^{e}	77.17^{fg}	19.14	2.79^{e}	77.02^{g}	20.19^{efg}	1.60^{e}
C_LFED	77.43	20.26^{ef}	1.29^{efg}	76.38^{e}	19.45	3.33^{f}	76.40^{efg}	20.55^{g}	2.15^{f}
SEM[d]	.29	.32	.16	.24	.25	.14	.24	.12	.16
Initial Slaughter Group	79.13	18.69	1.17	78.68	18.45	1.73	78.46	19.04	1.24

[a] Mo=% moisture, P=% protein, F=% fat.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d] SEM=standard error of least squares means.

TABLE IV-7 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON CHEMICAL COMPOSITION OF THE LONGISSIMUS AND SEMITENDINOSUS MUSCLES

Treatment Group[c]	Muscle[ab]					
	Longissimus			Semitendinosus		
	Mo	P	F	Mo	P	F
B	76.34^{efg}	20.82	1.73^{e}	77.76^{fg}	19.50	2.06^{ef}
C	75.72^{ef}	20.59	3.03^{fg}	76.42^{eg}	18.97	3.50^{g}
C_LDHT	75.41^{ef}	20.89	2.82^{fg}	76.20^{e}	19.17	3.27^{g}
C_HDHT	75.29^{e}	20.99	3.17^{g}	77.24^{efg}	19.13	2.61^{efg}
C_LTEST	77.02^{g}	20.39	1.44^{e}	77.59^{g}	19.52	1.82^{e}
C_LFED	76.07^{efg}	20.50	2.17^{ef}	76.84^{efg}	19.49	2.50^{efg}
SEM[d]	.36	.30	.29	.40	.20	.36
Initial Slaughter Group	78.52	19.61	1.02	78.89	18.55	1.56

[a] Mo=% moisture, P=% protein, F=% fat.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d] SEM=standard error of least squares means.

TABLE IV-8 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE OR DIHYDROTESTOSTERONE TO CASTRATED MALE PIGS UPON CHEMICAL COMPOSITION OF TRICEPS BRACHII, BRACHIALIS AND PECTINEUS MUSCLE

Treatment Group[c]	Muscle[ab]								
	Triceps Brachii			Brachialis			Pectineus		
	Mo	P	F	Mo	P	F	Mo	P	F
B	75.65^{f}	21.85^{h}	1.39^{f}	76.67	20.82^{f}	1.98^{ef}	75.78^{e}	21.60^{fg}	1.45^{ef}
C	75.34^{ef}	20.59^{e}	3.59^{g}	76.35	19.45^{e}	3.36^{h}	75.46^{e}	20.80^{e}	2.31^{g}
C_LDHT	75.85^{f}	20.71^{ef}	3.14^{g}	76.54	19.78^{ef}	3.32^{h}	75.39^{e}	21.67^{fg}	1.88^{fg}
C_HDHT	74.80^{e}	21.52^{gh}	2.80^{fg}	75.99	20.04^{ef}	2.86^{gh}	75.47^{e}	21.49^{fg}	2.05^{fg}
C_LTEST	75.56^{f}	21.59^{gh}	2.04^{ef}	76.77	20.24^{ef}	2.48^{fg}	75.22^{e}	22.06^{g}	1.57^{ef}
C_HTEST	75.79^{f}	21.43^{fh}	1.55^{e}	77.18	20.39^{ef}	1.45^{e}	76.61^{f}	21.16^{ef}	1.12^{e}
C_LFED	75.82^{f}	21.00^{efg}	2.72^{fg}	76.31	19.89^{ef}	3.05^{h}	75.23^{e}	21.87^{fg}	1.65^{ef}
SEM[d]	.20	.24	.32	.33	.34	.18	.19	.20	.18
Initial Slaughter Group	76.29	21.16	1.69	77.19	20.11	2.20	76.37	21.49	1.47

[a] Mo=% moisture, P=% protein, F=% fat.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d] SEM=standard error of least squares means.

TABLE IV-9 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON CHEMICAL COMPOSITION OF LONGISSIMUS AND SEMITENDINOSUS MUSCLES

Treatment Group[c]	Muscles[ab]					
	Longissimus			Semitendinosus		
	Mo	P	F	Mo	P	F
B	74.22	23.58^{ef}	1.70^{efg}	76.08^{fg}	20.64^{fg}	2.27^{ef}
C	74.34	22.53^{e}	2.55^{h}	74.80^{e}	20.78^{fg}	3.47^{g}
C_LDHT	74.36	22.58^{e}	2.50^{h}	75.65^{f}	20.60^{fg}	2.80^{fg}
C_HDHT	74.05	23.11^{ef}	2.31^{gh}	75.86^{fg}	20.20^{ef}	2.85^{fg}
C_LTEST	74.39	23.87^{f}	1.46^{eg}	75.48^{ef}	20.67fg	2.65fg
C_HTEST	74.94	23.12^{ef}	1.25^{e}	76.70^{g}	21.17^{g}	1.39^{e}
C_LFED	74.50	22.59^{e}	2.10^{fh}	75.89^{fg}	19.95^{e}	3.47^{g}
SEM[d]	.24	.38	.20	.26	.21	.29
Initial Slaughter Group	75.84	21.82	1.67	76.79	20.33	1.87

[a] Mo=% moisture, P=% protein, F=% fat.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B-boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d] SEM=standard error of least squares means.

In order to assess protein and fat deposition in selected muscles, the percentage moisture, fat or protein of each muscle was multiplied by the muscle weight to obtain total moisture, fat or protein in each muscle. These totals are given in tables IV-10 and 11 for the PREP study and tables IV-12 and 13 for the POSTP study.

As expected from the percentage composition of the selected muscles, the total moisture, protein and fat content of the muscles was similar to that of the percentage composition. The total ether extractable lipid content of muscles from the initial group of pigs was subtracted from the total lipid of the muscles in the treatment groups, to obtain muscle intramuscular lipid accretion. When accretion of all treatments is expressed as a percentage of the castrates, the relative effect of castration and administration of TEST or DHT can be readily visualized. The muscle lipid percentage accretions relative to castrates are presented in figures IV-3 and IV-4 for the PREP and POSTP studies, respectively. Castration had a marked effect on intramuscular lipid accretion in both PREP and POSTP pigs but the effect was more pronounced in the POSTP pigs. As shown in figure IV-3, PREP boars had an average of 34% lower intramuscular lipid accretion relative to castrates. Figure IV-4 indicates that POSTP boars had an average of 48.7% less intramuscular lipid accretion than castrates. It appeared

TABLE IV-10 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL MOISTURE, PROTEIN AND FAT OF THE TRICEPS BRACHII, BRACHIALIS AND PECTINEUS MUSCLES

Treatment Group[c]	Muscles[ab]								
	Triceps			Brachialis			Pectineus		
	Mo	P	F	Mo	P	F	Mo	P	F
B	415.93^{fg}	109.95^{g}	4.63^{e}	62.53^{hi}	15.20^{gh}	1.97^{e}	55.34^{f}	14.12^{g}	1.17^{efg}
C	354.70^{e}	88.47^{e}	8.31^{f}	50.05^{e}	12.21^{e}	2.31^{f}	45.67^{e}	11.74^{ef}	1.40^{f}
C_LDHT	403.68^{fg}	104.84^{fg}	8.93^{fg}	58.94^{gh}	14.22^{fg}	2.46^{fg}	50.13^{ef}	13.44^{fg}	1.10^{eg}
C_HDHT	391.36^{ef}	98.86^{eg}	9.88^{g}	60.84^{gi}	14.88^{gh}	2.73^{g}	43.65^{e}	11.30^{e}	1.55^{g}
C_LTEST	382.21^{ef}	98.11^{ef}	7.03^{fg}	55.05^{fg}	13.39^{ef}	2.53^{fg}	46.70^{e}	12.18^{ef}	1.05^{e}
C_HTEST	437.53^{g}	110.02^{g}	6.40^{ef}	63.58^{i}	15.80^{h}	2.26^{ef}	48.34^{ef}	12.65^{efg}	.99^{e}
C_LFED	381.61^{ef}	99.88^{efg}	6.35^{ef}	52.60^{ef}	13.40^{ef}	2.28^{ef}	50.42^{ef}	13.56^{fg}	1.38^{fg}
SEM[d]	12.91	3.78	.86	1.28	.39	.11	2.40	.63	.10
Initial Slaughter Group	135.23	31.94	2.00	20.38	4,78	.45	15.14	3.67	.27

[a]Total moisture (Mo), protein (P), and fat (F) per muscle in grams.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

TABLE IV-11 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL MOISTURE, PROTEIN AND FAT OF THE LONGISSIMUS AND SEMITENDINOSUS MUSCLES

Treatment Group[c]	Muscle[ab]					
	Longissimus			Semitendinos s		
	Mo	P	F	Mo	P	F
B	1001.11^{f}	273.09^{fg}	22.37^{ef}	221.62^{f}	55.51^{h}	5.93^{ef}
C	832.51^{e}	226.47^{e}	33.74^{fg}	183.03^{e}	45.38^{ef}	8.35^{g}
C_LDHT	1025.54^{fg}	284.28^{fg}	38.01^{fg}	203.40^{ef}	51.20^{eg}	8.57^{g}
C_HDHT	1005.70^{f}	280.29^{fg}	41.93^{g}	200.32^{ef}	49.62^{efg}	6.77^{fg}
C_LTEST	941.39^{f}	257.48^{f}	26.91^{ef}	202.84^{ef}	50.73^{efgh}	7.15^{fg}
C_HTEST	1121.90^{g}	297.78^{ef}	27.48^{ef}	188.85^{e}	47.91^{ef}	6.14^{ef}
SEM[d]	32.39	10.76	3.65	7.32	1.83	.83
Initial Slaughter Group	276.47	69.05	3.59	62.48	14.69	1.24

[a]Total moisture (Mo), protein (P), and fat (F) per muscle in grams.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

TABLE IV-12 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL MOISTURE, PROTEIN AND FAT OF THE TRICEPS BRACHII, BRACHIALIS AND PECTINEUS MUSCLES

Treatment Group[c]	Muscle[ab]								
	Triceps Brachii			Brachialis			Pectineus		
	Mo	P	F	Mo	P	F	Mo	P	F
B	1307.27^{f}	377.60^{f}	24.04^{e}	202.54^{g}	54.99^{g}	5.19^{f}	144.10^{f}	40.95^{f}	2.79^{ef}
C	1000.52^{e}	273.16^{e}	48.51^{f}	153.65^{ef}	39.27^{ef}	6.68^{g}	125.60^{e}	34.64^{e}	3.87^{g}
C_LDHT	1104.56^{e}	302.18^{e}	45.07^{f}	160.84^{ef}	41.56^{ef}	6.96^{g}	122.40^{e}	35.20^{ef}	3.05^{efg}
C_HDHT	1134.06^{ef}	325.66^{ef}	42.40^{ef}	169.50^{f}	44.67^{f}	6.33^{fg}	133.70^{ef}	37.94^{ef}	3.58^{fg}
C_LTEST	1145.38^{ef}	327.19^{ef}	30.75^{e}	168.98^{f}	44.49^{f}	5.47^{fg}	132.14^{ef}	38.72^{ef}	2.77^{ef}
C_HTEST	1118.54^{e}	317.00^{e}	22.67^{e}	154.89^{ef}	41.05^{ef}	2.83^{e}	142.29^{ef}	39.28^{ef}	2.08^{e}
C_LFED	1047.93^{e}	290.54^{e}	37.04^{ef}	148.28^{e}	38.59^{e}	5.88^{fg}	133.16^{ef}	38.67^{ef}	2.94^{efg}
SEM[d]	54.60	16.74	5.28	5.78	1.90	.47	6.71	1.90	.32
Initial Slaughter Group	714.07	198.06	15.82	107.14	27.91	3.18	82.94	23.34	1.60

[a] Total moisture (Mo), protein (P), and fat (F) per muscle in grams.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d] SEM=standard error of least squares means.

TABLE IV-13 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL MOISTURE, PROTEIN AND FAT OF THE LONGISSIMUS AND SEMITENDINOSUS MUSCLES

Treatment Group[c]	Muscle[ab]					
	Longissimus			Semitendinosus		
	Mo	P	F	Mo	P	F
B	2997.02	950.00	69.69^{eg}	660.14^{g}	179.25^{g}	19.43^{f}
C	2728.36	828.44	92.16^{fh}	535.09^{e}	148.60^{e}	25.33^{g}
C_LDHT	3016.70	918.08	100.93^{gh}	587.39^{ef}	160.00^{fg}	21.93^{fg}
C_HDHT	3153.86	981.64	98.37^{fh}	623.13^{fg}	165.00^{fg}	23.04^{fg}
C_LTEST	3060.84	981.26	60.93^{eg}	616.17^{fg}	168.94^{fg}	21.34^{f}
C_HTEST	3026.76	936.27	50.42^{e}	636.04^{fg}	175.42^{g}	11.77^{e}
C_LFED	2762.43	836.92	78.02^{fg}	527.22^{e}	138.92^{e}	23.58^{fg}
SEM[d]	126.86	45.63	7.59	23.87	7.13	1.84
Initial Slaughter Group	1763.70	507.44	38.84	370.67	98.13	9.03

[a]Total moisture (Mo), protein (P), and fat (F) per muscle in grams.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

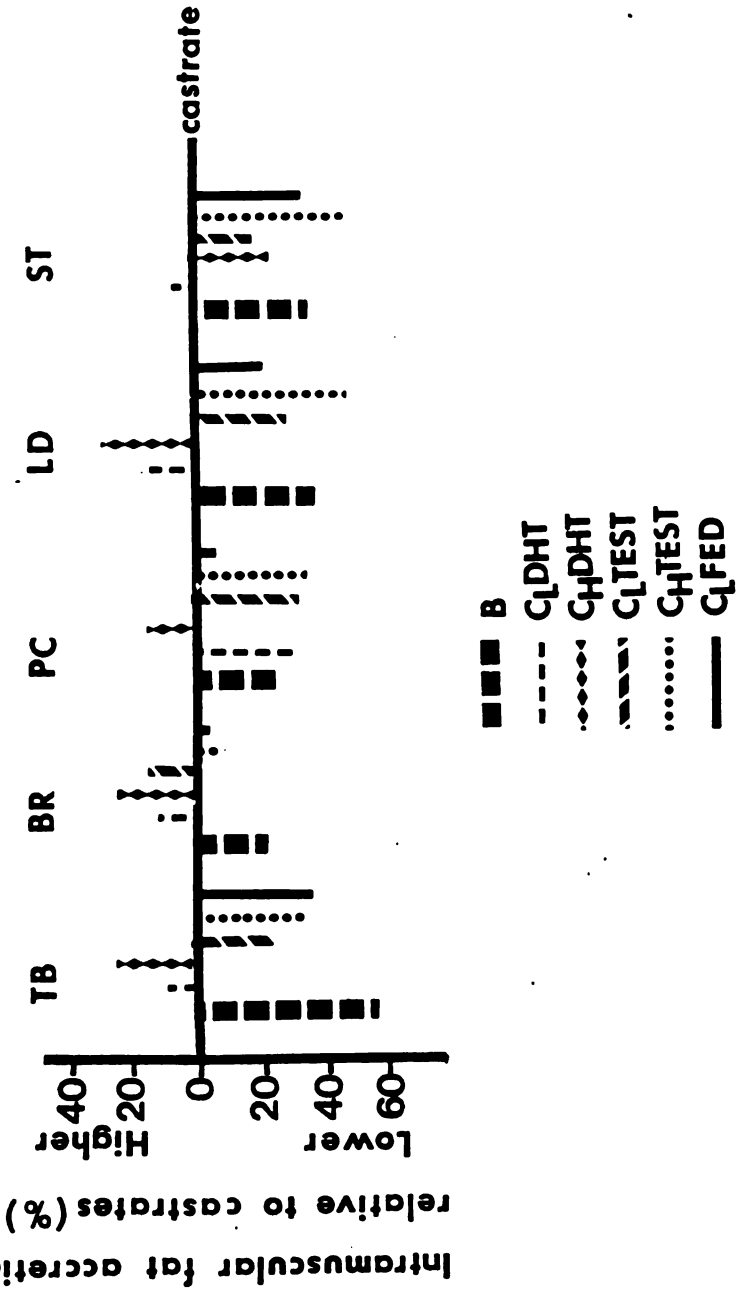


FIGURE IV-3. Intramuscular fat accretion in the triceps brachii, brachialis, pectineus, longissimus and semitendinosus muscles of boars, androgen implanted castrates and limit-fed castrates of the prepubertal study as a percentage of castrates.

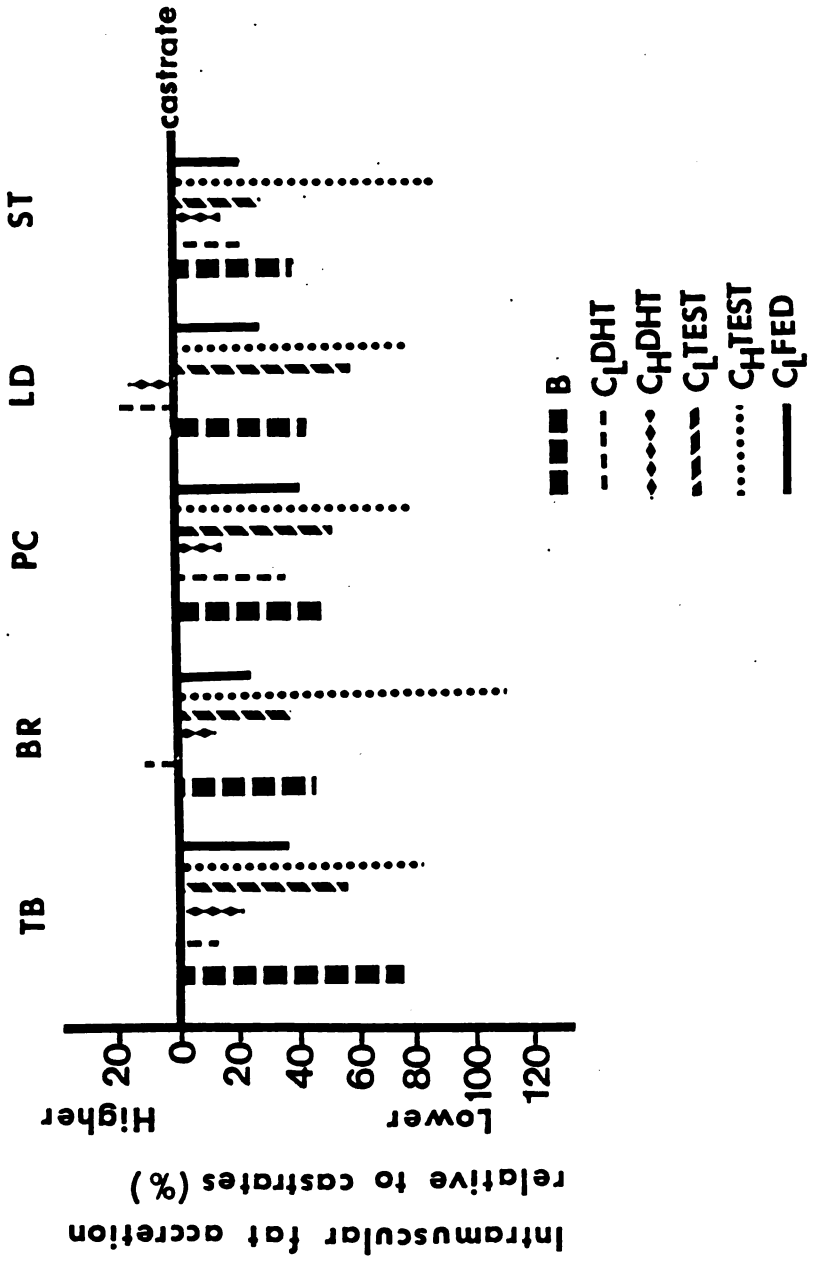


FIGURE IV-4. Intramuscular fat accretion in the triceps brachii, brachialis, pectineus, longissimus and semitendinosus muscles of boars, androgens implanted castrates and limit-fed castrates of the pubertal study as a percentage of castrates.

that both implants levels of DHT were ineffective in preventing intramuscular fat deposition but the high dose of TEST reduced intramuscular fat deposition. This indicates that TEST and DHT differ in their effect upon intramuscular fat deposition and supports the hypothesis that testosterone must be aromatized to estrogen metabolites to mediate effects on fat deposition (Wade and Gray, 1979). However, another alternative hypothesis is that reduction of TEST to DHT is minimal, whereas DHT is rapidly converted to diols (Warrenski and Almon, 1983). Limit-feeding castrates also depressed the castration associated lipid accretion to a level similar to C_HTEST in PREP pigs; however, C_HTEST in the POSTP study was more effective than the limit-fed castrates.

In order to assess the effects of castration and TEST or DHT upon fat-free muscle accretion or essentially protein plus water accretion, the quantities of fat in tables IV-10 through 13 were subtracted from the muscle weights presented in tables IV-2 and 3. These fat-free muscle weights (FFMW) are presented in tables IV-14 and 15 for the PREP and POSTP studies, respectively. Fat-free muscle weights of initial pigs were subtracted from the treatment groups of pigs and these net values are expressed as a percentage of the net change incurred by castrates. These relative individual muscle FFM acretion rates are illustrated in figures IV-5 and 6 for the PREP and POSTP

TABLE IV-14 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON FAT-FREE TRICEPS BRACHII, BRACHIALIS, LONGISSIMUS, PECTINEUS AND SEMITENDINOSUS MUSCLE WEIGHTS

Treatment Group[c]	Muscle[ab]				
	Triceps Brachii	Brachialis	Longissimus	Pectineus	Semitendinosus
B	531.2^{fg}	78.5^{fg}	1288.7^{f}	70.2^{f}	279.1^{f}
C	448.4^{e}	62.8^{e}	1066.2^{e}	58.1^{e}	231.1^{e}
C_LDHT	514.2^{fg}	73.6^{fg}	1321.8^{fg}	64.4^{ef}	258.1^{ef}
C_HDHT	495.8^{ef}	75.9^{fh}	1283.4^{f}	55.7^{e}	252.6^{ef}
C_LTEST	485.4^{ef}	68.9^{eg}	1203.6^{f}	59.8^{e}	255.5^{ef}
C_HTEST	553.6^{g}	80.1^{h}	1436.1^{g}	61.7^{ef}	273.0^{f}
C_LFED	486.5^{ef}	66.6^{e}	1235.3^{f}	64.6^{ef}	239.6^{e}
SEM[d]	16.33	1.65	42.28	3.04	8.95
Initial Slaughter Group	168.9	25.4	348.5	19.0	77.96

[a] Fat free muscle weight in grams.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d] SEM=standard error of least squares means.

TABLE IV-15 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON FAT-FREE TRICEPS, BRACHIALIS, LONGISSIMUS, PECTINEUS AND SEMITENDINOSUS MUSCLE WEIGHTS

	Muscle[ab]				
Treatment Group[c]	Triceps Brachii	Brachialis	Longissimus	Pectineus	Semitendinosus
B	1703.5^{f}	258.9^{g}	3967.3	198.2^{f}	848.2^{f}
C	1280.1^{e}	194.7^{ef}	3578.4	162.6^{ef}	690.5^{e}
C_LDHT	1411.3^{e}	203.2^{ef}	3957.1	159.4^{e}	754.5^{ef}
C_HDHT	1472.9^{ef}	216.6^{f}	4158.0	173.5^{ef}	798.0^{f}
C_LTEST	1485.0^{ef}	214.5^{f}	4053.6	172.9^{ef}	794.8^{f}
C_HTEST	1453.9^{e}	198.0^{ef}	3988.9	183.6^{ef}	817.7^{f}
C_LFED	1345.2^{e}	188.3^{e}	3628.1	174.0^{ef}	671.2^{e}
SEM[d]	70.75	7.41	167.66	8.76	30.88
Initial Slaughter Group	920.2	135.6	2286.8	107.00	473.7

[a]Fat free muscle weight in grams.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least squares means.

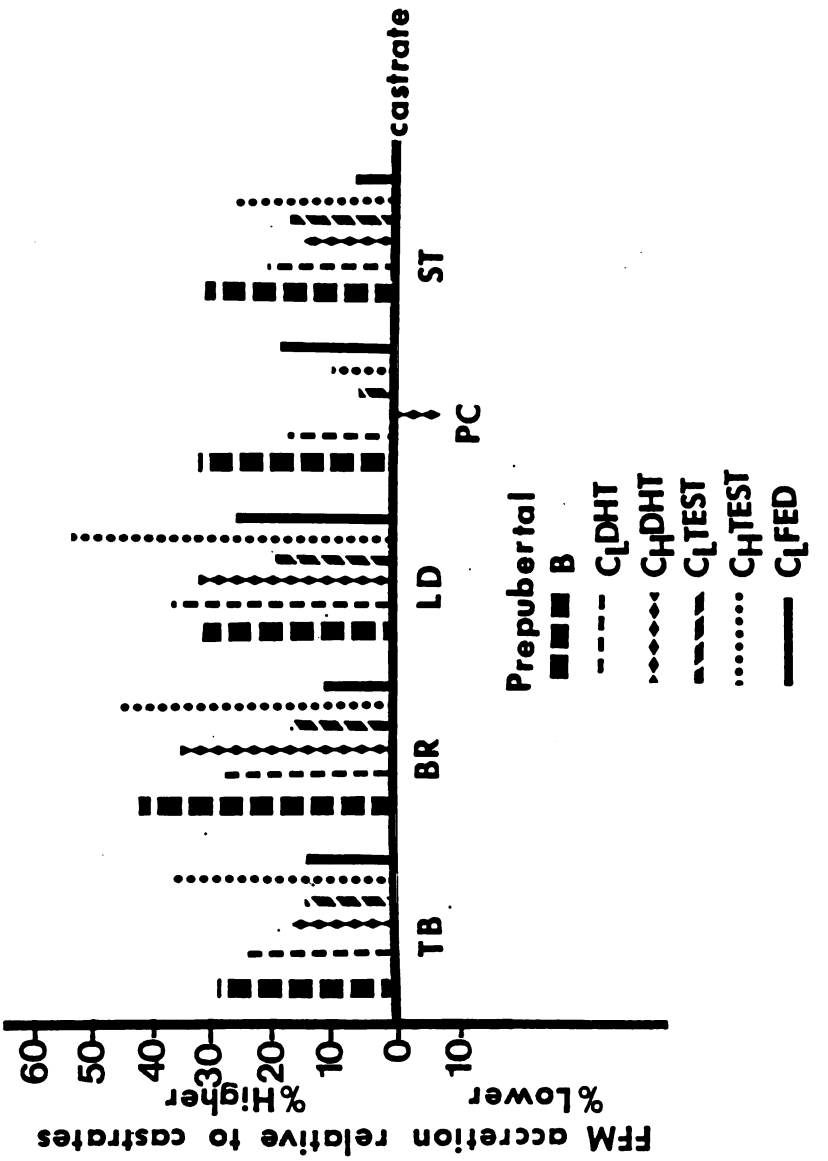


FIGURE IV-5. Fat-free triceps brachii, brachialis, longissimus, platineus and semitendinosus muscle accretion of boars, androgen implanted castrates and limit-fed castrates of the prepubertal study as a percentage of castrates.

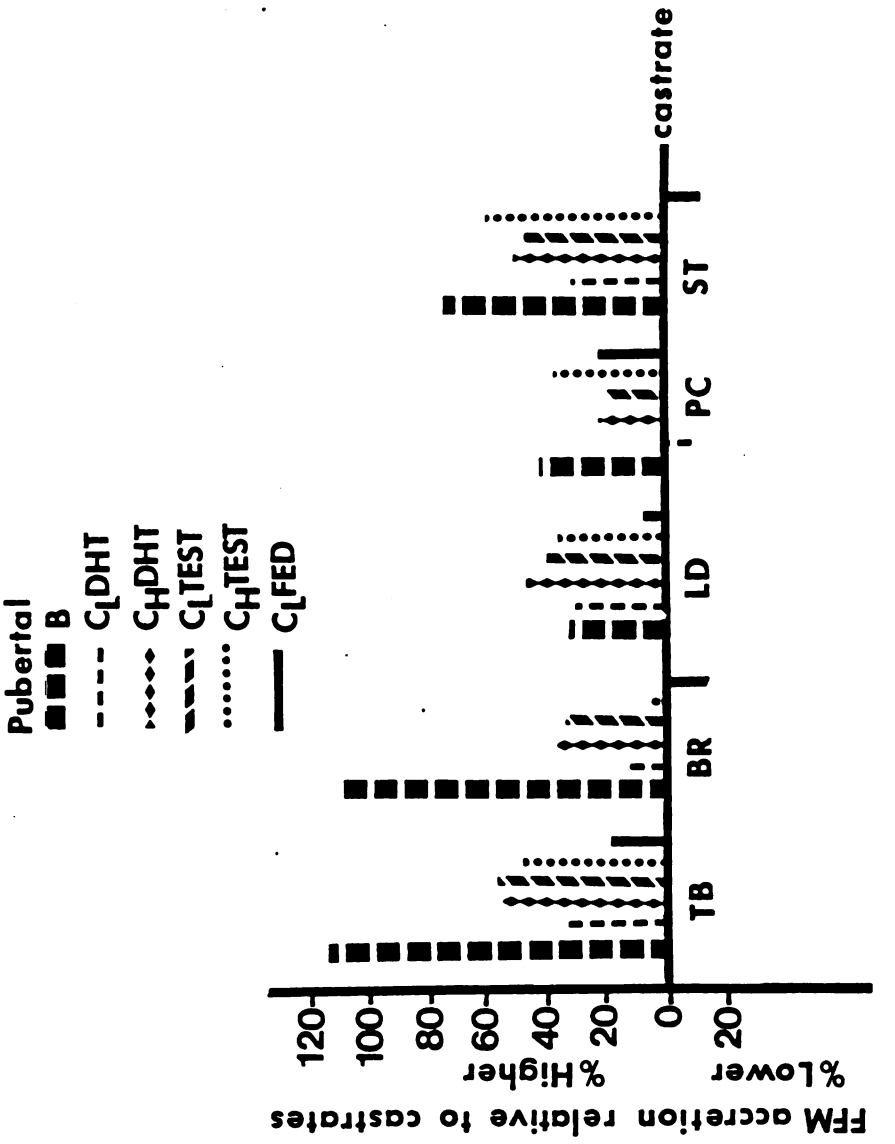


FIGURE IV-6. Fat-free triceps brachii, brachialis, longissimus, pectineus and semitendinosus muscle accretion of boars, androgen implanted castrates and limit-fed castrates of the pubertal study as a percentage of castrates.

studies, respectively. It appears that castration prepubertally and pubertally affected FFM accretion for the TB, BR, LD, PC and ST muscles. The effect of castration was much more evident in the POSTP pigs than for PREP pigs. All androgen treated groups had greater FFM weights (except for the PC) than castrates. However, muscles varied in the degree of response.

The effect of castration in this study resulted in a depression of FFM accretion in selected muscles. This observation is not consistent with data of Knudson (1983) who reported that 105 kg boars and castrates did not differ in fat-free LD, ST or BR muscles. However, there were tendencies for boars in that study to have 13 and 12% heavier fat-free ST and BR muscles, respectively, than castrates. Muscles have differential growth patterns and some muscles exhibit these patterns at different stages of growth (Richmond and Berg, 1982). Increased growth in the neck and shoulder muscles has been observed for bulls (Brannang, 1971), rams (Lohse, 1973) and boars (Walstra, 1980). Whether the age of castration influences differential muscle growth patterns has not been tested in pigs. Since muscles have individual growth patterns, genetic, nutritional and endocrine influences on muscle growth may be different depending upon the stage of development of a muscle or muscle group at the time of imposing the particular treatment.

In the present study, the response to castration was observed on selected muscles both prepubertally and pubertally. The response to exogenous testosterone on selected muscles has not been previously demonstrated in pigs. However, similar types of responses on muscles have been observed in testosterone treated guinea pigs (Kochakian et al., 1956). In contrast, previous studies with testosterone administration to pigs have indicated only minimal responses to overall body composition (Sleeth et al., 1953; Bratzler et al., 1954). Differences in the mode of hormone delivery (injection versus implant) may explain some of the differences in total carcass composition between this study and other.

Production of as much lean as is economically possible is important to the swine industry. The boar is clearly one alternative for this goal because of their enhanced muscle deposition over castrates. However, the boar is not compatible with the current marketing scheme in the United States due to boar taint at typical market weights. Alternatively, boars could be marketed at lighter weights or castrates could be implanted with androgens.

Summary

In the 3 wk study, the triceps brachii and semimembranosus muscles were 1.1- and 1.2-fold heavier than castrates. In both 5 wk studies, boars had heavier triceps

brachii, brachialis, longissimus, pectineus, teres major and semitendinosus muscles than castrates. Androgen treatment of prepubertal pigs appeared to be more effective in significantly increasing muscle weights over castrates than in pubertal pigs. Of the muscles examined, only the semitendinosus was significantly increased in weight over castrates by the high testosterone implant.

High testosterone implanted pigs as well as boars had less intramuscular fat than castrates. However, the fat-free muscle weights followed similar trends between treatments as wet muscle weights. All androgen treated pigs tended to have heavier fat-free triceps brachii, brachialis, longissimus and semitendenosus weights were significantly heavier. These data suggest that some muscles are more responsive to androgens than others and that testosterone may be more effective in stimulating muscle growth than dihydrotestosterone.

CHAPTER V

EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGEN UPON IN VITRO PROTEIN SYNTHESIS AND DEGRADATION RATES

Introduction

Practical and efficient means of enhancing skeletal muscle protein accretion in growing meat animals is of considerable interest. To study protein accretion, precise measurements of protein synthesis and degradation rates must be determined. The most appropriate methods for measuring skeletal muscle protein synthesis rates are by continuous infusion of radiolabeled amino acids (Waterlow et al., 1978) or a flooding dose (tracer + unlabeled amino acid) method (McNurlan et al., 1982; Goldspink et al., 1983). For experiments with large animal species, these methods can be costly. Recently, in vitro muscle incubation methods (Fulks et al., 1975) have been employed to study changes in protein synthesis and degradation rates during compensatory (Goldspink et al., 1983) and normal (Hentges et al., 1983) growth situations, as well as, in animals varying in endocrine and nutritional status (Goldberg, 1980). Moderate to large differences in muscle growth rates are readily detectable by this method.

Intact male cattle, sheep and swine have greater muscle accretion rates relative to castrated males (Galbraith and Topps, 1981). In addition, boars have higher metabolizable energy expenditures for maintenance than castrates (Fuller et al., 1980; Walach-Janiak, et al., 1980). Presence of the testes or administration of exogenous testosterone enhances incorporation of labeled amino acids into skeletal muscle proteins (Grigsby et al., 1976) and growth of skeletal muscles (Kochakian, 1976).

Protein deposition is associated with elevated protein turnover (Kielanowski, 1976; Reeds et al., 1980). Administration of exogenous testosterone also mobilizes amino acids (Kochakian, 1976). A synthetic analog of testosterone, trenbolone acetate, apparently decreases both skeletal muscle protein synthesis and degradation rates in female rats but accretion of protein is increased due to a disproportionate depression of degradation rates (Vernon and Buttery, 1976; 1978). Testosterone and trenbolone acetate may mediate their effects by different mechanisms.

It was the objective of this study to investigate the effects of castration and administration of testosterone or dihydrotestosterone to castrated prepubertal and pubertal male pigs upon in vitro skeletal muscle protein synthesis and degradation rates.

Materials and Methods

In Vitro Protein Synthesis and Degradation. Immediately after cessation of bleeding of pigs at slaughter, the left semitendinosus muscle was exposed and strips of muscle bundles were obtained by blunt dissection. Clamps were constructed from welding 2 Mueller alligator clips to a metal wire to separate the clamps by about 1 cm. The muscle strips were clamped to maintain passive tension and then excised. The muscle strips restrained within the teeth of the clamp weighed approximately 100 mg and were less than 2 mm thick. The method described by Fulks et al. (1975) were used to estimate protein synthesis and degradation rates. All strips were preincubated in oxygenated Krebs Ringer bicarbonate (KRB) buffer for .5 h. Incubation (2.5 h) in oxygenated KRB buffer (pH 7.4) containing insulin (.1U/ml), glucose (10mM), amino acids (5X plasma concentration; Appendix B-1) and U-[^{14}C] L-tyrosine (.2 uCi/ml) was used for measurement of protein synthesis rates. For determination of degradation rates, strips were incubated for 3 h in the same buffer as described above except that no labeled or unlabeled tyrosine was added and cycloheximide (.5mM) was added to inhibit protein synthesis. At the end of the incubation, incubation vials were placed on ice, the muscle strips weighed to the nearest milligram and then placed in corex tubes containing 1 ml of phosphate buffer (Appendix B-2).

Muscle strips were homogenized by a Brinkman polytron (setting 7) by giving 3 intermittent 20 sec bursts. The polytron tip was rinsed with 1 ml of phosphate buffer into the homogenate tube to which 500 ul of cold 50% TCA were added. Homogenates were centrifuged at 30,000 g for 20 min and then the supernatants or the intracellular pool was decanted and saved. The precipitates were washed two additional times with 5% TCA and the washings pooled with the original intracellular (IC) fraction. Precipitate and IC fractions were washed 3 times with anhydrous ether to extract TCA. Aliquots of the IC pool for synthesis were counted for radioactivity. Dried protein precipitates from synthesis incubations were digested in 500 ul NCS tissue solubilizer (Amersham) and counted for radioactivity. Media or the extracellular pool (EC) from degradation incubations were precipated with 50% TCA and handled similarly to the IC pools. Aliquots of the IC pool for synthesis and degradation and EC pools for degradation were analyzed for tyrosine contents by the fluorometric procedure described by Ambrose (1974).

Results and Discussion

Figure V-1 illustrates results of preliminary work for in vitro protein synthesis. Incorporation of radiolabel into protein was linear for the 2.5 h time period routinely used in the experiments. In addition, the intracellular

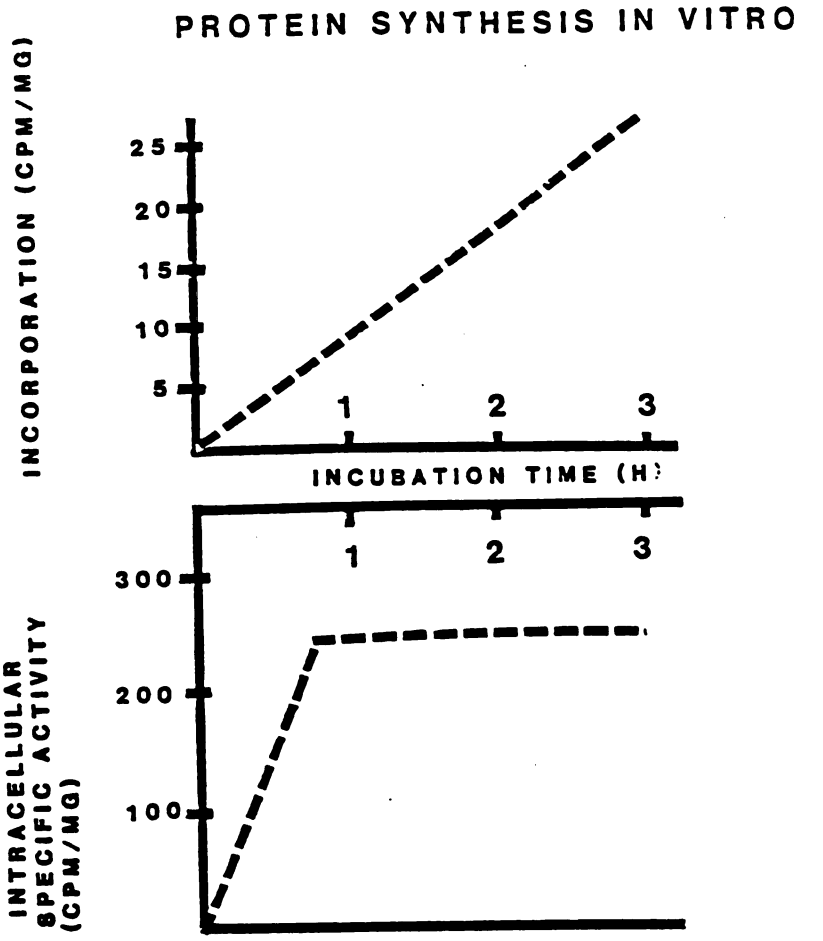


FIGURE V-1. Time course incorporation of label into protein and specific radioactivity of intracellular pool.

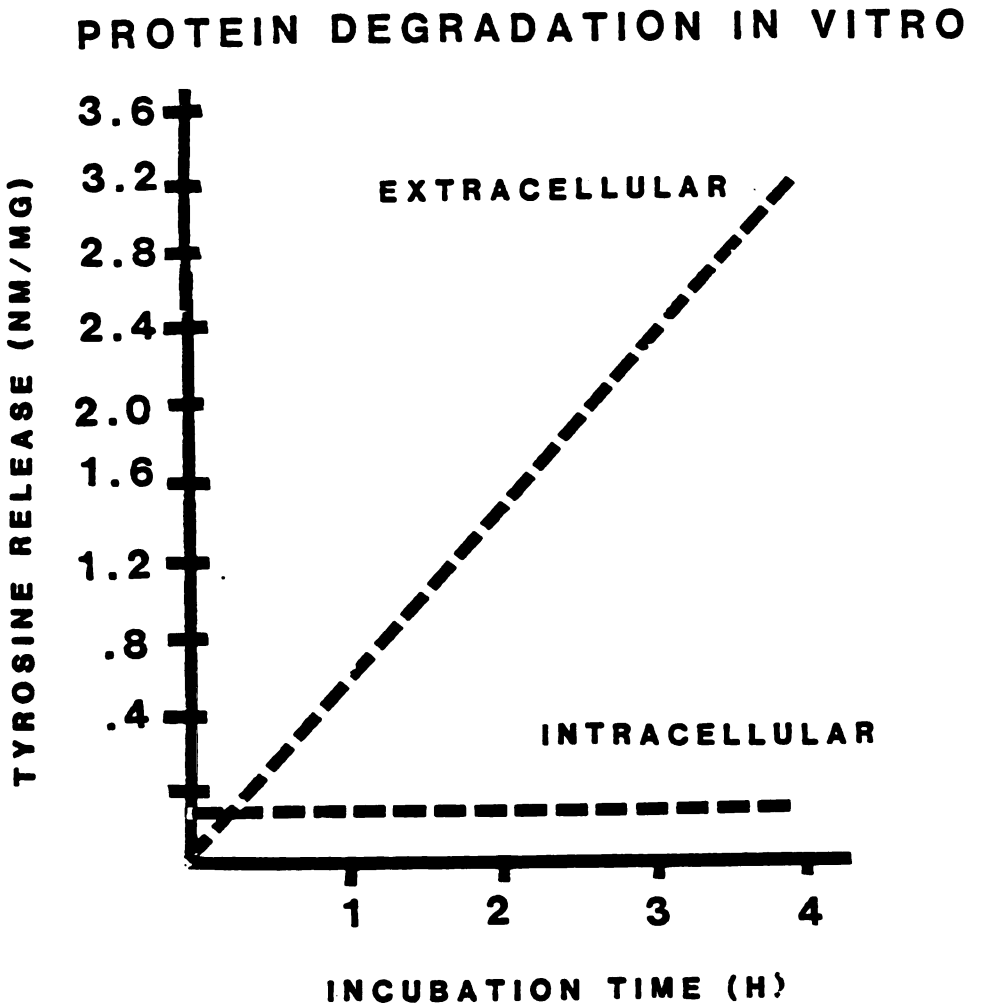


FIGURE V-2. The linear time release of tyrosine from muscle strips.

pool was in equilibrium with the extracellular pool and specific radioactivities were constant (after an initial lag) for the period of incubation. Figure V-2 illustrates the linear time release of tyrosine from muscle strips for over 3 h with essentially no change in the intracellular content of tyrosine.

The effects of castration and administration of testosterone (TEST) or dihydrotestosterone (DHT) to castrated male pigs upon in vitro protein synthesis and degradation rates in prepubertal (PREP) and pubertal (POSTP) pigs are presented in tables V-1 and 2, respectively. In the PREP study, castration resulted in a 49 and 61% reduction in in vitro protein synthesis rates for the white and red portions of the semitendinosus (ST) muscle, respectively. Due to differences in synthesis rates between red and white muscle strips, interpretation of hormone administration is complicated. In general, red portion strips had 3 to 5% higher synthesis rates than white strips from castrates and the implanted groups. However, red strips from boars had 34% higher synthesis rates than white strips. This could possibly mean the presence of the testes and factors other than testosterone differentially affect red muscle more than white muscle fibers. Only the high TEST (C_HTEST) implanted pigs had synthesis rates similar to boars, but they had 76% higher rates than castrates for white muscle strips. Although

TABLE V-1 EFFECT OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON RED AND WHITE SEMITENDINOSUS MUSCLE PROTEIN SYNTHESIS AND DEGRADATION RATES MEASURED IN VITRO[a,b]

Treatment Groups[c]	Protein synthesis rate		Protein degradation rate	
	White portion	Red portion	White portion	Red portion
B	55.89^{g}	75.12^{h}	479.06^{f}	517.78^{g}
C	28.32^{e}	29.17^{e}	402.67^{f}	501.67^{g}
C_LDHT	32.35^{e}	37.62^{ef}	220.77^{e}	295.84^{f}
C_HDHT	42.77^{efg}	44.60^{fg}	178.90^{e}	234.47^{ef}
C_LTEST	36.10^{ef}	35.76^{ef}	166.13^{e}	220.78^{ef}
C_HTEST	49.76^{fg}	52.13^{g}	130.20^{b}	147.21^{e}
SEM[d]	5.06	4.30	62.53	35.56

[a]Data expressed as picomoles of tyrosine incorporated into protein or released to media/mg muscle/h.
[b]Means within columns with different superscripts differ ($P < .05$).
[c]B=boars, C=castrates, C_L=low implants, C_H=high implants.
[d]SEM=standard error of least square means.

TABLE V-2 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON RED AND WHITE SEMITENDINOSUS MUSCLE PROTEIN SYNTHESIS AND DEGRADATION RATES MEASURED IN VITRO[a,b]

Treatment Groups[c]	Protein synthesis rate		Protein degradation rate	
	White portion	Red portion	White portion	Red portion
B	31.69^{f}	50.45^{f}	455.12^{g}	398.89^{g}
C	20.77^{e}	34.02^{e}	334.08^{f}	301.00^{f}
C_LDHT	24.81^{e}	36.00^{ef}	257.39^{ef}	223.96^{e}
C_HDHT	35.69^{f}	31.81^{e}	209.76^{e}	312.50^{f}
C_LTEST	30.18^{f}	33.77^{e}	216.67^{e}	276.63^{ef}
C_HTEST	31.87^{f}	38.47^{ef}	229.66^{e}	208.97^{e}
SEM[d]	1.99	4.50	24.59	17.71

[a]Data expressed as picomoles of tyrosine incorporated into protein or released to media/mg muscle/h.

[b]Means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[d]SEM=standard error of least square means.

nonsignificant, other treatments tended to have slightly higher protein synthesis rates in the white portion. For the red portion strips, high DHT (C_HDHT) and (C_HTEST) pigs had 53 and 79% higher synthesis rates, respectively, than castrates but 41 and 31% lower rates, respectively, than boars. The synthesis rates for the red muscle strips for C_HDHT and C_HTEST were 19 and 31% higher, respectively, than the corresponding low implant treatment groups. These data indicate a dose response trend for DHT and TEST with TEST also being more effective than DHT in stimulating synthesis rates in red muscle of PREP pigs.

Protein degradation rates were not different for either white or red muscle strips from PREP boars or castrates. However, red strips from boars and castrates had 8 and 25% higher degradation rates than white, respectively. This trend for higher degradation rates in red relative to white muscle strips was also observed for the androgen treated groups and is consistent with reports by Millward (1980). Administration of TEST in the present studies depressed degradation rates relative to boars and castrates. While degradation in red muscle strips of low DHT (C_LDHT) implanted pigs was 2-fold higher than C_HTEST pigs, no other differences existed between the implanted groups. Nevertheless, subtle dose response trends toward decreased degradation rates were apparent for both red and white muscle strips. C_HDHT implanted PREP pigs had about 20%

lower ($P < .05$) degradation rates than C_LDHT pigs and muscle strips from C_HTEST pigs had 22 and 33% lower ($P < .05$) degradation rates for white and red strips, respectively.

For POSTP pigs (table V-2), castrates had 34.5 and 32.6% lower synthesis rates than boars in white and red muscle strips, respectively. This castration associated reduction is lower than the 49 to 61% reduction shown in the PREP pig muscles. Synthesis rates in red muscle strips were 59 to 64% higher than white muscle strips for boars and castrates, respectively. Proportionately, these differences between red and white strips within a treatment group are greater than were observed in PREP pigs indicating the age of castration may be influencing these differences. Except for red strips from C_HDHT pigs, red muscle strips of androgen implanted pigs tended to have higher ($P < .05$) synthesis rates than castrates but all implanted groups had lower rates than boars. Except for white muscle strips of C_LDHT pigs, implanted pigs had about 45 to 72% higher white muscle strip synthesis rates than castrates. POSTP castrates had 26.6 and 24.5% lower degradation rates than boars for the white and red muscle strips, respectively. For degradation rates of white strips, implanted pigs had lower degradation rates than either castrates or boars. No dose dependency was observed but white strips from C_HDHT, C_LTEST and C_HTEST pigs,

were approximately 34.5 and 52% lower than castrates and boars, respectively, and red strip synthesis rates were consistently 36% lower than boars. Only red strips from C_LDHT and C_HTEST pigs were different (25.6 and 30.6% lower, respectively) from castrates. Degradation rates appeared to be depressed by androgen treatment. Even though, red and white strips differed ($P < .05$) in synthesis and degradation rates for boars and castrates, these data were averaged and the averages are presented in tables V-3 and 4 for PREP and POSTP pigs, respectively. The averaged data showed similar trends to those previously discussed for separate red and white muscle strips. In order to compare treatment effects, the data of each treatment group were expressed as a percentage of the boar values. The synthesis and degradation rates relative to boars are presented in table V-5. The percentages for synthesis and degradation rates of castrates and implanted pigs were all lower than boars (negative). The lower the percentage, the closer the rate was to that of boars. Low implant, PREP pigs were not different from castrates, and high androgen implanted pigs had higher synthesis rates than low implants but they were still lower than boars. A dose response trend appeared to have occurred. Implanting PREP pigs with DHT or TEST decreased degradation rates relative to boars or castrates. In addition, a subtle dose response trend was detectable.

TABLE V-3 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON PROTEIN SYNTHESIS AND DEGRADATION RATES MEASURED IN VITRO AVERAGED FOR THE RED AND WHITE PORTIONS OF THE SEMITENDINOSUS MUSCLE[a,b]

Treatment Group[c]	Protein Synthesis Rate	Protein Degradation Rate
B	65.5^{h}	498.42^{f}
C	28.74^{e}	452.17^{f}
C_LDHT	34.99^{ef}	258.30^{e}
C_HDHT	43.68^{fg}	206.69^{e}
C_LTEST	35.93^{ef}	193.45^{e}
C_HTEST	50.94^{g}	138.70^{e}
SEM[d]	3.97	39.87

[a]Data expressed as picomoles of tyrosine incorporated into protein or released to media/mg muscle/h.

[b]Means within columns with different superscripts differ ($P < .05$)

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[d]SEM=standard error of least square means.

TABLE V-4 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON PROTEIN SYNTHESIS AND DEGRADATION RATE MEASURED IN VITRO AVERAGED FOR THE RED AND WHITE PORTIONS OF THE SEMITENDINOSUS MUSCLE[a,b]

Treatment Group[c]	Protein synthesis rate	Protein degradation rate
B	41.07^{g}	427.00^{g}
C	27.40^{e}	317.54^{f}
C_LDHT	30.40^{ef}	240.68^{e}
C_HDHT	33.75^{ef}	261.13^{e}
C_LTEST	31.98^{ef}	246.64^{e}
C_HTEST	35.17^{fg}	219.32^{e}
SEM[d]	1.75	12.78

[a]Data expressed as picomoles of tyrosine incorporated into protein or released to media/mg muscle/h.

[b]Means within columns with different superscripts differ ($P < .05$)

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[d]SEM=standard error of least square means.

TABLE V-5 EFFECTS OF CASTRATION AND ANDROGEN ADMINISTRATION TO CASTRATED MALE PIGS UPON IN VITRO MUSCLE PROTEIN SYNTHESIS (PSR) AND DEGRADATION (PDR) RATES OF PREPUBERTAL AND PUBERTAL MALE PIGS[a,b,c]

Treatment Group[d,e]	Prepubertal		Pubertal	
	PSR[a]	PDR[b]	PSR[a]	PDR[b]
B	65.5	498.4	41.1	427.0
	---------Rates relative to boars, %[c]---------			
C	-56.2	-9.3	-33.3	-25.6
C_LDHT	-46.6	-48.2	-26.0	-43.6
C_HDHT	-33.3	-58.5	-18.0	-38.8
C_LTEST	-45.2	-61.2	-22.1	-42.2
C_HTEST	-22.3	-72.2	-14.4	-48.6

[a]Data for boars expressed as picomole tyrosine incorporated into protein or released to media/mg muscle/h.

[b]Other treatments besides boars are expressed as a percentage of boars.

[c]Rates for white and red portions of the semitendinosis were averaged.

[d]DHT=dihydrotestosterone, TEST=testosterone.

[e]B=boars, C=castrates, C_LDHT=low DHT implants, C_HDHT=high DHT implants, C_LTEST= low TEST implants, C_HTEST=high TEST implants.

For POSTP pigs, the averaged red and white muscle strip synthesis and degradation rates are presented in tables V-4 and 5. In general, synthesis rates were only 33% lower than boars. As indicated by lower percentages in table V-5, a dose response trend appeared to have occurred even though only the synthesis rates of C_HTEST pigs were different ($P < .05$) from castrates and similar ($P > .05$) to boars. In general, these protein synthesis data are consistent with the increased muscle ribosome activity of testosterone treated castrate rats compared to castrated rats observed by Florini (1970). Degradation rates were also 26% lower in POSTP castrates than boars and implanted pigs were lower than either boars or castrates. In contrast to the PREP pigs, no dose responses for TEST or DHT upon protein degradation rates were apparent in this study. In comparing PREP synthesis rates to POSTP rates, it appeared that synthesis rates were 37 and 5% lower (significance not tested) for POSTP boars and castrates, respectively, relative to their PREP counterparts. Degradation rates were 14 and 30% lower (significance not tested) in POSTP boars and castrates, respectively, relative to their PREP counterparts.

The effects of providing tension to in vitro incubated muscles alters responsiveness to nutrients in the incubation media and can increase synthesis and decrease degradation rates (Etlinger et al., 1980). While attempts

were made to be consistent in the amount of stretch or tension applied to strips used in this study, variation no doubt occurred in these studies. The degree of stretching alters prostaglandin synthesis in incubated muscles (Smith et al., 1983) and $PGF_2{}^{\alpha}$ and PGE_2 have been shown to influence protein turnover in in vitro muscle incubations (Rodemann and Goldberg, 1982).

Generally in vitro muscle protein synthesis and degradation rates are obtained by incubation of whole thin muscles. Our experiments and those of Hentges et al. (1983) involved muscles that had been cut. However, based upon preliminary data and inulin space, incubation of strips with the clamps to maintain tension had no adverse effects upon either synthesis or degradation rates. Strips incubated with the clamps had lower degradation rates than free floating muscle strips.

It appears that the muscle strip technique used in this study was sensitive enough to detect differences in rates of protein synthesis and degradation between boars, castrates and androgen implanted castrates. The tendency for higher synthesis and higher degradation rates and therefore higher protein turnover in boars relative to castrates is not inconsistent with higher metabolic rates of boars (Thorbek, 1975) relative to castrates nor for the model of increased protein turnover with increased heat production and greater protein deposition rates

(Kielenowski, 1976; Reeds et al, 1980). Fat-free muscle mass accretion rates were similar between PREP boars and castrates but were higher in boars in the POSTP study (Chapter III). Semitendinosus muscle accretion was highest in the boar in both studies (Chapter IV). Thus, is a definite correlation in this study between fat-free semitendinosis muscle accretion rates and both increased protein synthesis and degradation rates as measured in vitro. Since the magnitude of difference of in vitro protein synthesis and degradation rates between boars and castrates was greater for protein synthesis rates, it appears that greater semitendinosus muscle accretion occurred in boars because of higher synthesis rate relative to degradation rate. In fact, if a ratio of synthesis rate to degradation rate is calculated, boars had higher ratios than castrates in both studies.

Castration decreased in vitro protein synthesis and degradation rates but synthesis rates were decreased to a greater extent than degradation. TEST and DHT were shown to partially counteract the castration associated fall in synthesis rates but implants also appeared to decrease degradation rates. These responses need to be verified by in vivo mesurements. However, if these in vitro measurements are a true qualitative reflection of the in vivo action of androgen (either direct or indirect) then it would appear that the mechanism of action of these two

androgens is mediated differently than trenbolone acetate which decreases both in vivo synthesis and degradation rates (Vernon and Buttery, 1978). In addition, the differences between boars and androgen implanted castrates in in vitro protein turnover indicate that other factors are probably involved in regulating protein turnover in the boar. Based upon the data from this study, implanting castrated male pigs with androgen may have the potential benefit of increasing the efficiency of muscle protein deposition.

Failure to show direct effects of testosterone upon protein turnover in muscle cell cultures (Allen et al., 1983; Ballard and Francis, 1983) suggest that in vivo testosterone effects are apparently mediated via some indirect mechanism.

Summary

In both prepubertal and pubertal studies, boars had higher in vitro protein synthesis and degradation rates than castrates. This indicates that the greater semitendinosus muscle growth rate may be associated with higher protein turnover rates. Androgens tended to increase protein synthesis rates to castrates. These data suggest that both forms of androgen are mediated by similar mechanisms for affecting muscle protein turnover and would appear to increase protein deposition by increasing protein synthesis and reducing degradation.

CHAPTER VI

EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGENS UPON SKELETAL MUSCLE PROTEINASE ACTIVITY

Introduction

Protein accretion rates in skeletal muscle are dependent upon the net difference between synthesis and degradation rates. While the mechanisms of protein synthesis and measurement of protein synthesis rates can be assessed by a variety of methods (Waterlow et al., 1978), skeletal muscle protein degradation is a poorly understood process (Millward et al., 1981). The rate of skeletal muscle protein degradation is higher during periods of rapid muscle growth than in adult animals (Waterlow et al., 1978). Attempts to correlate protein accretion and turnover to catabolic enzymes of lysosomal and nonlysosomal origin have met with limited success. Millward et al. (1981) illustrated a variety of different growth situations which are correlated to changes in cathepsin D (lysosomal enzyme) activity. Other workers (Mayer et al., 1974, 1980; Noguchi et al, 1974; Dahlmann et al., 1978, 1980, 1981) have demonstrated a high relationship between protein degradation and activity of alkaline proteinases which are closely associated with the myofibrils. In contrast,

McElligott and Bird (1981) reported that these alkaline serine proteinases are of mast cell origin and play a minor role in the degradative process of muscle proteins. However, Dahlmann et al. (1980) and Dahlmann and Reinauer (1981) treated castrated male rats with testosterone and attenuated a castration associated increase in alkaline proteinase activity in skeletal muscles.

The objective of this investigation was to observe the effects of castration and testosterone or dihydrotestosterone administration to prepubertal and pubertally castrated male pigs upon acidic, neutral and alkaline proteinase activity in crude skeletal muscle homogenates.

Materials and Methods

The amount of enzymatic hydrolysis of ^{14}C hemoglobin at pH 3.8, 7.0 and 8.5 was used as indicator of proteolytic activity of semitendinosus muscle extracts. Labeling of porcine hemoglobin was prepared according to Roth et al. (1971). Small batches of hemoglobin were labeled and later pooled. Five hundred milligrams of porcine hemoglobin were dissolved in 15 ml of mega pure water and adjusted to pH 6.1 with .1 N NaOH. Fifty microcuries of $K^{14}CNO$ (52 mCi/mmol; Amersham) dissolved in mega pure water were added to the dissolved hemoglobin. The mixture was incubated at 50 C for 2 h and then stored overnight at 4 C. Two mililiters of mega pure water containing 20 umol of

cysteine hydrochloride (pH 6.1) were added and the mixture incubated at 37 C for 2 h. The solution was transferred to dialysis tubing (12,000 MW cutoff) and dialyzed for 48 h against 8 to 10 changes of deionized water. At the end of the dialysis, .1 ml was counted in 10 ml ACS (liquid scintillation cocktail; Amersham) and resulted in 7000 to 10,000 cpm and after precipitation with 5% TCA, 200 to 500 cpm were observed in the acid soluble fraction. The labeled hemoglobin was stored at -20 C until used.

Skeletal muscle was extracted by methods described by Rothig et al. (1978). Approximately 200 to 300 mg of powdered muscle were transferred into previously weighed, rubber stoppered corex tubes and after equilibration of the sample to room temperature (about 5 min), the quantity of muscle was determined to the nearest .1 mg. Samples were placed on ice and 5 ml of cold .05 M tris-HCL (pH 7.4) buffer containing 1 M KCL and .2% (w/v) Triton x-100 were added. The mixture was then homogenized with a Brinkman polytron at the 7 setting by giving three 20 sec bursts. Homogenates were centrifuged at 3000 X g for 10 min at 4 C and supernatants decanted through a single layer of cheesecloth. Homogenization of the remaining precipitate was repeated as above and after centrifugation, supernatants were pooled. The muscle extracts (supernates) were brought to equal volumes (such that protein concentrations were less than 5 mg/ml) with buffer and

stored at -20 C until assayed for proteolytic activity and protein (Lowry et al., 1951).

After preliminary testing, a standard incubation system was used for determination of proteolytic activity in the muscle extracts. Routinely, 200 ul of muscle extract, 125 ul of labeled hemoglobin and 200 ul of citrate-phosphate-borate buffer (appendix B-2) were incubated at 37 C in a shaking water bath for 2 h. The reaction was stopped by addition of 200 ul of cold 50% (w/v) TCA. Precipitated protein was spun down at approximately 10,000 X g for 10 min. Control mixtures or blanks containing all components except enzyme or muscle extract were incubated in parallel with the assay and then muscle extract was added. The acid soluble supernatants resulting after centrifugation were extracted 4 times with 5 ml of ether to rid the system of TCA and then aliquots were counted in 10 ml of ACS (Amersham) counting scintillant. Powdered muscle extracts from the red and white portions of the semitendinosus muscle were assayed at pH 3.8, 7.0 and 8.5. Choice of these pH environments was based upon preliminary studies which are explained in the results section. Activities were expressed as blank corrected cpm per milligram protein of muscle extract added.

Results and Discussion

In preliminary experiments, the pH dependency of proteolytic activity in the crude muscle extracts obtained from a fasted and a normal ad libitum fed castrated male pig was assayed. Figure VI-1 shows the pH dependence of proteolytic activity in which similar trends for peak activities at specific pH values were observed for both the fasted and normal pig. Even though activities in the acidic, neutral and alkaline range were higher for the fasted pig relative to the normal pig, this preliminary experiment was not replicated and definitive statements concerning fasting can not be made. Nevertheless, Rothig et al. (1978) reported similar results in rats and even a larger relative difference of activity at pH 9.0 between diabetic and normal rats. Based upon the preliminary pH dependency data, the major peaks were detected at pH 3.8, 7.0 and 8.5 and thus these 3 pH values were selected for all subsequent assays.

In other preliminary work, 4 ratios of ^{14}C-hemoglobin to crude muscle extract were incubated for .5, 1, 2 and 3.5 h. These data are shown in figure VI-2 and indicated some substrate limitation by 3.5 h when the amount of extract exceeded that of labeled substrate by 2-fold. However, for a 2 h incubation, linear activity was observed at all ratios of substrate to enzyme tested. Thus, all subsequent incubations were conducted for a 2 h period.

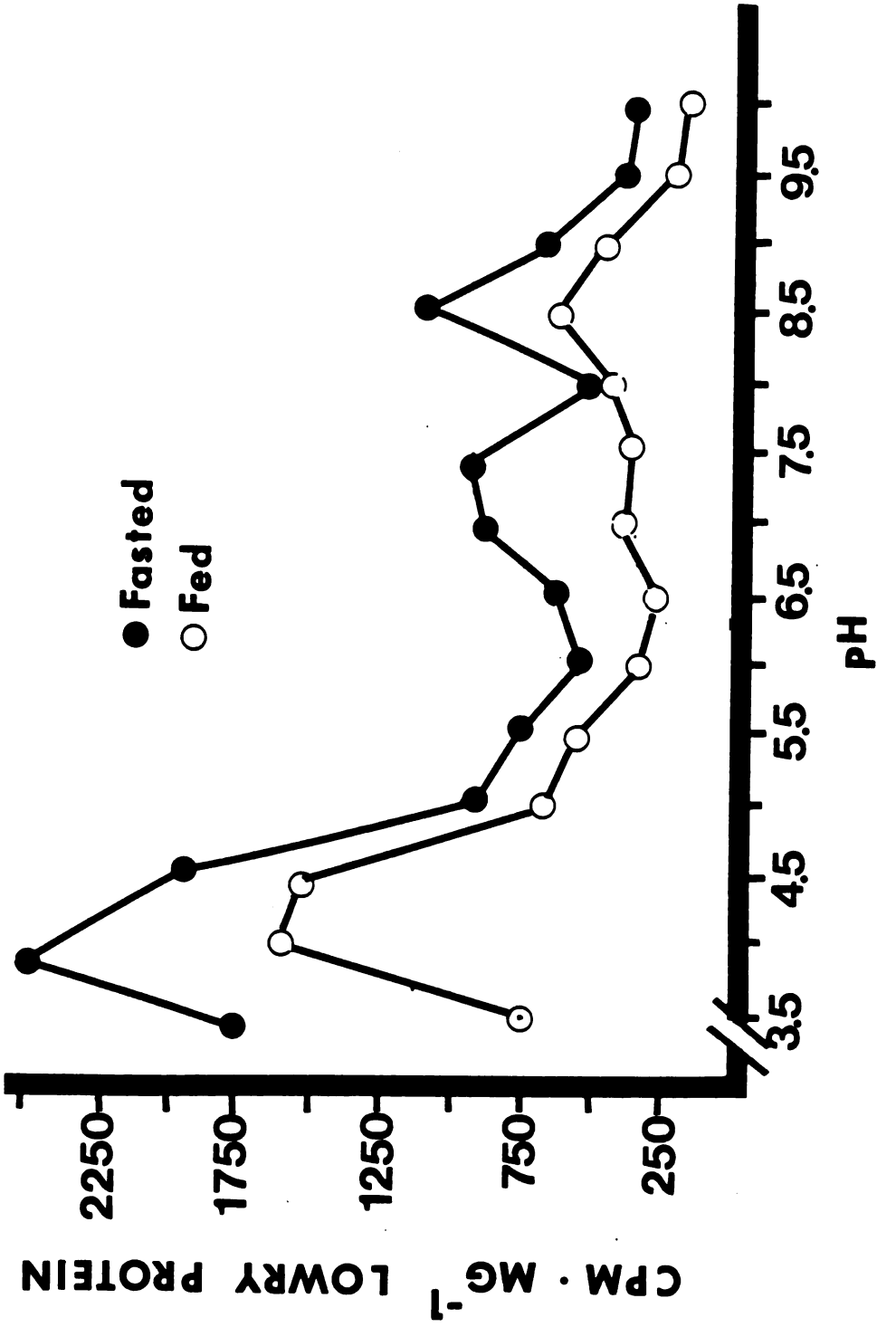


FIGURE VI-1. pH dependence of proteolytic activity of muscle extracts from fasted and fed pigs.

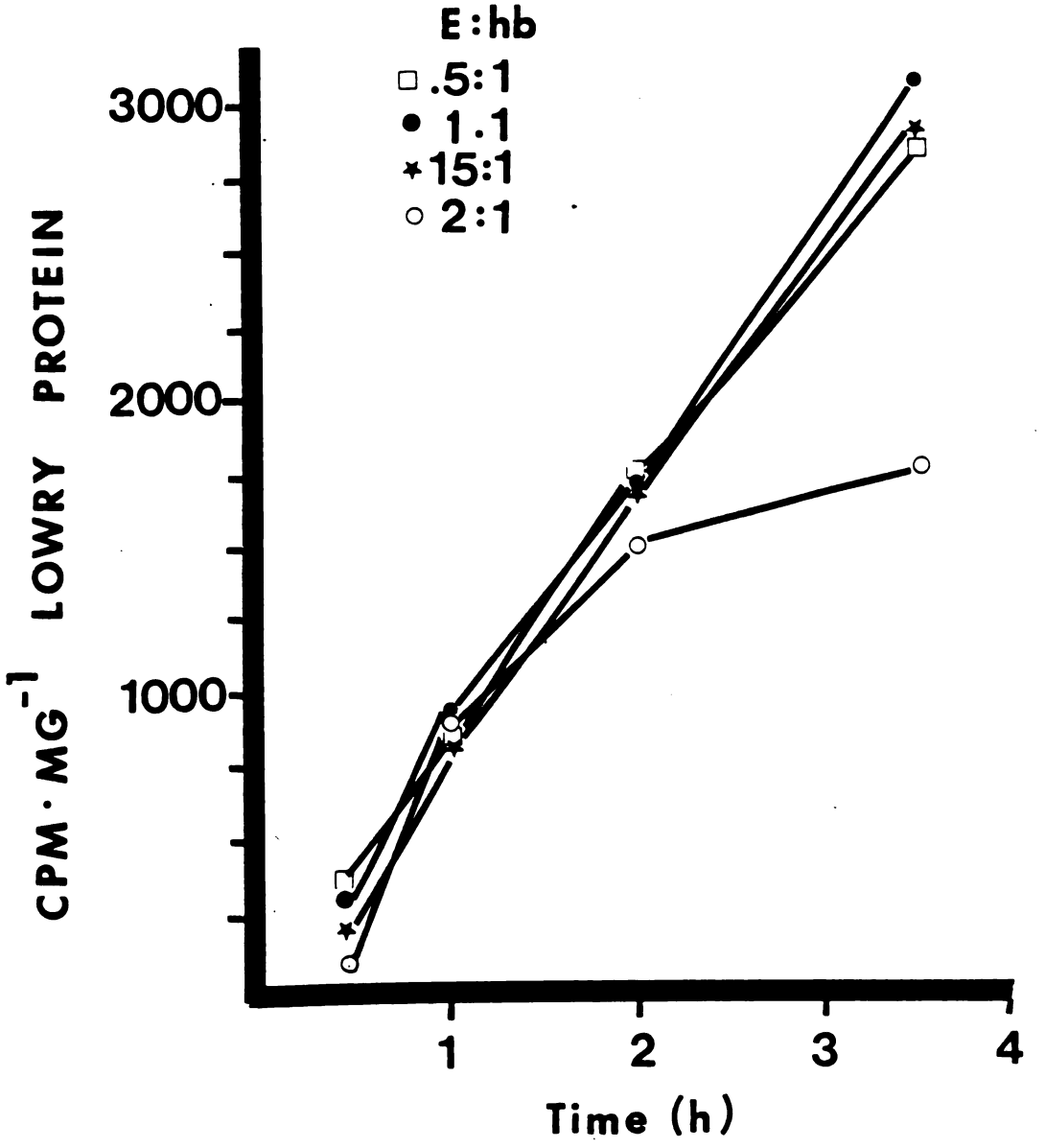


FIGURE VI-2. Effects of incubation time and ration of extract to hemoglobin substrate (v/v) upon proteinase activity at pH 3.8.

Acidic, neutral and alkaline proteinase activities were assessed in the red or white portions of the semitendinosus muscle. Table VI-1 presents the least square means for prepubertal (PREP) boars, castrates, low dihydrotestosterone (C_LDHT), high DHT (C_HDHT), low testosterone (C_LTEST) and high TEST (C_HTEST) group for the proteinase activity in the acidic (AP), neutral (NP), and alkaline (AKP) pH ranges for the red portion of the ST muscle. PREP boars had 10.5 and 9.4% higher ($P < .05$) AP and NP activities than castrates. No difference ($P > .05$) in AKP activity was observed for PREP boars and castrates (although boars had 12% lower activity). For the implanted groups, only C_LDHT pigs differed from castrates with 13.7% higher ($P < .05$) AP activity. For neutral proteinase activity in the red muscle extract, PREP implanted pigs tended to have 4 to 14% lower activity than castrates; however, only C_HDHT and C_LTEST differed ($P < .05$) from castrates. Alkaline proteinase activity of the red muscle extract from PREP implanted pigs tended to be lower than castrates and similar to boars which is consistent with work by Dahlmann et al (1980). High DHT implanted pigs, C_LTEST and C_HTEST pigs had 15.8, 23.2 and 21.7% lower ($P < .05$) AKP activity, respectively, in the red muscle extract than castrates which is in agreement with data of Dahlmann et al. (1980) for testosterone treated male castrated rats.

TABLE VI-1 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON ACIDIC, NEUTRAL AND ALKALINE PROTEINASE ACTIVITY OF THE RED PORTION OF THE SEMITENDINOSUS MUSCLE[a]

Treatment Group[b]	Proteinase Fraction		
	Acidic	Neutral	Alkaline
B	1897.8^{e}	1031.3^{f}	666.2^{de}
C	1699.2^{d}	934.4^{e}	745.8^{e}
C_LDHT	1932.3^{e}	843.5^{de}	664.2^{de}
C_HDHT	1844.9^{de}	799.1^{d}	628.0^{d}
C_LTEST	1672.7^{d}	804.1^{d}	572.3^{d}
C_HTEST	1674.1^{d}	896.2^{de}	583.9^{d}
SEM[c]	70.68	38.88	33.03

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

Proteinase activity determined on the white portion of the ST muscle of PREP pigs is presented in table VI-2. In general, acidic proteinase activities for white muscle extracts were 17% lower than activities in the red fraction. For the acidic fraction of the white muscle extract, castrates had 24.5% lower activities ($P < .05$) than boars. Pigs implanted with DHT tended to have higher AP activities than castrates but TEST implanted pigs were similar to castrates. The same trends existed for red or white AP activity in PREP pigs. NP activity in the white portion also followed similar treatment trends to those of the red portion. Activities were about 22% lower for the white portion than the red. High DHT implanted pigs had elevated NP activities relative to boars or castrates (54 and 87%, respectively) but $C_L$test and C_HTEST had about 37% lower NP activities ($P < .05$) than boars and tended to have lower activities than castrates. Sample mixing of red and white portions does not explain the higher NP activities for C_HDHT implanted pigs since the red portion was 30% lower than that observed for the white portion. Castration elevated AKP activities by 74% and as already shown for the red portion of the ST muscle, testosterone at both doses and C_LDHT significantly lowered AKP activities. This reduced AKP activity is consistent with the findings of Dahlman et al. (1980).

TABLE VI-2 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON ACIDIC, NEUTRAL AND ALKALINE PROTEINASE ACTIVITY OF THE WHITE PORTION OF THE SEMITENDINOSUS MUSCLE[a]

Treatment Group[b]	Proteinase Fraction		
	Acidic	Neutral	Alkaline
B	1721.1^{e}	743.6^{e}	294.2^{d}
C	1300.0^{d}	612.0^{d}	512.1^{e}
C_LDHT	1686.9^{e}	725.2^{e}	301.9^{d}
C_HDHT	1562.0^{de}	1146.3^{f}	407.5^{de}
C_LTEST	1335.0^{d}	471.2^{d}	284.8^{d}
C_HTEST	1282.7^{d}	457.6^{d}	310.2^{d}
SEM[c]	93.00	82.04	42.99

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

Proteinase activities in the red muscle portion of the ST muscle for pubertal (POSTP) pigs are shown in table VI-3. Castration caused a 49% decrease in AP activities, a 54% increase in NP activities and a 43% increase in AKP activities. Neither TEST nor DHT had any influence upon the AP activities relative to castrates as the implanted groups were similar in activities to those of castrates. However, for NP activities, administration of TEST or DHT resulted in decreased activities relative to castrates and activities were similar to those found in the boar. No dose response trend was observed. AKP of androgen implanted pigs had activities which were similar to boars and therefore 30 to 48% lower than castrates. Since C_HTEST were 25% lower than C_LTEST and C_HDHT had 17% ($P < .05$) lower AKP activity than C_LDHT, a slight dose response trend was obtained.

Table VI-4 presents proteinase activities for the white portion of the ST muscle of POSTP pigs. No large differences existed between activities in the red and white ST muscle portions. In fact, NP activities were about 7% higher in white than red and AKP activity was 34% higher. AP activity was 46% lower in castrates relative to boars while NP and AKP activities were 25 and 31% higher, respectively, in castrates compared to boars. These trends between boars and castrates were similar to those in the red muscle portion. Low DHT or TEST had no effect relative

TABLE VI-3 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON ACIDIC, NEUTRAL AND ALKALINE PROTEINASE ACTIVITY OF THE RED PORTION OF THE SEMITENDINOSUS MUSCLE[a]

Treatment Group[b]	Proteinase Fraction		
	Acidic	Neutral	Alkaline
B	2126.8[e]	500.4[d]	452.6[e]
C	1078.1[d]	773.4[e]	647.6[f]
C_LDHT	1184.5[d]	592.5[d]	454.6[e]
C_HDHT	1097.9[d]	462.9[d]	375.7[de]
C_LTEST	1172.5[d]	473.3[d]	447.2[e]
C_HTEST	1069.5[d]	577.4[d]	336.2[d]
SEM[c]	118.2	51.35	29.34

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

TABLE VI-4 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON ACIDIC, NEUTRAL AND ALKALINE PROTEINASE ACTIVITY OF THE WHITE PORTION OF THE SEMITENDINOSUS MUSCLE[a]

Treatment Group[b]	Proteinase Fraction		
	Acidic	Neutral	Alkaline
B	1492.0[f]	570.8[d]	558.8[d]
C	810.7[d]	713.5[e]	730.0[f]
C_LDHT	1083.7[e]	704.8[e]	611.4[e]
C_HDHT	1197.1[e]	456.2[d]	547.7[d]
C_LTEST	1110.4[e]	723.4[e]	650.0[e]
C_HTEST	1164.5[e]	457.1[d]	544.4[d]
SEM	61.40	32.14	13.70

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

to castrates for NP activities of the white muscle portion but the C_HDHT and C_HTEST had activities similar to boars and about 36% lower activities than castrates. Alkaline proteinase activities in the white muscle portion followed the same trend as observed for the red portion. Administration of DHT or TEST reduced AKP activities relative to castrates and in a dose dependent manner.

A qualitative evaluation of these data indicates that boars and castrates differ in proteinase activities. Since the AP activities were higher and AKP activities lower in boars compared to castrates, suggesting that the rate and type of protein degradation was also different. Since semitendinosus muscles of boars were heavier in boars than castrates (Chapter IV), a relationship may exist between higher AP and lower AKP activities and muscle growth. This could be an anabolic increase in AP activities as observed by Millward et al. (1981). The reductions of AKP in androgen implanted castrates is consistent with the data of boars. The inconsistency for androgen implants to elevate AP activity suggest that other factors besides TEST or DHT probably are influencing proteinase activity in boars.

Activities in the acidic, neutral and alkaline proteinase assays were totaled for the red and white muscle portions, respectively. Total proteinase AP activities for PREP pigs are presented in table VI-5. Only C_LTEST and C_HTEST pigs had significantly lower activities than boars

TABLE VI-5 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON COMPOSITE PROTEINASE ACTIVITY IN THE RED AND WHITE PORTIONS OF THE SEMITENDINOSUS MUSCLE[a]

Treatment Group[b]	Muscle Portion	
	Red	White
B	3595.4^{f}	2758.9^{ef}
C	3379.4^{df}	2425.0^{de}
C_LDHT	3440.1^{e}	2714.0^{e}
C_HDHT	3272.0^{df}	3115.9^{f}
C_LTEST	3049.0^{d}	2091.0^{d}
C_HTEST	3154.2^{de}	2050.5^{d}
SEM	113.43	131.24

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

in the red portion of the ST muscle. Even though boars tended to have elevated activities relative to castrates in both red (6%) and white (14%) portions, these differences were not significant. For the total proteinase activity in the white portion, C_HDHT pigs had 28% higher activity and C_LTEST and C_HTEST pigs had 13.8 and 15.5%, respectively, lower ($P < .05$) activities relative to castrates. Both TEST implanted groups had 25% lower total activity than boars.

Table VI-6 presents the total proteinase activity assayed in the red or white portions of the ST muscle of POSTP pigs. Castrates had 18.2% and 15.8% lower total proteinase activity in the red and white portions, respectively, than boars. In the red portion, implanted pigs tended to have lower total proteinase activity than castrates ($P < .05$) and significantly lower (25 to 41%) activities than boars. Pigs implanted with high doses of androgen tended to have lower activities than those pigs implanted with low doses. In the white portion, the DHT implanted and C_HTEST pigs were not different from castrates. In addition, C_LTEST implanted pigs had higher total proteinase activities than castrates and did not differ from boars.

A summation of the total proteinase activities in the red and white muscle extracts is presented in table VI-7. These data indicate that boars had 9 and 20% greater

TABLE VI-6 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON COMPOSITE PROTEINASE ACTIVITY IN THE RED AND WHITE PORTIONS OF THE SEMITENDINOSUS MUSCLE[a]

Treatment Group[b]	Muscle Portion	
	Red	White
B	3164.6[e]	2653.9[g]
C	2587.6[d]	2233.7[de]
C_LDHT	2365.1[d]	2383.4[ef]
C_HDHT	1873.6[d]	2202.7[de]
C_LTEST	2133.4[d]	2475.6[fg]
C_HTEST	1939.2[d]	2167.0[d]
SEM	191.81	66.20

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

TABLE VI-7 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED PREPUBERTAL AND PUBERTAL MALE PIGS UPON COMPOSITE PROTEINASE ACTIVITY OF THE SEMITENDINOSUS MUSCLE[a]

Treatment Group[b]	Pig Group	
	Prepubertal	Pubertal
B	6354.3^{f}	5701.5^{e}
C	5804.4^{e}	4753.3^{d}
C_LDHT	6154.1^{ef}	4631.5^{d}
C_HDHT	6387.9^{f}	4137.6^{d}
C_LTEST	5140.0^{d}	4576.8^{d}
C_HTEST	5204.7^{d}	4149.2^{d}
SEM	172.8	213.3

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

combined activities than castrates for pigs in the PREP and POSTP studies, respectively. Implanting pigs with DHT prepubertally elevated combined red and white proteinase activities relative to castrates. Implanting with TEST prepubertally depressed the combined red and white portion proteinase activities relative to boars and castrates. Implanting POSTP pigs with DHT or TEST depressed combined proteinase activities relative to boars but the decrease in activities were not significantly different than castrates. These data indicate that androgen implanted pigs had lower proteinase activity than castrates. If the proteinase activity is related to protein turnover, the mechanism of TEST and DHT appear to be similar to trenbolone acetate. Sinnett-Smith et al. (1983) reported decreased cathepsin D activity in muscles of male castrated and female lambs implanted with trenbolone acetate.

The percentage of total proteinase activity assayed at acid, neutral or alkaline pH of the red or white muscle portions for PREP and POSTP pigs, respectively, are presented in tables VI-8 and 9. In both the PREP and POSTP studies, there appeared to be a trend for DHT and TEST to prevent a shift in proteinase activity from the acidic to the alkaline fraction associated with castration. An analysis of the total proteinase activity in tables VI-8 and 9 further indicates that no one group of enzymes would be totally responsible for skeletal muscle protein degradation (Dayton et al. 1981).

TABLE VI-8 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED PIGS UPON THE PERCENTAGE OF TOTAL PROTEINASE ACTIVITY IN ACIDIC, NEUTRAL AND ALKALINE FRACTION OF THE RED AND WHITE PORTION OF THE SEMITENDINOSUS MUSCLE[a]

	Proteinase Fraction					
	Acidic		Neutral		Alkaline	
Treatment Group[b]	Red	White	Red	White	Red	White
B	52.8^{de}	62.2^{e}	28.7^{e}	27.1^{d}	18.5^{d}	10.7^{d}
C	50.2^{d}	53.8^{d}	27.6^{e}	25.2^{d}	22.1^{e}	20.9^{e}
C_LDHT	56.4^{e}	62.3^{e}	24.4^{d}	26.6^{d}	19.2^{d}	11.1^{d}
C_HDHT	56.4^{w}	50.6^{d}	24.4^{d}	36.1^{e}	19.2^{d}	13.3^{d}
C_LTEST	54.8^{e}	63.7^{e}	26.4^{de}	22.6^{d}	18.8^{d}	13.8^{d}
C_HTEST	52.9^{de}	61.9^{e}	28.5^{e}	23.0^{d}	18.9^{d}	15.1^{d}
SEM[c]	1.20	2.08	.84	2.10	.65	.47

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

TABLE VI-9 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTERONE (DHT) TO CASTRATED PIGS UPON THE PERCENTAGE OF TOTAL PROTEINASE ACTIVITIES IN THE ACIDIC NEUTRAL OR ALKALINE FRACTIONS OF THE RED AND WHITE PORTIONS OF THE SEMITENDINOSUS MUSCLE[a]

	Proteinase Fraction					
	Acidic		Neutral		Alkaline	
Treatment Group[b]	Red	White	Red	White	Red	White
B	69.2^{f}	58.6^{f}	16.0^{d}	20.5^{d}	14.8^{d}	20.9^{d}
C	43.0^{d}	35.7^{d}	31.1^{f}	31.6^{e}	25.9^{g}	32.6^{f}
C_LDHT	53.0^{e}	45.1^{e}	27.0^{ef}	29.4^{e}	19.9^{f}	25.5^{e}
C_HDHT	55.7^{e}	54.2^{f}	24.8^{e}	20.9^{d}	19.5^{ef}	24.8^{e}
C_LTEST	56.3^{e}	45.1^{e}	22.2^{e}	28.3^{e}	21.5^{f}	26.6^{e}
C_HTEST	53.6^{e}	53.1^{f}	29.4^{ef}	21.8^{d}	17.1^{de}	25.1^{e}
SEM[c]	2.27	1.88	1.78	1.84	1.06	.63

[a]Data reported as CPM per milligram of Lowry protein.

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants.

[c]SEM=standard error of least square means.

In summary of the proteinase data, it appears that castration reduced acidic proteinase activity. Neither DHT nor TEST significantly altered activity in the acidic fraction. Castration reduced neutral proteinase activity prepubertally but elevated activity in this fraction postpubertally. TEST and DHT either tended to reduce proteinase activity in this NP fraction relative to boars and castrates or else they had little effect. Castration elevated proteinase activity in the alkaline range and TEST and DHT inhibited the castration associated elevation. Santidrian et al. (1982) found that castration of male rats decreased 3-methylhistidine excretion. In addition, testosterone administration appeared to decrease 3-methylhistidine excretion in corticosterone treated rats. This latter observation is consistent with work reported by Dahlmann and Reinauer (1981).. In their work on male rats, castration elevated alkaline proteinase activity by 50% and this elevation was suppressed back to normal with daily injections of .5 mg of testosterone. They also found that basal alkaline proteinase activity was suppressed by 70% by testosterone administration. Their data plus the data in this chapter suggest that alkaline proteinase activity probably plays a relatively insignificant role in in vivo protein turnover of boars. In fact, data of McElligott and Bird (1981) indicated that the alkaline proteinase activities observed by Dahlmann et al. (1979)

were of mast cell origin and are not involved in muscle protein breakdown. Nevertheless, the observation that AKP activity was altered by treatment in this study and elevated in obese mice (Trostler et al., 1982) and their ability to degrade myofibrillar proteins (Sanada et al., 1978; Yasogawa et al., 1978) deserves further attention. On the other hand, acidic proteinases are probably responsible for the higher skeletal muscle protein degradation data reported in chapter V for boars compared to castrates.

Schwartz and Bird (1977) demonstrated that cathepsins B and D are capable of hydrolyzing actin and myosin. Cathepsins H (Bird and Carter, 1980) and L (Sohar et al., 1979) have also been shown to degrade myosin and possibly other myofibrillar proteins. The decreases in degradation rates reported in Chapter V for the implanted groups do not appear to be reflected by the proteinase assay data presented in this chapter. It is possible that neither the proteinase assay nor the in vitro muscle strip incubation assay reflect true relative degradation rate differences between treatments. The in vitro muscle incubation procedure has inherent problems, particularly exaggerated degradation compared to in vivo data (Mulvaney, 1981). The proteinase assay used in these studies provides only a crude measure of degradation because a myofibrillar protein, hemoglobin, served as the substrate. Thus,

interpretation of these data and their relationship to in vivo muscle protein turnover are limited and should only be considered to provide a relative indication of treatment effects. Since the in vitro muscle incubations were assayed at a neutral pH, the NP fraction may be the fraction to examine further. Neutral proteinases could serve as a nonlysosomal system for nicking proteins which possibly enhances degradation by lysosomal enzymes (Bird et al., 1980). However, these results are inconsistent between the two assays because castration stimulated proteinase activity in the NP fraction of pubertal pigs but the in vitro muscle incubation data indicated that castration was associated with a decrease in degradation.

While differences between treatments were detected with both the proteinase assay and the in vitro muscle strip incubations, whether true biologic in vivo effects or differences were measured is questionable. Perhaps the only conclusion to be derived from the proteinase data is that the acidic proteinase activity accounted for most of the activity measured and that boars had the highest activities in this pH range. Therefore, the increased (ST) muscle accretion for boars relative to castrates is correlated with high acidic proteinase activity. The reduced in vitro muscle strip degradation rates in androgen implanted pigs is not reflected by significant decreases in the acidic activity relative to castrates.

Summary

Based upon proteinase activities measured, it appears that boars had greater proteolytic activity than castrates. In addition, acid proteinase activities accounted for the bulk of proteinase activity assessed. It is suggested that further investigation on the relationship of proteolytic activity and muscle growth should focus on the acidic proteinases.

CHAPTER VII

EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGENS UPON ADIPOSE TISSUE ACCRETION AND IN VITRO LIPOGENIC AND LIPOLYTIC ACTIVITIES

Introduction

The pig has a high capacity for fat deposition (Allen et al., 1976). Knudson (1983) reported that 105 kg castrated male pigs had 25.4% carcass fat, whereas littermate boars had 17.0% carcass fat. Numerous studies of growth and body composition differences between intact male and castrated male pigs have been reported and were summarized by Walstra and Kroeske (1968), Wismer-Pederson (1968), Martin (1969), Turton (1969), Field (1971), Galbraith and Topps (1981) and Seideman et al. (1982). The recurring dichotomy between castrated and intact male pigs is in the differences in the extent of fatness. While the ontogeny of lipogenic (Allee et al., 1971; Anderson et al., 1973; Hood and Allen, 1973; Mersmann et al., 1973) and lipolytic (Mersmann et al., 1976; Steffen et al., 1978) capacity in castrated male pig adipose tissue has been investigated, neither the lipogenic nor lipolytic activity differences between castrated and intact male pig adipose tissue have been characterized.

Hansen et al. (1980) observed that testosterone treatment of male rats and estrogen treatment of female rats depressed adipose tissue fatty acid synthesis and increased lipolysis. Wade and Gray (1979) hypothesized that gonadal steroids influenced adiposity by direct actions on adipose tissue. Gray et al. (1979) found that treatment of castrated male rats with testosterone propionate depleted cytoplasmic estrogen receptors and reduced lipoprotein lipase activity in epididymal fat pads. These effects were blocked by inhibition of the aromatization of testosterone to estradiol via treatment with androsta-1,4,6-triene-3,17-dione.

The objectives of this study were to investigate the effects of castration and administration of testosterone or its nonaromatizable form, dihydrotestosterone, to castrated males upon adipose tissue fatty acid synthesis rates, lipoprotein lipase and intracellular lipase activity in both prepubertal and pubertal pigs.

Materials and Methods

At the initiation of each experiment, four to six pigs were slaughtered and were physically dissected as described in Chapter II. Determination of total carcass fat and perirenal fat allowed calculation of carcass fat (CFA) and perirenal fat (PRA) accretion over the duration of the experiment.

Fatty Acid Synthesis (FAS) Activities. Immediately after exsanguination, subcutaneous and perirenal adipose tissue samples were obtained as described for LPL and HSL assays for determination of FAS activities. Adipose tissue samples were placed in oxygenated KRB buffer (one-half the usual calcium; see Appendix B-3) at 37 C. Slices (.3 mm thick and weighing about 100 mg) were made on a Stadie Riggs microtome using saline solution as a lubricant. Slices were blotted and weighed on either a Cahn or a roller balance to the nearest milligram. Slices were chopped with scissors to increase surface area and transferred to 25 ml Erlenmeyer flasks containing 3 ml of assay buffer, gassed with oxygen-carbon dioxide mixture (95:5), were stoppered and the flask plus contents placed in a shaker (80 strokes/min) water bath at 37 C for 2 h. The assay buffer consisted of oxygenated (95:5; O_2:CO_2) KRB buffer (pH 7.4) (one-half the usual calcium) which included porcine insulin (Sigma; .1 U/ml), glucose (10 mM) and tritiated water (.1 mCi/ml). At the end of the 2 h incubation, slices were removed from the flasks, rinsed 3 times in saline solution and transferred to 50 ml culture tubes containing 10 ml of a KOH:ethanol solution (3:7; 30% KOH: 95% Ethanol) for saponification. The tubes were heated at 60 C for 2 to 4 h or until the tissue was dissolved and then for an additional 30 min. The samples were then extracted twice with 5 ml of petroleum ether with

vortexing for 1 full min. The petroleum ether which contained the nonsaponifiable lipids was aspirated and discarded. Approximately 1.5 to 2 ml (one disposable pipette full) of 12 N HCL were added to the sample tubes to bring the pH to about 2 (checked with pH paper). The HCL converted soaps to fatty acids which were extracted 3 times with the addition of 5 ml aliquots of petroleum ether followed by 1 min of vigorous vortexing. After each extraction, the petroleum ether (top layer) was aspirated off and transferred to scintillation vials. The ether was driven off under a stream of air and then 10 ml of .4% Omnifluor (New England Nuclear) ethanol:toluene (1:4) liquid scintillation cocktail were added. Assuming pure water in the incubation media to have a molarity of approximately 55 and after counting aliquots of the media, the specific radioactivity (DPM/mol) of the media was determined. The nanomoles converted to fatty acid (FA) per minute per gram of tissue was calculated as follows:

$$FA = C \times \frac{1}{E} \times \frac{1}{t} \times \frac{1000}{w} \times \frac{1}{SA}$$

where C was the counts per minute of the samples counted, E the efficiency of counting determined from channel ratio of tritium standards and counter external standard, t the time of incubation in minutes, w the weight of the adipose

tissue slice in milligrams and SA the specific activity of the media.

Lipoprotein Lipase (LPL) and Hormone Sensitive Lipase (HSL) Assay. Immediately after exsanguination, subcutaneous adipose tissue (SQAT) samples from the middle fat layer were obtained approximately 3 cm lateral to the dorsal median plane in the neck region. Immediately after resection of the SQAT sample, an incision was made caudal to the umbilicus and perirenal adipose tissue (PRAT) samples were obtained. Samples were placed in ice cold .9% saline solution and then cut into .3mm slices.

LPL was determined from 1 g of adipose tissue slices prepared by homogenization in 3 ml of ice cold buffer which consisted of .25 M sucrose -50 mM Tris-heparin (80 U/ml) (pH 8.5) using a Brinkman polytron (three 20 sec bursts at setting 7). The resultant homogenate was centrifuged at 10,000 X g for 30 min at 0 C. The resulting infranatant (liquid layer below the solid fat cake) was aspirated and strained through a single layer of cheesecloth. The infranatant fraction was kept on ice until used as the LPL enzyme source. For preparation of the HSL source, adipose tissue was treated similarly except that the homogenization buffer consisted of .25 M sucrose - 50 mM HEPES - 10 mM sodium phosphate - 1 mM EDTA (pH 6.8). Homogenates were centrifuged at 1200 X g for 30 min a 0 C. The infranatant fraction served as a source of HSL enzyme activity (Steffen et al., 1978).

Enzyme activity was assayed as outlined by Bensadoun et al. (1974) with modifications of Steffen et al. (1978). The routine assay system for LPL activity involved the following components in a total volume of 1 ml: .2 mmol of Tris buffer (pH 8.5), 1 mmol of NaCl, 10 umol of $CaCl_2$, 20 mg BSA (Sigma, fatty acid free), .1 ml of normal pig serum (pooled from several pigs; see below), 2.5 mg of gum arabic and 13.45 umol of glyceryl tri (^{14}C) oleate (.02 uCi/umol of triolein). Substrate was added to the reaction as a gum arabic-triolein emulsion prepared by sonication. ^{14}C-triolein (Amersham) plus appropriate amounts of cold triolein (Sigma) were gassed with nitrogen for 30 min after the odor of solvents were no longer detected. Gum arabic and mega pure water were added to the triolein mixture and sonicated. Substrate, assay buffer and pig serum were preincubated for 20 min before initiation of the assay with .1 to .2 ml of enzyme. Reactions were continued for 30 min at 30 C with shaking (80 strokes/min) using 50 ml culture tubes (to provide larger surface area for enzyme-substrate interaction).

The reaction was terminated by addition of 6 ml of Dole's extraction solution isopropanol: heptane: 3N H_2SO_4 in the ratio of 40:10:1 v/v). After vortexing (30 sec) and standing for 5 min, 5 ml heptane (containing .4 umol of triolein) and .5 ml water were added and the mixture vortexed for 30 sec and the phases allowed to

separate overnight (12 to 15 h). Fatty acids were isolated from a 3 to 4 ml aliquot of the heptane phase by a resin method of Kelly (1968) as modified by Baginsky (1981). Ion exchange resin (Sigma, Amberlite, IRA 400, 20 to 50 mesh, hydroxyl charged form) was prepared by suspending resin in 2.5 M NaOH (45:1, w/v). After equilibration for 10 h with occasional stirring, the resin was washed with deionized water (until water tested neutral with pH paper) and resuspended in isopropyl alcohol (.7 ml/g). After 24 h, the isopropanol was replaced with an isopropyl alcohol: water (9:1) mixture (.7 ml/g) and the resin equilibrated for 6 h with occasional stirring. This last step was repeated twice more before the resin was washed several times with n-heptane until the odor of isopropanol was no longer detected. The resin was then stored in dark brown jars at 4 C until used.

The heptane aliquot from the reaction tube was added to 20 ml scintillation vials with 1 g of wet resin (previously washed with 2 ml heptane containing triolein (Sigma, 40 mg/ml) to decrease nonspecific adsorption of labeled triolein to the resin) and the vials were vortexed for 1 min. Excess solvent was aspirated from the vials and the resin washed 3 times with 5 ml of heptane. One milliliter of NCS solubilizer (Amersham) was added to each vial and the resin-NCS suspension incubated at 60 C for 20 min to displace the fatty acids from the resin. Fatty acid

radioactivity was determined by a Beckman (Model LS-3133P) liquid scintillation counter in 10 ml of .4% Omnifluor (New England Nuclear) - toluene scintillation mixture. Counting efficiency was determined by addition of ^{14}C-toluene added to vials containing resin and was generally 76%. Recoveries were determined from a standard solution of ^{14}C-oleic acid bound to albumin which was included in the same assay mixture as the samples except emulsions of unlabeled triolein were used. Recovery was calculated from the ratio of the ^{14}C-oleic acid counts obtained with the standard solution included in the assay and the counts obtained with the same amount of standard solution added directly to counting vials containing resin which had been previously washed with heptane and solubilizer as in the assay. Recovery ranged between 50 and 55%.

HSL activity was assayed in a total volume of 1 ml containing .2 mmol HEPES buffer (pH 6.8), 10 mg of BSA (Sigma, fatty acid free), 2.5 mg of gum arabic and 22.6 umol, of glyceryl tri-^{14}C-oleate (.013 uCi/umol of triolein) as a gum arabic-triolein emulsion (see above). The reaction was started with .1 to .2 ml of enzyme and continued for 60 min at 30 C with shaking (80 strokes/min). Fatty acids liberated were isolated as described above for the LPL assay.

The nanomoles of fatty acid (FA) liberated per milligram infranatant protein were calculated as follows:

FA = (C x Fp x R) SAapp where C was the counts per minute obtained for the experimental sample (average of triplicates minus counts per minute of blank tubes, Fp was the factor used to correct the volume of infranatant added to the assay to milligrams protein (Lowry et al, 1951), R was the percent recovery and SAapp was the apparent specific activity. The apparent specific activity compensated for quenching due to the resin and solubilizer and was determined for each bath of resin and triolein preparation. For determination of SAapp, aliquots of a ^{14}C-triolein solution was counted in vials of properly treated resin and expressed as counts per minute per nanomole of fatty acid.

Blood was collected from 48 h fasted pigs by venipuncture and serum harvested. The serum was heated for 1 h at 50 C in a water bath to inactivate basal lipolytic activity, then centrifuged at 480 X g to remove particulates and stored in 20 ml aliquots at -20 C until used. The amount of serum needed in the LPL assay to produce maximal LPL activiation was determined by adding increasing amounts of serum directly to typical assay tubes. Maximal activation for the serum batch used was determined to be .1 ml.

Results and Discussion

Assay Optimization. In preliminary work involving optimization of the LPL and HSL assays, a variety of assay components and conditions were studied in mice and pig adipose tissue. These included effects of varying concentrations of heparin (in LPL homogenization), salts, BSA, gum arabic and triolein. Maximal LPL activity was observed with 60 to 70 U of heparin/ml of homogenization buffer; 200 U/ml appeared to be inhibitory. Variations of $CaCl_2$ from 0 to 50 mM concentrations resulted in a asymptotic curve with a peak activity around 10 to 20 mM. At least .05 mmol of NaCl were needed but decreased activity was observed at .25 mmol, and at .7 mmol complete inhibition occurred. Both BSA and gum arabic concentrations affected LPL activity. Maximal activity occurred with 20 to 40 mg/ml of BSA which was 60 and 40% higher than that at 0 and 10 mg/ml of BSA. Gum arabic concentratons of 5 to 20 mg/ml indicated similar activities, whereas concentrations of 2 to 2.5 mg/ml resulted in the greatest activity. The effects of heat treated serum and BSA together were tested such that maximal activity was observed when .1 ml of serum and 20 mg/ml of BSA were used. It should be noted that different batches of serum resulted in variation of activity and therefore sufficient quantities of serum were obtained and pooled to complete all assays with the same batch of

serum. The effects of incubation time and amount of enzyme present upon LPL activity is illustrated in figure VII-1. Based upon these results .1 to .2 ml of the enzyme source was used in 30 min incubations. The effect of increasing triolein concentration was tested and indicated that maximal occurred activity at 8 to 10 nM and essentially no change up to 20 nM concentrations. Varying pH from 7.0 to 10.0 indicated maximal activity occurred between pH 8 and 9.

Preliminary studies with the HSL assay involved the variation of pH, buffer substrate concentrations and incubation time. When the assay media was adjusted to a pH less than 6.5 (5 to 8.5 tested) or greater than 7.0, less activity was observed. Homogenates were prepared with 50mM HEPES, 50 mM HEPES-10 mM phosphate buffer or 50 mM phosphate buffer alone, and the greatest activity was observed with the combination buffers. In addition, maximal activity was observed at triolein concentrations exceeding 20 nM. Figure VII-2 shows a linear relationship of fatty acids released for up to 60 min at enzyme concentrations ranging from .05 to .3 ml.

FAS Activities. Results of fatty acid synthesis activities from the pigs of the pilot study are presented in table VII-1. FAS activities determined on SQ biopsy samples from the middle backfat layer revealed that castration had a definite effect on activities by 10 d

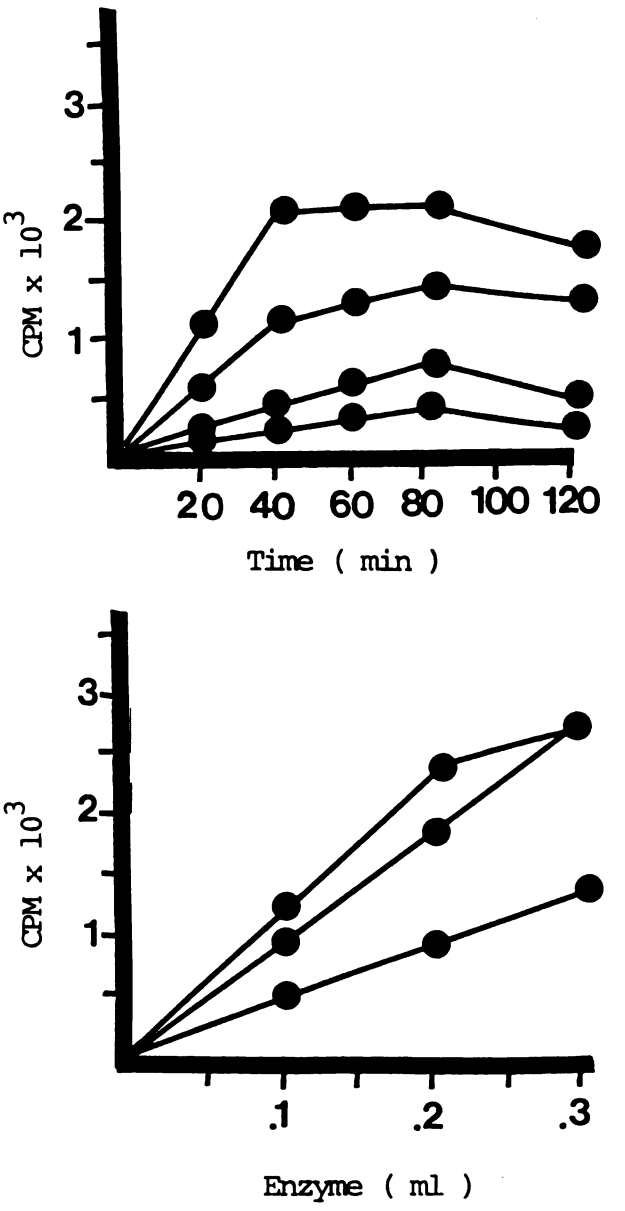


FIGURE VII-1. Effects of incubation time and amount of enzyme on LPL activity.

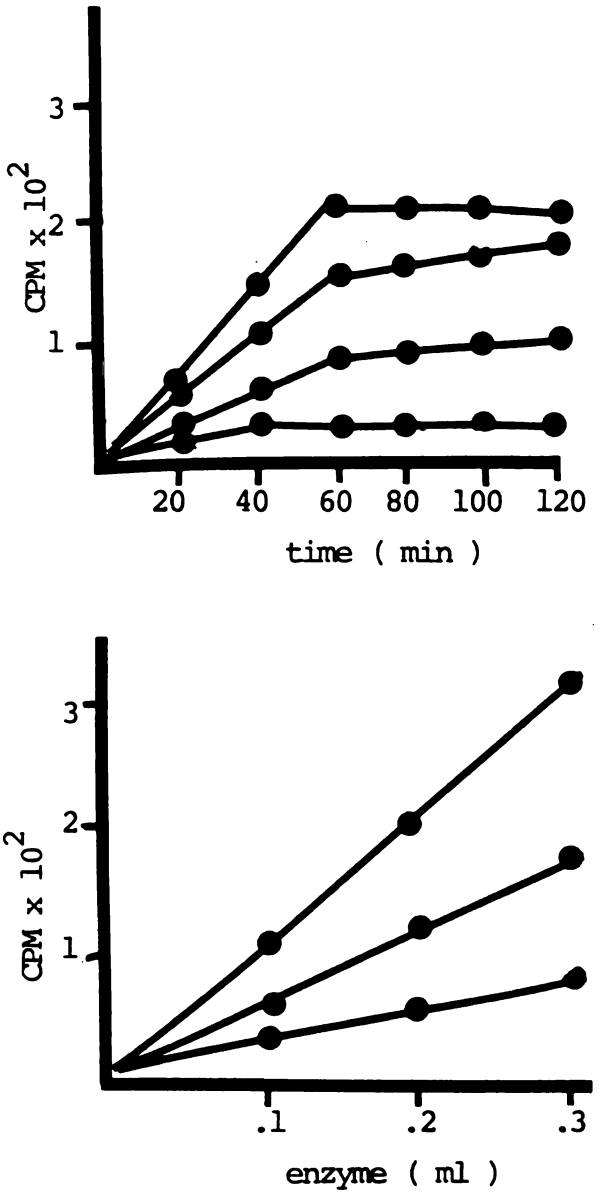


FIGURE VII-2. Effects of incubation time and amount of enzyme on HSL activity.

TABLE VII-1 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE FATTY ACID SYNTHESIS AND LIPOPROTEIN LIPASE ACTIVITY[abc]

Treatment Group[d]	Biopsy Samples		Slaughter Samples			
	SQFAS	SQLPL	SQFAS	SQLPL	PRFAS	PRLPL
B	10.6^{h}	1625.9^{g}	8.6^{f}	1536.3^{f}	19.7^{f}	1788.7^{g}
C	20.2^{i}	3143.3^{h}	13.9^{g}	2567.8^{g}	32.2^{g}	2995.5^{h}
C_LTEST	12.8^{gh}	1968.7^{g}	9.8^{f}	2051.1^{fg}	21.8^{f}	2242.6^{g}
C_ITEST	7.4^{fg}	1552.2^{g}	8.9^{f}	2103.5^{g}	24.4^{f}	2065.1^{g}
C_HTEST	5.3^{f}	856.9^{f}	7.8^{f}	1137.7^{f}	25.9^{f}	1042.2^{f}
SEM[e]	2.2	255.6	1.6	231.1	3.6	257.7

[a] FAS=fatty acid synthesis activity reported as nmol of $^{3}H_2O$ converted to fatty acid/g of adipose tissue/min.

[b] LPL=lipoprotein lipase activity reported as nmol of fatty acids liberated/mg of Lowry protein/h.

[c] Treatment means within columns with different superscripts differ ($P < .05$).

[d] B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[e] SEM=standard error of least squares means.

after castration. FAS activities were 90% higher in castrates relative to boars. Testosterone implants were effective in reducing the increase in FAS activities due to castration. There was also a testosterone dose response effect as indicated by the intermediate implanted (C_ITEST) and high implanted (C_HTEST) pigs having lower incorporation of ^{3}H-water into fatty acids than either boars or castrates. After 3 wk (at slaughter) the magnitude (61% higher for castrates) of the difference between boars and castrates was less than the biopsy sample 10 d removed before slaughter. Part of this reduced magnitude of difference was due to an 18 to 31% fall in FAS between the two samplings. The decrease in FAS activity between biopsy and slaughter samplings may be a overshoot response for the biopsied samples which by the time of slaughter was reduced due to readjustments of the endocrine system which were disturbed by castration. For the C_ITEST and C_HTEST pigs, the FAS values were higher at slaughter than at biopsy. Since FAS rates have been shown to be affected by feed intake (O'Hea and Leveille, 1969) part of the depression in FAS observed in this study for boars or testosterone treated castrate relative to castrates may be explained by lower feed intake (Chapter III).

FAS activities for perirenal adipose tissue obtained at slaughter are presented in table VII-1. In general, the

FAS activities expressed on a gram of perirenal adipose tissue basis were 2- to 3-fold higher than those in subcutaneous adipose tissue. Since this was only a 3 wk study, the number of cells per gram of adipose tissue probably was similar between treatments (Anderson, 1972). If this assumption is correct, then the relative differences between treatments reflect real differences in adipocyte lipogenic activity. Boars had 39% lower FAS activities relative to castrates. Testosterone treated castrates had 20 to 32% lower FAS activities relative to castrates. Anderson (1972) indicated that adipose tissue depots differ in their capacity to synthesize fatty acids and triglyceride. In general, lipogenic activity expressed on a gram of tissue basis decreases with age of the pig after about 4 mo of age.

The differences in subcutaneous and perirenal FAS and LPL activities do not appear to be correlated with differences in the quantity of the fat accretion for the pilot study (see Chapter III). While there appeared to be definite responses in FAS and LPL activities in both depots due to castration and administration of testosterone to castrated male pigs, the duration of the pilot study (3 wk) was apparently not sufficiently long to enable detection of differences in carcass fat. It should be emphasized that the castrates in this study and subsequent studies were left as boars up to the initiation of the study. Another

factor in the pilot study is that the pigs weighed less than 40 kg when the experiment was initiated and weighed approximately 56 kg at the termination, a time which corresponds with increased testosterone secretion (Lapwood and Florcruz, 1978; Colenbrander et al., 1978) in boars. Based upon the results observed in the pilot study, 5 wk experiments were conducted for the prepubertal (less than 50 kg at slaughter) and pubertal (heavier than 70 kg at initiation) studies.

Subcutaneous (SQ) and perirenal (PR) adipose tissue FAS activities of the pigs in the prepubertal (PREP) and pubertal (POSTP) studies are presented in table VII-2. The effect of castration was apparent both prepubertally and pubertally. Both PREP and POSTP boars had 63% lower subcutaneous FAS activities than castrates. While there were no differences in SQ FAS activities of DHT implanted castrates, C_LDHT and C_HDHT implanted pigs had 2.4- and 2.1-fold higher SQ FAS activities than boars. C_HDHT implanted pigs also had 20% lower SQ activities than castrates but the activities were similar to C_LTEST implanted pigs. The greatest SQ FAS response to testosterone in PREP pigs was observed for the C_HTEST group which was similar to boars with 53% lower SQ FAS activities than castrates.

Administration of DHT to POSTP castrated pigs had no effect on diminishing the castration associated increase in

TABLE VII-2 EFFECTS OF PREPUBERTAL AND PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE FATTY ACID SYNTHESIS[ab]

Treatment Group[c]	Prepubertal		Pubertal	
	SQFAS	PRFAS	SQFAS	PRFAS
B	13.4^{e}	32.0^{e}	10.4^{e}	19.5^{e}
C	36.3^{h}	70.0^{f}	28.3^{g}	39.5^{f}
C_LDHT	32.6^{gh}	66.9^{f}	26.3^{g}	48.8^{f}
C_HDHT	28.5^{fg}	71.1^{f}	28.2^{g}	48.7^{f}
C_LTEST	25.9^{f}	39.0^{e}	18.0^{fg}	24.4^{e}
C_HTEST	17.1^{e}	28.7^{e}	15.5^{f}	16.2^{e}
SEM[d]	2.2	4.67	1.5	3.8

[a] FAS=fatty acid synthesis activity reported as nmol of $^{3}H_2O$ converted to fatty acids/g of adipose tissue/min.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants.

[d] SEM=standard error of least squares means.

SQ FAS activity. While there were no differences in SQ FAS activities between C_LTEST and C_HTEST pigs these values were 36 and 45% lower than castrates, respectively, but still 73 and 49% higher than POSTP boars, respectively. In general, the SQ FAS activities were 22% lower (significance not tested) in POSTP than in PREP boars and castrates.

The same trends for treatment difference in PR FAS were observed in PREP and POSTP pigs. Boars had 54 and 51% lower PR FAS activities than PREP and POSTP castrates, respectively. DHT was ineffective in altering PR FAS activities from that of castrates and DHT implanted castrates had 2- to 2.5-fold higher PR FAS activities than boars. However, testosterone implants reduced the increases in PR FAS activities associated with untreated castrates. Testosterone implanted groups did not differ from each other or boars in either the PREP or POSTP pigs. Nevertheless, C_HTEST pigs tended to have 26 to 33% lower PR FAS activities than C_LTEST pigs, thus indicating a possible dose response trend.

In an attempt to decrease variation in the data due to adipose tissue composition between the treatment groups, the milligrams of protein per gram of adipose tissue (Chapter III) were multiplied by the SQ FAS or PR FAS activities to express the data on a nanamole/mg protein/min basis. While not presented in table VII-2, the expression of the data on this basis had no effect on treatment

differences. Hood and Allen (1973) discussed the various methods for expression of adipose tissue enzyme data. When enzyme data were expressed on a per gram wet tissue basis, enzyme activity decreased with increasing live weight. This is consistent with the data in the present study as POSTP pigs had approximately 22 and 40% lower enzyme activities for the SQ and PR depots, respectively, than PREP pigs. When Hood and Allen (1973) expressed enzyme data on an equal number of adipose cells or soluble protein basis, a general increase in enzyme activity was observed in 28 to 109 kg pigs. However, the greatest differences between live weight groups were observed when their data were expressed on a cell basis.

The reduction in FAS activities observed in boars and testosterone implanted male castrates compared to castrates is in agreement with data on rats provided by Hansen et al. (1980). In that study, male rats were injected with 40 mg of testosterone or 1 mg of estradiol 3 d before adipocytes were prepared and assayed for lipogenic activities. Both testosterone and estradiol reduced lipogenic activities. The observation in the present study that DHT was unable to reduce an increased lipogenic activity associated with castration lends support to the theory that aromatization of testosterone to estrogen is required for metabolic effects of testosterone upon adipose tissue (Gray et al., 1979). Burch et al. (1982) reported a 50% decrease in

sheep subcutaneous adipose tissue fatty acid synthetase activity but only an 18% decrease in acetyl-CoA carboxylase activity due to implanting with a trenbolone acetate-estradiol combination.

LPL Activities. LPL activities for SQ and PR depots in PREP and POSTP pigs are presented in table VII-3. When the data were expressed on a soluble protein basis, PREP boars had 3.4- and POSTP boars had 1.7-fold lower SQ LPL activities than castrates. In neither the PREP nor the POSTP study did DHT affect LPL activity relative to castrates. Even though C_LTEST PREP pigs had 47% higher SQ LPL activities than boars the C_LTEST pigs had over 40% lower activities than castrates and DHT implanted pigs. C_HTEST implanted PREP pigs were not different from either boars or C_LTEST pigs but they had 54% lower SQ LPL activities than castrates.

The magnitude of the difference in LPL activities between POSTP boars and castrates was not as large as in the PREP study. In addition, the C_LTEST group of the POSTP study had SQ LPL activities similar to castrates and DHT implanted pigs. However, C_HTEST Pigs had 61% lower SQ LPL activities than castrates and 33% lower (nonsignificant) activities than boars. The SQ LPL data reported in this study are slightly higher than the SQ LPL data obtained from pigs as reported by Steffen et al. (1978).

TABLE VII-3 EFFECTS OF PREPUBERTAL AND PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE LIPOPROTEIN LIPASE ACTIVITY[ab]

Treatment Group[c]	Prepubertal		Pubertal	
	SQLPL	PRLPL	SQLPL	PRLPL
B	903.8^{e}	525.4^{e}	1365.0^{e}	2158.9^{fg}
C	3103.1^{g}	2432.1^{g}	2357.6^{f}	2619.7^{g}
C_LDHT	3686.9^{g}	2553.2^{g}	2330.9^{f}	2756.2^{g}
C_HDHT	2906.7^{g}	2116.1^{g}	2903.9^{f}	2585.6^{g}
C_LTEST	1698.6^{f}	1127.6^{f}	2224.8^{f}	1785.5^{f}
C_HTEST	1415.0^{ef}	898.3^{ef}	815.0^{e}	841.4^{e}
SEM[d]	139.8	101.6	322.2	257.8

[a] FAS=fatty acid synthesis activity reported as nmol of $^{3}H_2O$ converted to fatty acids/g of adipose tissue/min.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants.

[d] SEM=standard error of least squares means.

In analyzing the PR LPL data in table VII-3, prepubertal boars had 4.6-fold lower PR LPL activities than castrates. In both studies, the testosterone treated pigs had PR LPL activities closest to boars with the C_HTEST pigs exhibiting a significant depression (almost 3-fold) in their activities relative to castrates. These data from this earlier maturing depot are difficult to interpret since boars had higher adipose tissue protein than castrates. If it can be assumed from the adipose tissue protein data (Chapter III) that boars had a greater number of cells per unit tissue than castrates the effects of castration upon LPL activity will become more apparent as well as the effect of testosterone administration. The depressed LPL activity observed in boars and testosterone implanted male castrates compared to castrates is consistent with data reported by Gray et al. (1979) and Slusser and Wade (1981). In those studies, LPL activities were reduced in male rats and hamsters when animals were treated with testosterone or estradiol. In addition, the 5α reduced forms of testosterone such as DHT were unable to reduce LPL activity (Slusser and Wade, 1981).

HSL Activities. Opposing the lipogenic activities of fatty acid synthesis and the uptake of fatty acid via LPL are the intracellular lipases or as assayed in this study, the hormone sensitive lipase (HSL). Basal lipolysis is low in swine adipose tissue (Mersmann et al., 1976) and

decreases dramatically after 150 d of age (Steffen et al., 1978).

HSL activities in SQ and PR depots of PREP and POSTP pigs are presented in table VII-4. SQ HSL activities of PREP and POSTP boars were 74 and 40% higher, respectively, than castrates. Testosterone implants but not DHT, resulted in greater HSL activities in castrates. SQ HSL activities in DHT implanted pigs were not different from castrates. The HSL data in these studies are similar to those observed in pigs by Scott et al. (1981) and only slightly lower than those reported by Steffen et al. (1978).

The combination of increased FAS and LPL activities and lower HSL activities after castration are consistent with the difference in quantity of total carcass fat of PREP and POSTP boars and castrates (Chapter III). As shown in tables III-10 and 11, PREP boars had 15.5% less and POSTP boars had 21% less total fat (excluding perirenal fat) in their respective carcasses at slaughter than castrates. Both the C_LTEST and C_HTEST groups had less (4 to 5% less for C_LTEST and 14 to 36% less for C_HTEST) total carcass fat compared to sham implanted castrates. Total carcass fat of DHT implanted pigs either was similar to castrates or intermediate to that of the C_LTEST pigs and castrates. Even though HSL activities were elevated in boars and C_HTEST pigs compared to castrates, these

TABLE VII-4 EFFECTS OF PREPUBERTAL AND PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE HORMONE SENSITIVE LIPASE ACTIVITY[ab]

Treatment Group[c]	Prepubertal		Pubertal	
	SQHSL	PRHSL	SQHSL	PRHSL
B	222.6^{g}	135.5^{e}	213.8^{f}	118.4^{gi}
C	128.2^{e}	106.5^{efg}	512.7^{e}	63.3^{ef}
C_LDHT	125.3^{e}	94.0^{ef}	137.6^{e}	81.3^{efg}
C_HDHT	135.7^{e}	82.8^{e}	152.2^{e}	57.6^{e}
C_LTEST	155.7^{ef}	109.3^{efg}	181.0^{ef}	111.7^{fgh}
C_HTEST	178.4^{f}	134.5^{fg}	198.5^{ef}	142.3^{hi}
SEM[d]	16.4	16.1	22.2	20.7

[a] HSL-hormones sensitive lipase activity reported as nmol fatty acids liberated/ mg Lowry protein/h.

[b] Treatment means within columns with different superscripts differ ($P < .05$).

[c] B=boars, C=castrates, C_L=low implants, C_H=high implants.

[d] SEM=standard error of least square means.

differences become relatively small when data are expressed on a total adipose tissue mass basis since castrates had more total adipose tissue mass than boars or C_HTEST pigs. Testosterone accelerates fat mobilization (Laron and Kowaldo - Silbergeld, 1964). and norepinephrine stimulated lipolysis in rats (Hansen et al., 1980).

From the data presented in this chapter, it appears that testosterone depressed FAS activity and the uptake of circulating lipid, and increased the mobilization of fat. These observations are in agreement with the data of Hansen et al. (1980) who reported that both the incorporation of ^{14}C-glucose into fatty acids and the lipolytic activity were affected by testosterone administration to male rats. Based upon the relationship of the adipose tissue enzyme assays of the present study and total quantity of carcass fat between treatments (Chapter III), it appears that DHT has little effect on controlling lipid deposition when compared to boars or the TEST implanted pigs. Even though POSTP castrates implanted with DHT had slightly less carcass fat than castrates these data suggest DHT has little or no effect in regulating fat deposition. DHT also was essentially ineffective in reducing feed intake, whereas TEST and boars voluntarily consumed less feed than castrates (Chapter III).

Based upon the higher total carcass fat content of limit-fed castrates (pair fed controls) relative to boars

in this study (Chapter III), the fattening process may be more closely controlled by direct or indirect effects of testosterone upon adipose tissue rather than by a mere food intake response. This observation needs further investigation. These data lend credence to the hypothesis that testosterone affects feed intake and fat metabolism only after having been aromatized to estrogens (Wade and Gray, 1979). Mendelson et al. (1983) reported that there is significant conversion of androstenedione to estrone and to a lesser extent 17-β estradiol in adipose tissue, and the conversion may be indiced by glucocorticoids. The basis for this hypothesis is due to a dose dependent 20- to 60-fold increase in the conversion of androstenedione to estrone when dexamethasone was added to adipose tissue cultures. This is consistent with the anti-insulin effects of glucocorticoids described by Baxter (1976). Massively obese human males have reduced circulating concentrations of testosterone and increased concentrations of estrogens (Kley et al., 1980). Ideal body weights for humans appear to be highly correlated (r=.8) with estrogen concentrations. Hamosh and Hamosh (1975) and W ilson et al. (1976) found reduced adipose tissue LPL activity in estrogen treated male and female rats. In contrast, Prior et al. (1983) reported increased FAS activities in 17-β estradiol implanted steers and bulls relative to nonimplanted steers and bulls when the data were expressed on a gram of tissue basis and only subtle increases when expressed on a cell basis. Lipolytic

activities were unaffected by estradiol treatment. However, nonimplanted bulls had 50% lower acetyl CoA carboxylase activities than steers. There also was a trend for bulls to have greater basal lypolytic activities rates than steers, and for the estradiol implants to have slightly enhanced lipolytic activity in steers. It is possible the dose used in their study far exceeded that which would be optimal for reducing lipogenic and increasing lipolytic activities.

Summary

Measurement of fatty acid synthesis, lipoprotein lipase and lipolytic activities in subcutaneous and perirenal adipose tissue yielded data that complement the fat accretion data. In the 3 wk study, boars had 38 to 47% lower fatty acid synthesis activities than castrates. Testosterone reduced fatty acid synthesis rates by 43 to 74% in the biopsied subcutaneous samples and by 29 to 44% in the slaughter samples when data are compared to castrates. Fatty acid synthesis activities in the perirenal depot of testosterone implanted pigs were reduced by approximately 20 to 32% compared to castrates. Similar trends were observed in both 5 wk studies but it appeared that high dihydrotestosterone reduced subcutaneous fatty acid synthesis activities in prepubertal pigs. This suggest that age may influence the response to androgens. Fatty acid synthesis activities suggest that castration elevated activities by

approximately 2-fold. Administration of testosterone but not dihydrotestosterone effectively prevented this castration associated increase in fatty acid synthesis activities.

Lipoprotein lipase activities were approximately 40% lower in boars than castrates in the 3 wk study. Lipoprotein lipase activities were 3.4- and 4.6-fold higher in subcutaneous and perirenal adipose tissue samples, respectively, for prepubertal castrates compared to boars. Activities in pubertal castrates were 1.7- and 1.2-fold higher than boars for subcutaneous and perirenal samples, respectively. The difference between boars and castrates of the 2 studies indicate that castration had a greater response in prepubertal pigs compared to pubertal pigs. In all 3 studies, testosterone administration reduced lipoprotein lipase activities compared to the data of castrated pigs. Dihydrotestosterone implants had no effect upon lipoprotein lipase activities when compared to castrates.

Magnitudes of difference between treatments were less for lipolytic activities; however, prepubertal boars had 1.7- and 1.3-fold higher activities in subcutaneous and perirenal depots, respectively, than castrates. Minimal effects due to androgens were observed except that high testosterone tended to elevate lipolytic activites relative to castrates and other implanted groups.

These data suggest that castration increases fat deposition in pigs by elevating fatty acid synthesis and lipoprotein lipase activities and reducing hormone sensitive lipase activities. Administration of dihydrotestosterone was ineffective in altering these aspects of adipose tissue metabolism. Administration of testosterone reduced fattening primarily by reducing fatty acid synthesis and lipoprotein lipase activities and subtle increases in hormone sensitive lipase activities. It is suggested that either testosterone acts directly, is aromatized to estrogens or that some antilipogenic factor is potentiated. Administration of testosterone could possibly be used to reduce fat deposition in castrated male pigs.

CHAPTER VIII

EFFECTS OF CASTRATION AND ADMINISTRATION OF ANDROGENS UPON BONE ACCRETION

Introduction

There is a definite paucity of knowledge on bone growth in meat animal species. Since male meat animals are generally castrated in this country, testosterone is not a prequisite for longitudinal bone growth. However, testosterone influences bone growth (Short, 1980). Castration of young male rats slows growth (Scow, 1952; Aschkenasy-Lelu and Aschkenasy, 1959) but small doses (.1mg/rat/d) of testosterone prevented this diminished growth (Bergstrand, 1950; Scow, 1952). Larger doses (1mg/rat/d) of testosterone were inhibitory to bone growth (Rubinstein and Solomon, 1941). However, the age of castration of rats may influence the response to testosterone (Werff Ten Bosch, 1977). Testosterone stimulates the physis to undergo cell division and widening during growth (Ogden, 1980).

The relationships between muscle growth and bone growth are poorly understood. Intact male cattle, sheep and pigs have greater muscle mass as well as more bone than their castrated counterparts (Galbraith and Topps, 1981). Percentage bone in carcasses of boars will be about 2% more

than in male castrates (Field, 1971). Bones of meat animal species grow in a coordinated pattern but differential bone growth can occur (Richmond et al., 1979). If differential bone growth can be induced by endogenous or exogenous testosterone and if this induced bone growth is measurable, then our understanding of the relationships of bone growth to muscle growth would be enhanced. In addition, since bone is an endocrine sensitive tissue, increased understanding of the mechanisms of action of growth promoting agents can be facilitated.

The objectives of this study were to assess the effects of castration and administration of testosterone or dihydrotestosterone to castrated male pigs upon bone accretion and linear growth rates of bone in male pigs castrated prepubertally and pubertally. Besides gravimetric and linear measurements on selected bones, tetracycline was used as an intravital marker to assess subtle differences in longitudinal bone growth.

Materials and Methods

The scapula, radius-ulna, humerus, femur and tibia-fibula were removed from the right side of each carcass, cleaned of adhering tendon, fat and muscle, weighed to the nearest one-tenth of a gram and then frozen at -20 C until further analysis. The radius-ulna and tibia-fibula were weighed as bone pairs due to fusion of

these respective bones. Length measurements of the scapula, ulna and fibula were made to the nearest millimeter at the time of slaughter. A meter stick adapted with a stationary back drop on one end and a sliding back drop on the other end was used to measure lengths of the radius, humerus, femur and tibia bones. The radius and tibia were sawed into two longitudinal halves such that a 3 mm thick longitudinal center section could be obtained. Total length and diaphysis length measurements were made on these center sections. On 1 d and 2 d after the initiation of each of the two studies, 20 mg/kg of oxytetracycline was administered intravenously to all pigs. The tetracycline becomes localized in replicating cells near the epiphyseal plate and remains in those cells even after ossification (Hansson, et al., 1972). Therefore, when the center sections were placed under ultraviolet light, a fluorescent tetracycline band was detected and marked with a lead pencil for subsequent measurements. The distance from the tetracycline band boundary proximal to the epiphyseal plate was measured to the proximal boundary of the epiphyseal plate under a dissecting microscope equipped with an 8 x eyepiece and calibrated in millimeters. Similarly, the distal end of each of these bones also was measured. These measurements were referred to as either proximal or distal end growth. It should be noted that these measurements do not represent total longitudinal bone growth for 3

reasons. Firstly, the tetracycline band was too diffuse on the boundary closest to the marrow cavity to enable precise marking of that boundary. Secondly, the tetracycline band was in some cases as much as 1 cm wide. Thirdly, some longitudinal growth occurred in the metaphyses.

Proximal and distal epiphyseal plate widths on both surfaces of the longitudinal center sections were measured in micrometers under the same dissecting microscope at approximately 32 x magnification. Due to variability in plate widths within an epiphyseal plate, ten to twenty individual observations were made on each plate and averaged. Observations which were 2-fold wider than the mean were deleted from the averaged values.

After the humerus and femur bones were measured for total length, the mid-length point was determined and the bones were sawed perpendicular to the diaphysis at distances equivalent to 10% of the total length of each side of the mid-length point. The outside and inside boundaries of the diaphysis wall on both the proximal and distal surfaces were traced on acetate paper. A planimeter was then used to measure the outer and inner diaphysis wall circumferences and subtraction of the inner circumference from the outer circumference resulted in an arbitrary diaphysis wall area on both the proximal and distal ends. This wall area measurement was used as an indicator of changes in bone thickness.

Results and Discussion

Bone Weight. The weights of the atlas, first thoracic vertebra, pelvic, scapula, humerus, radius-ulna, tibia-fibula and total bone weight from the right side of the carcasses of the pigs in the pilot study are presented in table VIII-1. There were no significant differences between treatments for any bone weights. However, for most bones weighed, boars and testosterone (TEST) implanted pigs had heavier bones indicating a positive effect of testosterone upon bone growth. For subsequent studies, the scapula, humerus, radius-ulna, femur, tibia-fibula and total bones from the right side of each carcass were removed and weighed. Total bone weight as well as scapula, radius-ulna, tibia-fibula, humerus, and femur weights removed from the right sides of the carcass of PREP and POSTP pigs are presented in tables VIII-2 and 3, respectively. These total bone weight data differ from those presented in Chapter III in that the latter were calculated from the percentage bone in the right side times the hot carcass weight. Splitting errors may have under- or overestimated the total bone weight data presented in tables VIII-2 and 3. Since right side weights did differ between some treatments (Chapter III) it is difficult to interpret total bone weight data from the right side only. However, the trends appear to be similar between total bone in the carcass (Chapter III) and that in the right side

TABLE VIII-1 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON SELECTED BONE WEIGHTS REMOVED FROM THE RIGHT SIDE OF THE CARCASS[ab]

	Bone[c]								
Treatment Group[c]	Atlas	First Thoracic	Pelvic	Scapula	Humerus	Radius-Ulna	Femur	Tibia-Fibula	Total[d] Bone
B	24.32	43.91	183.93	159.42	223.93	167.83	240.83	193.83	2.75
C	21.97	42.88	152.41	147.01	195.81	146.22	209.50	164.63	2.34
C_LTEST	21.60	38.40	171.02	150.14	212.13	166.16	243.12	180.20	2.55
C_ITEST	18.65	37.22	174.72	160.85	216.03	170.40	220.16	183.45	2.64
C_HTEST	22.33	45.08	168.26	153.87	205.50	158.96	209.28	180.39	2.51
SEM[f]	2.29	4.13	8.49	7.02	9.53	7.5	14.99	9.48	.10
Initial Slaughter Group	18.70	17.72	109.20	92.60	144.78	114.40	158.06	123.08	1.79

[a]Three week pilot study.

[b]Least square means within columns do not differ ($P < .05$).

[c]Individual bone weights are reported in grams.

[d]Total bone weight is reported in kilograms.

[e]B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[f]SEM=standard error of least square means.

TABLE VIII-2 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL BONE, SCAPULA, RADIUS-ULNA, TIBIA-FIBULA, HUMERUS AND FEMUR WEIGHTS REMOVED FROM THE RIGHT SIDE OF THE CARCASS[a]

	Bone[c]					
Treatment Group	Total Bone	Scapula	Radius-Ulna	Tibia-Fibula	Humerus	Femur
B	1957.4^{fh}	105.2	119.3^{f}	142.8^{g}	144.5	177.7^{fh}
C	1827.6^{efg}	102.9	105.1^{e}	119.5^{e}	140.7	159.4^{ef}
C_LDHT	1760.5^{e}	101.3	111.2^{ef}	125.6^{eg}	142.4	156.4^{ef}
C_HDHT	1669.4^{e}	102.8	108.1^{ef}	114.2^{e}	135.0	151.2^{e}
C_LTEST	1775.6^{eg}	99.2	112.7^{ef}	121.2^{e}	141.8	154.8^{e}
C_HTEST	1934.6^{fg}	101.7	105.8^{e}	141.4^{fg}	145.1	168.9^{fg}
C_LFED	1747.3^{e}	103.5	102.5^{e}	124.2^{ef}	133.5	162.1^{eg}
SEM[d]	55.80	2.99	3.76	5.87	4.25	4.23
Initial Slaughter Group	800.6	37.1	56.3	60.9	64.9	76.1

[a]Least square treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Bone weights reported in grams.

[d]SEM=standard error of least square means.

TABLE VIII-3 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON TOTAL BONE, SCAPULA, RADIUS-ULNA, TIBIA-FIBULA, HUMERUS AND FEMUR WEIGHTS REMOVED FROM THE RIGHT SIDE OF THE CARCASS[a]

Treatment Group[b]	Bone[c]					
	Total Bone	Scapula	Radius-Ulna	Tibia-Fibula	Humerus	Femur
B	4694.1^{g}	314.8^{f}	292.9^{f}	294.9^{g}	403.0^{f}	400.6^{f}
C	4049.8^{e}	252.2^{e}	258.2^{e}	270.7^{e}	347.2^{e}	361.3^{e}
C_LDHT	4064.5^{e}	268.9^{e}	247.0^{e}	268.8^{e}	328.4^{e}	356.9^{e}
C_HDHT	4107.9^{ef}	277.1^{ef}	250.7^{e}	269.7^{ef}	342.6^{e}	357.5^{e}
C_LTEST	4156.3^{ef}	270.4^{e}	260.0^{e}	271.2^{ef}	337.8^{e}	376.4^{ef}
C_HTEST	4527.6^{fg}	261.2^{e}	265.3^{e}	289.8^{fg}	347.6^{e}	380.8^{ef}
C_LFED	4083.2^{e}	245.9^{e}	251.5^{e}	271.9^{ef}	317.4^{e}	362.0^{e}
SEM[d]	109.66	10.91	8.34	5.85	15.65	12.39
Initial Slaughter Group	3103.5	185.6	195.4	206.3	257.6	281.9

[a]Least square treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Bone weights reported in grams.

[d]SEM=standard error of least square means.

reported in this chapter. Only the right side data will be discussed in this chapter.

In the PREP study, total bone weight from the right side of boars and castrates were not different. Boars and castrates tended to have more bone than implanted pigs with the exception of the high testosterone (C_HTEST) group. Limit-fed PREP castrates had the least total bone weight of all treatments.

In the POSTP study, boars had 16% more bone in the right side than castrates and tended to have more bone than the androgen implanted groups. The C_HTEST pigs were closest to boars in total bone weight having only 3.5% less bone in the right side, but all implanted pigs in the POSTP study tended to have slightly more (3 to 4%) bone ($P < .05$) than castrates. Limit-fed castrates had bone masses similar to castrates. In order to assess the effects of castration and administration of testosterone and dihydrotestosterone to castrated male pigs upon bone accretion, the bone weights of the initial slaughter group of pigs were subtracted from the bone weights of pigs within each treatment group in tables VIII-2 and 3 and the accretion was expressed as a percentage of the castrate bone accretion. These data are presented in figures VIII-1 and 2 for the PREP and POSTP studies, respectively. As indicated in figure VIII-1, representing the PREP study, no one bone appears to be more responsive to TEST or DHT than

another but it appears that the C_HTEST treatment had relative bone accretion rates that were similar to those of boars. It appears that dihydrotestosterone at the high dose had an inhibitory effect on bone accretion relative to castrates. As illustrated in figure VIII-2 for the POSTP pigs, boars far exceeded castrates in bone accretion. When considering the radius-ulna, tibia-fibula, humerus and femur, it appeared that C_HTEST was more effective than the other implant groups in stimulating bone accretion relative to castrates. Unlike the PREP study, scapula weights were stimulated to a much greater extent by androgen in POSTP pigs with the C_HDHT group showing the greatest response. Limited-fed castrates were similar to or had less bone accretion than castrates during the 5 wk experiment.

Linear Bone Growth. The differences in bone weights should be related to changes in bone length or thickness. Lengths of the pelvic bone (pilot study only), scapula, humerus, radius, femur and tibula for the pilot study and the PREP and POSTP studies are presented in tables VIII-4, 5 and 6, respectively. While boars in the pilot study tended to have slightly longer bones than castrates, only the 5% difference in femur length was significant. No other detectable trends in bone length were evident in this 3 wk study.

TABLE VIII-4 EFFECTS OF CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) TO CASTRATED MALE PIGS UPON SELECTED BONE LENGTHS REMOVED FROM THE RIGHT SIDE OF THE CARCASS[a]

Treatment Group[b]	Bone[cd]					
	Pelvic	Scapula	Humerus	Radius	Femur	Tibia
B	21.2	16.4	15.7	12.0	17.6[g]	16.0
C	20.0	16.0	14.8	11.9	16.8[f]	15.0
C_LTEST	20.6	16.9	15.4	13.0	16.4[f]	15.1
C_ITEST	20.5	16.3	15.2	13.9	17.1[fg]	15.6
C_HTEST	20.2	16.6	14.7	12.9	16.7[f]	15.3
SEM[e]	.44	.34	.35	1.09	.27	.39
Initial Slaughter Group	18.0	14.7	13.9	9.7	14.3	12.0

[a]Three week pilot study.

[b]B=boars, C=castrates, C_L=low implants, C_I=intermediate implants, C_H=high implants.

[c]Individual bone lengths in centimeters.

[d]Treatment means within columns with different superscripts differ ($P < .05$).

[e]SEM=standard error of least squares means.

TABLE VIII-5 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SCAPULA, RADIUS, ULNA, TIBIA, FIBULA, HUMERUS AND FEMUR LENGTH[a]

Treatment Group[b]	Bone[c]						
	Scapula	Radius	Ulna	Tibia	Fibula	Humerus	Femur
B	14.3^{f}	10.2^{f}	9.7	14.0^{ef}	11.7	13.9^{f}	15.0^{fg}
C	13.8^{ef}	9.5^{e}	9.8	13.5^{e}	10.8	13.2^{ef}	14.3^{e}
C_LDHT	14.5^{f}	9.8^{ef}	10.0	13.9^{ef}	11.6	13.7^{ef}	15.4^{g}
C_HDHT	14.2^{f}	9.8^{e}	9.8	13.9^{ef}	10.9	13.5^{ef}	14.7^{ef}
C_LTEST	13.8^{ef}	9.8^{ef}	9.8	14.2^{ef}	11.1	13.7^{ef}	14.8^{eg}
C_HTEST	14.2^{f}	10.0^{ef}	9.7	14.3^{f}	11.6	13.5^{ef}	14.8^{eg}
C_LFED	13.1^{e}	9.9^{ef}	9.6	13.7^{ef}	11.8	13.7^{ef}	14.8^{eg}
SEM[d]	.28	.15	.13	.23	.33	.20	.19
Initial Slaughter Group	10.7	7.5	7.9	10.3	9.9	10.7	11.15

[a] Least square treatment means within columns with different superscripts differ ($P < .05$).

[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c] Bone lengths reported in centimeters.

[d] SEM=standard error of least squares means.

TABLE VIII-6 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON SCAPULA, RADIUS, ULNA, TIBIA, FIBULA, HUMERUS AND FEMUR LENGTH[a]

Treatment Group[b]	Bone[c]						
	Scapula	Radius	Ulna	Tibia	Fibula	Humerus	Femur
B	21.4^{f}	13.9^{f}	13.4	18.7^{fg}	16.1^{g}	19.5^{g}	21.5^{f}
C	19.4^{e}	13.3^{e}	13.2	18.2^{ef}	15.5^{ef}	18.2^{e}	20.1^{e}
C_LDHT	19.7^{e}	13.5^{ef}	13.4	18.5^{efg}	15.9^{fg}	17.9^{e}	20.2^{e}
C_HDHT	20.1^{e}	13.4^{e}	13.1	18.0^{ef}	16.0^{fg}	18.4^{ef}	20.3^{e}
C_LTEST	19.7^{e}	13.5^{ef}	13.3	18.2^{ef}	15.6^{ef}	17.8^{e}	20.2^{e}
C_HTEST	19.9^{e}	13.7^{ef}	13.5	19.0^{g}	16.0^{fg}	19.2^{fg}	20.7^{ef}
C_LFED	19.9^{e}	13.4^{e}	13.2	17.9^{e}	15.2^{e}	18.2^{e}	20.2^{e}
SEM[d]	.33	.15	.23	.22	.18	.29	.26
Initial Slaughter Group	17.2	12.0	11.5	16.6	14.2	16.2	18.1

[a]Least square treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Bone lengths reported in centimeters.

[d]SEM=standard error of least squares means.

For PREP boars only the radius, humerus and femur were significantly longer 6, 5 and 5%, respectively, than castrates ($P < .05$) and a similar trend ($P > .05$) existed for the scapula, tibia and fibula. Implanted pigs tended to have longer bones relative to castrates but none of them was significantly longer ($P < .05$) than castrates except for femur length of the C_LDHT group.

Postpubertally, boars tended to have longer bones than castrates but only the differences in scapula (5%), radius (4.5%), fibula (3.9%), humerus (7%) and femur (7%), respectively, were significantly ($P < .05$) different. Implanted pigs generally were not different from castrates and only the tibia (4.4%) and humerus (5.5%) for the C_HTEST pigs relative to castrates were longer ($P < .05$). Walstra (1980) reported that boars had longer bones than castrates when fed ad libitum. In addition, few differences in bone length between boars and castrates were observed before 4 mo of age.

The few detectable differences in linear bone growth in these studies either means that differences between treatments are so subtle that simple gross length measurements were not sensitive enough or that differences in bone accretion by these treatments is more of a function of density or thickening rather than length. Bone is a metabolically active organ with continual remodeling (Ham and Cormack, 1979) and it is feasible that castration and

testosterone administration may affect specific aspects of long bones without altering total length.

In order to assess the effects of castration and androgen administration upon bone growth, the diaphysis (from diaphyseal aspect of epiphyseal plate on the proximal end of the diphyseal aspect of the epiphyseal plate on the distal end) and proximal and distal epiphyseal plate widths were measured. In addition, tetracycline was used as an intravital fluorescent marker to enable measurement of subtle differences in linear bone growth.

The radius diaphysis length, growth determined by tetracycline marking on the proximal and distal ends and the proximal and distal epiphyseal plate widths of the radius for PREP and POSTP studies, respectively are shown in tables VIII-7 and 8. Because the radius was longer in boars than castrates so was the radius diaphysis length (by 3%) in the PREP study (table VIII-7). Even though the C_HDHT pigs had essentially the same ($P > .05$) total radius length, the radius diaphyseal length was 4.3% longer ($P < .05$) than castrates. The other implant groups had slightly longer ($P < .05$) radius diaphyseal lengths than castrates. Aside from a 42% (.29 cm) greater distal end growth as measured by tetracycline marking in the C_HTEST pigs no other differences in growth were observed. Nevertheless, castrates tended to have the least growth as determined from the tetracycline marked bone.

TABLE VIII-7 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON DIAPHYSIS LENGTH, PROXIMAL AND DISTAL END GROWTH MEASUREMENTS AND PROXIMAL AND DISTAL END EPIPHYSEAL PLATE WIDTHS OF THE RADIUS[a]

	Bone Measurements[d]				
Treatment Group[b]	Diaphysis Length	Proximal End Growth	Distal End Growth	Proximal Epiphyseal Plate Width	Distal Epiphyseal Plate Width
B	76.4^{g}	10.89	7.44^{f}	.90	$.73^{g}$
C	72.2^{f}	10.44	6.89^{f}	.86	$.63^{fg}$
C_LDHT	74.5^{fg}	11.10	7.40^{f}	.85	$.63^{fg}$
C_HDHT	75.3^{g}	10.99	7.03^{f}	.82	$.64^{fg}$
C_LTEST	74.1^{fg}	10.39	6.90^{f}	.88	$.65^{fg}$
C_HTEST	74.4^{fg}	11.13	9.81^{g}	.88	$.61^{f}$
C_LFED	7.12^{f}	10.21	6.21^{f}	.85	$.61^{f}$
SEM[e]	.10	.57	.72	.03	.03
Initial Slaughter Group	56.6	--	--	.95	.64

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Diaphysis length measured from epiphyseal plate on distal end to proximal end epiphyseal plate; Proximal and distal end growth measured from tetracycline boundary to epiphyseal plate.

[d]Data reported in millimeters. [e]SEM=standard error of least squares means.

TABLE VIII-8 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON DIAPHYSIS LENGTH, PROXIMAL AND DISTAL AND GROWTH MEASUREMENTS AND PROXIMAL AND DISTAL END EPIPHYSEAL PLATE WIDTHS OF THE RADIUS[a]

	Bone Measurements[cd]				
Treatment Group[b]	Diaphysis Length	Proximal End Growth	Distal End Growth	Proximal Epiphyseal Plate Width	Distal Epiphyseal Plate Width
B	105.0^{g}	6.71^{gh}	3.75^{h}	$.78^{h}$	$.45^{g}$
C	99.9^{f}	6.26^{fg}	2.88^{f}	$.71^{gh}$	$.41^{fg}$
C_LDHT	101.0^{f}	5.51^{f}	2.44^{fg}	$.52^{f}$	$.35^{f}$
C_HDHT	101.6^{f}	5.91^{fg}	2.14^{f}	$.72^{gh}$	$.45^{g}$
C_LTEST	101.2^{f}	7.28^{hi}	3.10^{gh}	$.72^{gh}$	$.35^{f}$
C_HTEST	101.9^{f}	7.76^{i}	3.15^{gh}	$.68^{g}$	$.37^{fg}$
C_LFED	101.9^{f}	6.30^{fg}	3.10^{gh}	$.64^{g}$	$.36^{f}$
SEM[e]	.16	.30	.22	28.24	27.26
Initial Slaughter Group	89.5	--	--	.79	.50

[a] Treatment means within columns with different superscripts differ ($P < .05$).

[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c] Diaphysis length measured from epiphyseal plate on distal end to proximal end epiphyseal plate; Proximal and distal end growth measured from tetracycline boundary to epiphyseal plate.

[d] Data reported in millimeters.

[e] SEM=standard error of least squares means.

The radii of POSTP pigs (table VIII-8), showed that boars had 5.1% (.5 cm) longer diaphysis lengths than castrates which accounts for most of the .6 cm advantage in total radius length. Implanted groups had slightly longer diaphysis lengths than castrates which correlates well with the slightly longer radii data presented in table VIII-6. No definite trends are apparent from the tetracycline data obtained on the radius from POSTP pigs except that C_LTEST and C_HTEST pigs had more proximal end growth ($P < .05$) than boars, castrates or DHT implanted pigs (table VIII-8). In both PREP and POSTP studies, the greatest growth of the radius occurred at the proximal ends. In addition, the ratio of the growth on the distal ends to that occurring on the proximal ends as determined by tetracycline marking was about .6 for PREP pigs and about .4 for POSTP pigs, indicating a shift in growth intensity with increased age of the pig.

In general the epiphyseal plate widths of the bones examined were greater for PREP pigs relative to POSTP pigs. While boars of both studies tended to have greater (6 to 10% on proximal ends and 10 to 16% on distal ends for PREP and POSTP studies, respectively) plate widths than castrates, none of the differences was significant. This trend is not consistent with the concept of wider epiphyseal plate widths observed in castrated male rats (Silberberg and Silberberg, 1971). No other trends due to

androgen treatment were detectable except that implanted pigs like castrates had narrower plate widths than boars which was not unexpected based upon reported effects of testosterone upon epiphyseal plate widths (Silberberg and Silberberg, 1971).

The tibia was selected as the representative long bone from the hindleg for longitudinal cross sectional measurements. However, this selection was made prior to statistical analysis of the bone length measurements. A better choice for these measurements might have been the femur for the hindleg and the humerus for the forelimb since these bones showed greater differences in total length between boars and castrates than the tibia.

The tibia diaphysis length, growth measurements on the proximal and distal end, and the proximal and distal end epiphyseal plate widths of PREP and POSTP studies are presented in tables VIII-9 and 10, respectively. No differences in tibia diaphysis length were detected for the PREP study but trends were the same as for total length measurements. In the POSTP study, C_HTEST pigs appeared to have greater tibia diaphysis lengths than castrates and C_LDHT pigs but not the other treatments. As indicated by the ratio of growth on the distal end to that on the proximal end determined from tetracycline marking, greater growth activity occurred in the tibia for PREP pigs compared with POSTP pigs. No distinct differences were

TABLE VIII-9 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON DIAPHYSIS LENGTH, PROXIMAL AND DISTAL END GROWTH MEASUREMENTS AND PROXIMAL AND DISTAL END EPIPHYSEAL PLATE WIDTHS OF THE TIBIA[a]

	Bone Measurements[cd]				
Treatment Group[b]	Diaphysis Length	Proximal End Growth	Distal End Growth	Proximal Epiphyseal Plate Width	Distal Epiphyseal Plate Width
B	111.8	14.78^{fg}	10.34^{fg}	$.99^{f}$	$.89^{g}$
C	105.9	13.44^{f}	10.12^{f}	$.94^{f}$	$.90^{h}$
C_LDHT	109.9	14.92^{fg}	11.24^{g}	1.01^{fg}	$.72^{f}$
C_HDHT	106.9	14.63^{fg}	10.83^{fg}	$.99^{f}$	$.72^{f}$
C_LTEST	110.8	15.77^{g}	11.18^{g}	1.08^{g}	$.83^{g}$
C_HTEST	111.6	14.45^{fg}	10.18^{f}	$.97^{f}$	$.74^{fg}$
C_LFED	103.9	13.19^{f}	10.10^{f}	$.91^{f}$	$.85^{h}$
SEM[e]	.39	.54	.33	.03	.032
Initial Slaughter Group	80.5	--	--	1.03	.94

[a] Treatment means within columns with different superscripts differ ($P < .05$).

[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c] Diaphysis length measured from epiphyseal plate on distal end to proximal end epiphyseal plate; proximal and distal end growth measured from tetracycline boundary to epiphyseal plate.

[d] Data reported in millimeters.

[e] SEM=standard error of least squares means.

TABLE VIII-10 EFFECTS OF PUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON DIAPHYSIS LENGTH, PROXIMAL AND DISTAL END GROWTH MEASUREMENTS AND PROXIMAL AND DISTAL END EPIPHYSEAL PLATE WIDTHS OF THE TIBIA[a]

	Bone Measurements[ed]				
Treatment Group[b]	Diaphysis Length	Proximal End Growth	Distal End Growth	Proximal Epiphyseal Plate Width	Distal Epiphyseal Plate Width
B	152.7^{gh}	10.51^{jk}	6.59^{g}	$.84^{g}$	.67
C	148.4^{fg}	8.62^{fg}	4.67^{f}	$.69^{f}$	.56
C_LDHT	146.2^{f}	8.69^{fg}	5.74^{g}	$.75^{fg}$	.54
C_HDHT	150.8^{fgh}	8.36^{f}	4.58^{f}	$.82^{fg}$	.63
C_LTEST	150.2^{fgh}	9.60^{hi}	6.02^{g}	$.87^{g}$	.60
C_HTEST	155.3^{h}	10.35^{ij}	6.76^{g}	$.85^{g}$	.59
C_LFED	146.2^{f}	9.30^{gh}	6.02^{g}	$.77^{fg}$	.53
SEM[e]	.20	.25	.29	.04	.05
Initial Slaughter Group	133.8	--	--	.91	.67

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[c]Diaphysis length measured from epiphyseal plate on distal end to proximal end epiphyseal plate; proximal and distal end growth measured from tetracycline boundary to epiphyseal plate.

[d]Data reported in millimeters. [e]SEM=standard error of least squares means.

visualized between treatments of PREP pigs for tetracycline marked growth measurements; however, there were differences in the POSTP study. Boars had 22% greater growth on the proximal and distal ends of the tibia than castrates. In addition, C_LTEST and C_HTEST pigs had greater (12 to 19%, respectively) proximal end growth than castrates or DHT implanted pigs. Similar trends occurred for growth at the distal end. These observations are consistent with the effects of testosterone upon bone growth in rats measured by tetracycline as an intravital marker (Jansson et al., 1983).

Tibia epiphyseal plate widths were greater in PREP pigs than in POSTP pigs. Small differences existed between treatments in either the proximal or distal end epiphyseal plate widths for PREP pigs. In POSTP pigs, boars had 21% greater proximal end plate widths and 20% greater ($P < .05$) distal end plate widths than castrates. Although nonsignificant, there appeared to be a trend for greater plate widths relative to castrates for the testosterone implant dosages used in this study.

Because no definite treatment trends in epiphyseal plate widths or tetracycline marked growth of the radius or tibia were detected from the individual observations, the proximal and distal end measurements were added together. These summed plate widths and texracycline marked growth measurements from the proximal and distal ends are

presented in tables VIII-15 and 16 for PREP and POSTP pigs, respectively. Based upon these summed data, essentially no differences existed for epiphyseal plate widths of the radius in PREP pigs. The summed tibia epiphyseal plate data, indicate that DHT at both doses and TEST at the higher dose may be decreasing the plate width relative to castrates and definitely relative to boars. The summed tetracycline marked growth measurements indicate that TEST and DHT at the low doses were stimulatory to tibia growth relative to castrates; however, the C_HTEST was more effective in stimulating longitudinal growth than C_LTEST for the radius and the C_LTEST was more effective than C_HTEST in the tibia. Higher microscopic magnifications should be used for epiphyseal plate width determinations and fine tuning of the tetracycline marking method is needed. This would involve more precise marking of the fluorescent bands and the use of higher microscopic magnification to ensure greater sensitivity of the measurements. The growth trends for both the radius and tibia were similar within each study. The greater growth in the tibia compared to the radius is not in agreement with the hypothesis of McMeekan (1940) that the bones of the forelimb grow at a faster rate than hindlimb bones.

Bone Thickness. Knudson (1983) used the ratio of bone weight to length as an indicator of bone thickening. The bone thickness estimates for the scapula, radius-ulna,

tibia-fibula, humerus and femur bones and are presented in tables VIII-11 and 12 for PREP and POSTP pigs, respectively. In the PREP study, there was no suggestion for bone thickening or increased density in boars and testosterone treated pigs relative to castrates based upon bone weight to length. Only the tibia-fibula of boars had a 16% ($P < .05$) higher ratio of weight to length than castrates. For POSTP boars the weight to length ratios were 13.4, 8.8 and 6.7% larger for the scapula, radius-ulna and tibia-fibula bones, respectively, and the ratios for the humerus and femur tended to be higher than castrates. This confirms observations of Walstra (1980). Based on this ratio of weight to length indication of density or thickening, no differences occurred due to either form of androgen.

As described in the methods section of this chapter, another indicator of diaphysis wall thickening was examined. The total circumferential area of the diaphysis wall on the proximal and distal ends of a center section of the humerus and femur are given in tables VIII-13 and 14 for the PREP and POSTP studies, respectively. With this method, greater thickening occurred in boars relative to castrates in both studies. Wall thicknesses were also almost 2-fold greater in POSTP than PREP pigs. After comparing the treatment group thickness measurements to the initial values for each study, it is apparent that the

TABLE VIII-11 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON RATIOS OF WEIGHT TO LENGTH OF THE SCAPULA, RADIUS-ULNA, TIBIA-FIBULA, HUMERUS AND FEMUR[ab]

Treatment Group[c]	Bone				
	Scapula	Radius-Ulna	Tibia-Fibula	Humerus	Femur
B	7.34	11.68^{f}	10.27^{g}	10.42^{ef}	11.84^{g}
C	7.50	10.93^{ef}	8.86^{ef}	10.61^{ef}	11.15^{fg}
C_LDHT	6.95	11.31^{ef}	9.03^{eg}	10.39^{ef}	10.18^{e}
C_HDHT	7.20	11.31^{ef}	8.26^{e}	9.98^{ef}	10.30^{e}
C_LTEST	7.18	11.53^{f}	8.57^{e}	10.33^{ef}	10.44^{ef}
C_HTEST	7.15	10.58^{e}	9.89^{fg}	10.74^{f}	11.38^{g}
C_LFED	7.89	10.30^{e}	9.01^{eg}	9.73^{e}	10.96^{eg}
SEM[d]	.27	.32	.42	.29	.30
Initial Slaughter Group	3.47	7.51	5.90	6.06	6.62

[a]Bone weight divided by bone length.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least square means.

TABLE VIII-12 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) AND DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON RATIOS OF WEIGHT TO LENGTH OF THE SCAPULA, RADIUS-ULNA, TIBIA-FIBULA, HUMERUS AND FEMUR[ab]

	Bone				
Treatment Group[c]	Scapula	Radius-Ulna	Tibia-Fibula	Humerus	Femur
B	14.72[f]	21.20[f]	15.81[f]	20.71[f]	18.70
C	12.98[e]	19.39[e]	14.82[e]	19.08[ef]	17.93
C_LDHT	13.62[ef]	18.32[e]	14.55[e]	18.33[ef]	17.66
C_HDHT	13.82[ef]	18.94[e]	14.97[ef]	18.61[ef]	17.62
C_LTEST	13.71[ef]	19.27[e]	15.02[ef]	18.93[ef]	18.59
C_HTEST	13.13[ef]	19.34[e]	15.26[ef]	18.13[ef]	18.37
C_LFED	12.32[e]	18.87[e]	15.15[ef]	17.37[e]	17.87
SEM[d]	.51	.52	.26	.83	.52
Initial Slaughter Group	10.79	16.28	12.43	15.90	15.57

[a]Bone weight divided by bone length.

[b]Treatment means within columns with different superscripts differ ($P < .05$).

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least square means.

TABLE VIII-13 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON THICKNESS MEASUREMENTS ON THE PROXIMAL AND DISTAL ENDS OF THE HUMERUS AND FEMUR

	Bone[ab]			
	Humerus		Femur	
Treatment Group[c]	Proximal Thickness	Distal Thickness	Proximal Thickness	Distal Thickness
B	1.98^{g}	2.09^{f}	1.90^{f}	1.92^{f}
C	1.39^{e}	1.58^{e}	1.36^{e}	1.48^{e}
C_LDHT	1.45^{ef}	2.12^{f}	1.42^{e}	1.54^{e}
C_HDHT	1.67^{eg}	1.81^{ef}	1.62^{ef}	1.63^{ef}
C_LTEST	1.79^{g}	2.00^{f}	1.73^{f}	1.55^{e}
C_HTEST	1.75^{fg}	1.91^{ef}	1.74^{f}	1.59^{e}
C_LFED	1.44^{e}	1.62^{e}	1.41^{e}	1.43^{e}
SEM[d]	.10	.12	.09	.10
Initial Slaughter Group	.71	1.07	.93	.69

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]Thickness measurement is the area in cm^2 of diaphysis wall.

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least square means.

TABLE VIII-14 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON THICKNESS MEASUREMENTS OF THE PROXIMAL AND DISTAL ENDS OF THE HUMERUS AND FEMUR

	Bone[ab]			
	Humerus		Femur	
Treatment Group[c]	Proximal Thickness	Distal Thickness	Proximal Thickness	Distal Thickness
B	3.50^{fg}	4.13^{i}	3.72^{g}	3.27^{g}
C	2.81^{e}	3.31^{e}	2.67^{e}	2.41^{e}
C_LDHT	2.92^{eg}	3.76^{gh}	3.04^{ef}	3.03^{fg}
C_HDHT	3.69^{g}	4.10^{hi}	3.01^{ef}	3.09^{fg}
C_LTEST	3.04^{ef}	3.74^{g}	3.30^{fg}	2.99^{fg}
C_HTEST	3.23^{eg}	3.66^{f}	3.42^{fg}	2.88^{eg}
C_LFED	2.69^{e}	3.38^{ef}	2.50^{e}	2.52^{ef}
SEM[d]	.16	.17	.19	.10
Initial Slaughter Group	2.48	2.38	2.69	3.38

[a]Treatment means within columns with different superscripts differ ($P < .05$).

[b]Thickness measurement is the area in cm^2 of diaphysis wall.

[c]B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.

[d]SEM=standard error of least square means.

greatest amount of thickening occurred prior to the initiation of the POSTP study or in other words pigs weighing less than 75 kg. TEST and DHT implanted pigs also tended to have greater bone thicknesses relative to castrates.

In addition to the summed proximal and distal end epiphyseal plate widths and tetracycline marked growth tables VIII-15 and 16 present a summation of the proximal and distal end measurements of the diaphyseal center section of the humerus and femur to indicate diaphyseal wall areas for the PREP and POSTP studies, respectively. In the PREP study, boars had 37 and 35% greater HU and FE diaphyseal wall thicknesses, respectively, than castrates. An overall trend for increased thickening is indicated for the testosterone treatments. The C_HDHT pigs had 20%, C_LTEST pigs 28% and C_HTEST pigs 23% thicker humerus diaphyseal walls than castrates. Since considerable thickening had already occurred prior to the initiation of the POSTP study, the changes in thickness from the initial slaughter group of POSTP pigs were less than in the PREP study. However, boars in the POSTP study had 25 and 38% thicker humerus and femur diaphyseal walls than castrates and C_HDHT implanted pigs had 27% greater humerus thicknesses than castrates. C_LDHT pigs had 19.5%, C_LTEST pigs 24% and C_HTEST pigs 24% thicker femur diaphyseal walls than castrates. The other implant groups

TABLE VIII-15 EFFECTS OF PREPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON AVERAGE PROXIMAL AND DISTAL END EPIPHYSEAL PLATE WIDTHS AND TETRACYCLINE MARKED GROWTH MEASUREMENTS OF THE TIBIA AND RADIUS AND DIAPHYSIS WALL THICKNESS OF THE HUMERUS AND FEMUR[a]

Treatment Group[b]	Epiphyseal Plate[c] Width		Tetracycline marked growth[d]		Diaphyseal wall thickness[e]	
	Radius	Tibia	Radius	Tibia	Humerus	Femur
B	1630.6	1873.5^{ij}	18.32^{gh}	25.11^{gi}	4.06^{i}	3.83^{h}
C	1485.5	1845.0^{hij}	17.33^{gh}	23.57^{g}	2.97^{g}	2.84^{g}
C_LDHT	1483.9	1729.2^{gi}	18.50^{gh}	26.16^{hi}	3.58^{hi}	2.96^{g}
C_HDHT	1464.7	1705.1^{g}	18.03^{gh}	25.46^{gi}	3.48^{gi}	3.26^{gh}
C_LTEST	1528.0	1905.5^{j}	17.28^{g}	26.95^{i}	3.79^{i}	3.28^{gh}
C_HTEST	1490.2	1713.2^{gh}	20.94^{h}	24.63^{gh}	3.66^{hi}	3.34^{gh}
C_LFED	1465.2	1764.7^{gij}	16.42^{g}	23.29^{g}	3.06^{gh}	2.85^{g}
SEM[f]	57.32	46.46	1.22	.74	.19	.19
Initial Slaughter Group	1586.7	1972.8	--	--	1.78	1.62

[a] Treatment means within columns with different superscripts differ ($P < .05$).
[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[c] Plate width in micrometers.
[d] Tetracycline marked growth in millimeters.
[e] Thickness measurement is the area in cm^2 of the diaphysis wall.
[f] SEM=standard error of least square means.

TABLE VIII-16 EFFECTS OF POSTPUBERTAL CASTRATION AND ADMINISTRATION OF TESTOSTERONE (TEST) OR DIHYDROTESTOSTERONE (DHT) TO CASTRATED MALE PIGS UPON POOLED PROXIMAL AND DISTAL END EPIPHYSEAL PLATE WIDTHS AND TETRACYCLINE MARKED GROWTH MEASUREMENTS OF THE TIBIA AND RADIUS AND DIAPHYSIS WALL THICKNESS OF THE HUMERUS AND FEMUR[a]

Treatment Group[b]	Epiphyseal Plate Width[c]		Tetracycline Marked Growth[d]		Diaphyseal Wall Thickness[c]	
	Radius	Tibia	Radius	Tibia	Humerus	Femur
B	1234.9^{j}	1506.7	10.46^{ij}	17.10^{l}	7.64^{h}	6.99^{h}
C	1118.3^{hij}	1251.2	9.15^{gj}	13.29^{g}	6.13^{g}	5.08^{g}
C_LDHT	867.3^{g}	1288.0	7.94^{g}	14.42^{hij}	6.68^{g}	6.07^{gh}
C_HDHT	1166.4^{ij}	1446.9	8.05^{gh}	12.94^{gh}	7.79^{h}	6.10^{gh}
C_LTEST	1073.0^{hi}	1472.3	10.39^{ij}	15.62^{ijk}	6.78^{g}	6.29^{hi}
C_HTEST	1048.6^{hi}	1446.2	10.91^{i}	17.01^{kl}	6.89^{gh}	6.30^{h}
C_LFED	1000.7^{h}	1307.4	9.40^{hj}	15.32^{j}	6.06^{g}	6.02^{g}
SEM[f]	44.23	83.39	.44	.44	.25	.30
Initial Slaughter Group	1295.0	1579.4	--	--	6.07	4.86

[a] Treatment means within columns with different superscripts differ ($P < .05$).
[b] B=boars, C=castrates, C_L=low implants, C_H=high implants, C_LFED=limit-fed castrates.
[c] Plate width in micrometers.
[d] Tetracycline marked growth in millimeters.
[e] Thickness measurement is the area in cm^2 of the diaphysis wall.
[f] SEM=standard error of least square means.

had thicker ($P < .05$) humerus and femur diaphyseal walls than castrates but they were less than the diaphyseal thicknesses of boars.

Short (1980) discussed the "double threshold" effect of testosterone upon bone growth. High concentrations initially stimulate bone but may also retard growth, whereas low concentrations are usually stimulatory. The effect of large doses of testosterone on growth suppression may not be on bone per se but mediated through inhibition of pituitary function (Silberberg and Silberberg, 1971). The effects of testosterone on bone growth in normal castrated and gonadohypopysectomized male rats was reported by Jansson et al. (1983). Testosterone caused a dose dependent increase in linear bone growth in castrated rats. However, in growth hormone deficient castrated rats, no increase in bone growth due to testosterone was observed.

In the present study, no consistent dose dependent effects upon linear bone growth were observed for either testosterone or dihydrotestosterone. The effect of castration upon bone accretion was less apparent in PREP pigs than POSTP pigs. In addition, bone accretion was more effectively stimulated by testosterone in POSTP pigs than in PREP pigs. The effects of castration reported here are less obvious than reported in rats (Spencer et al., 1976) but follow trends reported by Knudson (1983). While the

effects of castration or androgen administration upon linear growth were subtle in this study, it appears that the presence of the testes and testosterone administration (at these doese) stimulate bone lengthening and bone thickening in pigs. These effects on bone growth also coincide with a stimulation of selected muscles, particularly the semintendinosus (Chapter IV).

Summary

Weights of several bones of pigs in the 3 wk study did not differ due to treatment. In the prepubertal study, high testosterone implanted pigs did not differ from castrates but had more bone than high dihydrotestosterone implanted pigs. This latter difference was apparent in the tibia-fibula and femur bone weights. In the pubertal study, boars had greater total bone and individual bone weights than castrates. There was also a tendency for high testosterone implanted pigs to have more total bone than high dihydrotestosterone implanted pigs. A similar trend was observed for low testosterone compared to low dihydrotestosterone.

Boars tended to have longer bones than castrates in all studies. Tibia linear growth appeared to be stimulated by high testosterone in both 5 wek studies. This is supported by total length, diaphysis length and tetracycline marked growth measurements. Since dihydrotestosterone implanted

pigs were similar to castrates in these measurements, a differential effect due to the form of androgen was detected.

Androgen administration increased bone thickness compared to castrates but generally not to the same degree as the differences observed between boars and castrates. Testosterone implanted pigs tended to have thicker bones than dihydrotestosterone implanted pigs.

Based upon the bone data, it appears that castration decreased bone growth and that androgens can increase bone growth and thickening but testosterone was more effective than dihydrotestosterone. This indicates a difference in the mode of action of the two forms of the androgen. In terms of use as growth promotants, testosterone proved to be more suitable than dihydrotestosterone in stimulating linear bone growth. The effects observed in these studies may be different from those observed in a more chronic administration.

APPENDICES

TABLE A.1. Testosterone Assay

PREPARATION

1. Set up assay sheet (160 tube maximum - including standards, standard serum, samples).

2. Number extraction tubes (16 X 100 mm) for Tracers (TR), standard serum (SS) and blanks.

3. Number assay tubes (12 X 75 mm) for standards, zeros, SS and samples.

EXTRACTION EFFICIENCY

1. Add 10ul ^{3}H testosterone (refrigerator 3 - no. 3072) to each of 3 scintillation vials and 3 extraction tubes (TR). Dry with nitrogen. (use Hamilton syringe - clean with MeOH before and after).

2. To the scintillation vials add 5.0 ml ACS cocktail, cap and label "TTC" (tracer total counts). Set aside.

3. To tubes add appropriate amount of serum (200ul) from random samples to be assayed or a standard serum, allow to equilibrate 30 minutes and proceed with extractions.

SAMPLING AND EXTRACTION

1. Sampling - add 200ul serum to extraction tubes according to the assay sheet.
   a) Standard serum - low (200ul) and high (50ul) in triplicate.
   b) Unknowns - 200ul in duplicate.

2. Extraction - add 10 volumes (2 ml) Benzene: Hexane (1:2) to each tube.
   a) Vortex all tubes for 30 seconds.
   b) Freeze in the tubes with a MeOH: dry ice bath.
   c) Decant the supernatant:
      1) TR decant into scintillation vials, add 5.0 ml ACS, cap, label "TR" and set aside.
      2) Decant samples and standard serum into 12 X 75 mm tubes.
      3) Evaporate the solvent in vacuum oven in the hood.

STANDARD CURVES

1. With hamilton syringe, add appropriate amounts to tubes. Do in triplicate: 0, 5, 10, 15, 20, 25, 30, 40, 60, 80, 100 ul

Page 2 - TABLE A.1.

Standard curves - continued

These are taken from stock testosterone solution (10 ng/ml)

2. Allow to dry in vacuum drying oven.

ASSAY PROCEDURE

1. Add 200ul antibody*to all tubes (not background), vortex 2 seconds.

2. Allow to equilibrate 30 min at room temperature.

3. Add 200ul ^{3}H testosterone to all tubes and to 3 scintillation vials, vortex tubes. To scintillation vials add 5.0 ml ACS, cap and label "100%" or "TC". Set aside.

4. Incubate tubes 12-18 hours at 1-5^{o} (cooler, C).

Separation of Bound vs. Free hormone

(.5% charcoal, 1% dextran)

1. Put stock charcoal solution on magnetic stirrer for about 10 minutes.

2. Put assay tubes on ice bath for 10-15 minutes.

3. Aliquot enough charcoal for assay into small beaker with a stir bar and place in ice bath on stir plate.

4. Add 0.5 ml charcoal to all tubes with a Cornwall syringe.

5. Vortex and spin 15 min at 3000 rpm.

6. Put carriers into ice bath.

7. Decant the supernatants into scintillation vials and mix with 5.0 ml ACS.

These above steps must be done quickly and without interruption

COUNTING

1. Load counter according to the computer protocol.

2. Count all tubes for 4 min and record on magnetic tape.

*Antibody has cross reactivity with DHT of approximately 55%.

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Computer Protocol:

back ground
"0"
std curves
TTC
TR
samples - (blanks, High serum, low serum and samples)
100% or TC

TABLE B. 1 Stock amino acid solution

Amino Acid	g/l
Alanine	.6060
Arginine	.3640
Aspartie	.0538
Cysteine	.1078
Glutamine	.4310
Glycine	.5454
Histidine	.1482
Isolencine	.3570
Leucine	.4580
Lysine	.5926
Methionine	.1280
Phenylalanine	.1346
Proline	.5792
Valine	.3030
Threonine	.1548
Serine	.2290
Glutamine	.4512
Tryptophan	.2356
Asparagine	.0538
Chloramphenicol*	.0054

This mixture was calculated to be 5 X porcine plasma values plus corrected for dilution in KRB solution.

*Not an amino acid; added as a preservative.

TABLE B. 2 Phosphate buffer

Stock solution:

Monobasic Sodium phosphate ($NaH_2PO_4 \cdot H_2O$)	Dibasic Sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$)
27.5 g/l	53.6 g/l

Volumes of each plus 100 ml H_2O to give pH desired:

pH		
6.8	51	49
7.0	34	61
7.1	33	67
7.2	28	72
7.3	23	77
7.4	19	81
7.6	13	87

TABLE B. 3 Citrate - phosphate - borate buffer

Primary stock solutions:

1. 40 g NaOH per liter
2. 35 ml of 85% H_3PO_4 per liter
3. 70 g citric acid per liter
4. .1 N HCl

Mixture of above*:

100 ml H_3PO_4

100 ml Citric acid

343 ml NaOH

3.54 g Boric acid

Bring to 1 liter with water

*Take 20 ml of the mixture plus corresponding HCl to arrive at desired pH. For pH 6.7 this requires about 34.6 ml of HCl.

From: Teorell, T. and E. Stenhagen. 1938. Ein Universalpuffer furden pH-Bereich 2.0 to 12.0. Biochem. Z. 299:416.

TABLE B. 4 Krebs-Ringer bicarbonate buffer

Primary stock solutions		Volume used to make 5 X buffer**
NaCl	4.50g/100 ml	25 ml
KCL	5.75g/100 ml	1 ml
$CaCl_2$	6.10g/100 ml	.75 ml
KH_2PO_4	10.55g/100 ml	.25 ml
$MgSO_4$	9.25g/100 ml	.25 ml
or		
$MgSO_4 \cdot 7H_2O$	19.10g/100 ml	
$NaHCO_3$*	65.00g/1000 ml	5.25 ml

*Gassed with O_2:CO_2 (95:5) for 1 h

**On day of use, dilute with 130 ml H_2O and gas for 15 min.

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