Farm Animal Models for Cellular and Molecular Skeletal Muscle Research

Susan K. Duckett,KatherineM. Byrne',KirnL. Hossner 'andMichael V. Dodson[(i]*

University of Idaho, Moscow, (I) Washington State University, Pullman and (2) Colorado State University, Ft Col/ins

Abstract
The idea of using farm animals to serve mankind far exceeds their use as a source of meat. Farm animals make excellent models for investigating tissue growth, producing biological materials for human use, and developing surgical protocols, artificial organ-replacement devices and medication doses. Domesticated sheep have recently been identified that display selective muscle hypertrophy under the influence of a single gene. These animals, along with certain breeds of cattle that display double muscling as a result of hyperplasia, represent new domestic animal models applicable for cellular and molecular skeletal muscle research. Combined with recent advances in cloning and gene transfer, these animal models provide powerful tools for the investigation of cellular and molecular mechanisms that regulate muscle development.
Key words: muscle, hypertrophy, hyperplasia, cloning, transgenics.

Basic Appl. Myol. 8 (2):169-173, 1998

The Use of Farm Animals for Growth/Clinical Studies

Recently, new developments in human medicine have come about through the use of farm animals. Sheep and goats have been used to develop and test new surgical techniques such as dynamic cardiomyoplasty. Human medicinals are now being produced by transgenic farm animals. Cloning of farm animals has set the stage for future application in human medicine and muscle growth research. In this section, we will review the current status of using farm animals for clinical and growth studies.

Dynamic cardiomyoplasty

Failure to maintain normal cardiac output by damaged heart muscle results in compromised health of humans [39] and is one of the leading causes of patient death. Restoration of cardiac function by heart transplantation is usually the final option for treatment, but many complications arise in finding a compatible donor at the critical time period for clinical resolution. There are several options for short-term maintenance of cardiac function until a permanent donor heart can be found. These include limitation of mobility and use of medication to insure that cardiac output is proportional to patient activity [11], electro stimulation of remaining cardiac tissue, as well as use of artificial pumping chambers to bridge the gap between certainty of heart failure and initiation of the donor heart function following transplantation.

Another option, is the use of dynamic cardiomyoplasty [9, 45] in which the skeletal muscle ventricle is used to make the heart contract reversing the damaged structure of the failing heart [reviewed in 1], This method was developed and tested using animals (dogs, goats, sheep). Now, the patient's own Latissimus dorsi muscle is repositioned to envelop the heart, subsequently conditioned to withstand the consistent intermittent contractions required, then stimulated to augment cardiac contractility [I]. This method has been used successfully to maintain human life for as long as eight and one-half years following surgery [8].

Transgenics

Transgenic animals are those which possess a foreign gene in their genome. They have been used to study muscle development [2, 17, 33], identify the role of genes in cardiac muscle disorders [34], improve disease resistance [24, 42], serve as bioreactors [42], and test models of muscle gene therapy [19, 26]. Historically, the creation of transgenic animals was accomplished by microinjecting the gene of interest into a fertilized ovum [5, 23], More recently, though, viral vectors such as retrovinises and adenoviruses have been used to transfect embryos with specific gene [26]. These animals have a distinct advantage over cultured cells since they provide information on the influence of the gene in a complex multicellular system, in vivo. In addition, transgenic animals can sexually reproduce and pass on the alteration to offspring [15, 43].

Muscle researchers have used transgeiiic animals to study smooth muscle regulation in vascular disease [10, 34] and myofibril formation in skeletal muscle disease [18]. For animal scientists, transgenic animals provide a means to improve muscle development by increasing muscle mass, improving tenderness, and increasing feed efficiency. Expression of growth hormone, growth hormone releasing factor, and insulin-like growth factor-1 have been used as transgenes in farm animals [41,42]. Although these transgenic animals were shown to produce the growth factors, increased growth and muscle development was varied and limited [42]. The transgenic sheep and pigs that were produced also had physiologic dysfunctions including stomach ulcers, joint disease, kidney disease, and infertility. The primary problem with transgenic animals is an unregulated, continuous gene expression in all tissues of the body at all developmental stages. Thus, constant exposure of the fetus, neonate and adult to pharmacological levels of the transgene product leads to serious metabolic consequences for the affected animal. The phosphoenolpyruvate carboxykinase (PEPCK) gene promoter which is regulated by systemic glucose in a tissue-specific, development-dependent manner, was shown to effectively regulate Iransgene expression in mice [35] but has not been used successfully in ruminants.

Cloning

Individual animal variation Is the bane of animal researchers. Using animals of the same breed or strain reduces experimental variability, but determining the significance of relatively minor physiological changes or differences in treatment effects requires large numbers of animals. The idea of using cloned animals for research has been present for many years. The first studies to produce genetically identical vertebrates were reported by Briggs and King in 1952 [6], who transplanted frog nuclei into enucleated eggs and were able to induec development up to the tadpole stage. For mammals, relatively simple methods such as embryo splitting with mechanical tools provided identical embryos but is limited to the production of 2 or 4 clones at a time. This method has been used successfully with cattle [32, 38] and sheep [20, 48], However, a more refined technique for producing larger numbers of identical animals is the use of nuclear transplantation. This method uses nuclei from cells of preimplantation embryos which are transplanted into enucleated oocyles. Although early studies used a physical transfer of nuclei with micromanipulation/micro injection [25], the use of electrically-induced cell fusion is much more efficient [36], providing greater than 90% success versus the 30-40% success rate in nuclear transfer using strictly mechanical means. Nuclear transplantation has been used successfully in sheep [44, 47] and cattle [4, 40].

The above studies depend on the use of undifferentiated cells from embryos of unknown potential. Ideally, one would like to use differentiated cells from adult animals with known superior genetic traits for production of meat, wool or offspring. It has been a long-held tenet of development biology that as cells differentiate into the specialized functions of adults, they become more and more irreversibly specialized and incapable of producing all of the cells of an embryo or adult. Thus, cells which were differentiated past the morula stage of early embryo genesis were not totipotent and their use in cloning was inefficient and impractical. The recent publication of a method which used differentiated adult cells to make clones has altered our perceptions of totipotency, differentiation and animal cloning. This breakthrough in technology used mammary to enter the quiescent phase (Go) of the cell cycle before electrophoretie nuclear transfer into enucleated oocytes [49]. Synchronizing the adult cells to the Go phase of the cell cycle by serum starvation allows the differentiated nucleus to be "reprogrammed" to an undif-ferentiated, totipotent state by the oocyte cytoplasm. This final piece to the puzzle of differentiation and its reversibility now provides us with the means to replicate farm animals of superior genetic characteristics in order to enhance animal productivity. In addition, this provides the cell biologist with the ability to study the mechanisms involved in the control of cell processes in superior animals. Primary cell cultures derived from cloned animals can be compared to production characteristics of the genetically identical animal in vivo. In addition, a melding of transgenic technology with cloning allows the exact replication of superior transgenic animals which may not breed true but which may prove to have invaluable genetic characteristics. Thus, the true clones, derived from adult animals with proven genetic characteristics, will provide animal and cell biologists with a source of genetically identical tissues which can be analyzed in the lab without irreversibly destroying the transgenic animal.

New Animal Models

Farm animal models with increased muscle mass are often sought for skeletal muscle research and increased lean production. Two new animal models will be introduced that increase muscle mass through two either hyperplasia or hypertrophy of muscle fibers. The status of research studies on these models will be presented in this section. We suggest the investigation into skeletal muscle growth using these animal models will help elucidate the mechanisms of myogenesis.

Callipyge

Callipyge (Greek; calli-, beautiful; -pyge, buttocks) is the name given to a gene that promotes and a phenotype that exhibits selective muscle hypertrophy in sheep. The locus of the callipyge gene was mapped to ovine chromosome 18 [14]. Matings between normal ewes and heterozygous callipyge rams indicated that the callipyge phenotype was controlled by a single, dominant gene when inherited from paternal allele [27], More recent work [13] shows that the callipyge gene is transmitted through a non-Mendelian inheritance pattern referred to as polar overdominance. In this inheritance pattern, the callipyge gene acts as a normal, dominant gene when it is inherited from the sire; however.

the gene does not follow simple Mendelian principles when it is inherited from the dam [13], Thus, the callipyge phenotype is only expressed in heterozygous sheep which inherit the gene from the sire.

The callipyge phenotype does not become visually apparent in lambs until about 4 to 6 weeks after birth [27] but can be detected earlier through the use of molecular markers [14].

Phenotypic expression of the callipyge gene results in selective muscle hypertrophy. Muscles in the pelvic limb and torso of callipyge sheep are enlarged by 42-50%, whereas muscles in the thoracic limb are unaffected [28], The enlarged muscles have increased percentages of fast twitch glycolytic (FG) fibers and larger fiber diameter of FG and fast [witch oxidative-glycolytic (FOG) fibers [7]. The hypertrophied muscles have increased proteinrDNA without changes in protein; RNA or RNA:DNA [7]. These results suggest that the enlargement of the callipyge muscles was through hypertrophy rather than hyperplasia, and possibly the result of reduced protein turnover in these muscles.

Callipyge hypertrophied muscles have higher levels of calpaslatin activity [30]. Calpastatin is the endogenous inhibitor of the calpain proteinase system and strongly associated with reduced muscle protein degradation. Callipyge sheep have a lower excretion of urinary nitrogen further indicating a reduction in protein turnover [3], Dietary administration of a p-adrenergic agonist to callipyge lambs does not enhance muscle growth [31]. Thus, the effects of the callipyge gene appear to be mediated through similar intracellular events as p-adrenergic agonists. Satellite cells have been isolated from the serni mem bran osus muscle of four normal and four callipyge sheep. Crude, primary (growing) cultures of satellite cells from each animal per group were pooled, cloned and viable satellite strains established. Although studies are not finished, preliminary data using both initial primary cultures and subsequent tertiary cultures suggest differences exist between normal and callipyge-derived satellite cells in ability to proliferate and differentiate in vitro [16].

Double muscling

Double muscling (DM) is a phenotype in cattle, pigs, and sheep where there is a generalized increase in muscle mass and cellularity. Breeds where the DM phenotype occurs in high frequency are Belgian Blue and Piedrnontese in cattle, Pictrain in pigs, and Texel in sheep. In cattle, the locus of the gene responsible for the DM has been mapped to bovine chromosome 2 and named rnh for muscular hypertrophy [12]. The DM phenotype occurs in the homozygous condition, for the recessive animals (mh/mh) [12]. The location of this gene is within the same interval as myostatin (GDF-8), a member of the transforming growth factor-b superfamily [29]. Disruption of the myostatin gene in mice was recently shown to increase muscle mass by 2-3 fold through a combination of hypertrophy and hyperplasia [37].

Myostatin appears to function as a negative regulator of skeletal muscle growth in mice [37]. Belgian Blue DM cattle are homozygous for an 11 base pair deletion in the coding region [22,29]. This mutation removes the portion of the myostatin protein that was targeted for the disruption in the mouse study [29, 37], Piedmontese DM cattle have a base pair transition in the same region [29]. Thus, mutations in the bovine myostatin gene result in the DM phenotype in cattle [22, 29]. At present, DM pigs and sheep breeds have not been examined for mutations in the myostatin gene. Double muscled fetuses have approxi-matley twice the number of muscle fibers as normal fetuses [46]. This increase in muscle fibers of DM is evident before 90 d of gestation [46], Mean fiber diameter area does not differ between normal and double muscled fetuses [46], Gerrard and Judge [21] found that scrum-stimulated myoblast replication was higher for DM than normal fetuses during the time when secondary rnyofiber formation was greatest. Thus, the DM phenotype in cattle is the result of hyperplasia, and not hypertrophy, of muscle fibers that occurs in the fetus. This is in contrast to hypertrophy seen in the callipyge sheep.

Conclusions

In this paper, we suggest that farm animals offer an alternative model for use in cellular and molecular study of muscle growth. Farm animal models like callipyge and double muscled animals combined with transgenics and cloning techniques will help identify specific regulatory elements involved in both embryonic, and postnatal myogenesis. In addition to meat production, farm animals represent excellent models for use in cellular and molecular skeletal muscle research.

Address correspondence to:

Dr Susan K. Duckett, University of Idaho, 216 Ag Science Bldg, Moscow, ID 83844-2330, phone 208 885 7390, fax 208 885 6420, Email sduckett@uidaho.edu.

References

[1] Arpesella G, Mikus PM, Giannoni A, Lombard! P, Pierangeli A, Zampieri S, Cantini C, Carraro U: Daily work-rest stimulation regimen of sheep LD for cardiomyoplasty: Rationale and results up to six months. *Basic ApplMyol* 1996; 6 (5): 341-349.

[2] Benito M, Valverde AM, Lorenzo M. IGF-I: A mitogen also involved in differentiation processes in mammalian cells. *Int J Biochem Cell Bio}* 1996; 28(5): 499-510.

[3] Bemal G, Johnson DE, Snowder GD, Torrent J, Ramsey J, Larson E, McGinty G: Effect of callipyge gene on metabolism of wethers. *Proc West Sect, Am Soc Anim Set* 1995; 46: 483-485.

[4] Bondioli KR, Westhusin ME, Looney CR: Production of identical bovine offspring by nuclear transfer. *Therio* 1990; 33; 165-174.

[5] Brem G, Brenig B, Goodman HM, Selfden RC, Graf F, KruJTB, Springman K, Hondele J, Meyer J, Winnaker EL, Krausslich H: Produclion of transgenic mice, rabbits and pigs by micro inject ion into pronuclei. *Zuchthygiene (Berlin)* 1985; 20: 251.

[6] Briggs R and King TJ: Transplantation of living nuclei from blasmla cells into enucleated frogs' eggs. *Proc Nat Acad Sci USA* 1952; 38: 455-463.

[7] Carpenter CE, Rice OD, Cockett. NE, Snowder, GD: Hisfology and composition of muscles from normal and callipyge lambs. *J Anim Set* 1996; 74: 388-393.

[8] Carraro U, Chachques JC, Desnos M, Hagege A, Fontaliran F, Carpentier A: Eight-year human dynamic cardiomyoplasty: Preserved structure of myofibers and vessiles of the lalissimus dorsi. *Basic App/Myol* 1996; 6 (5): 333-336.

[9] Carraro U: Basic and Applied Myology: Perspectives for the 90's. *Basic and Applied Myology (Padova, Italy)* Unipress 1991.

[10] Casscells W: Smooth muscle cell growth factors. *Prog Growth Factor Res* 1991; 3 (3): 177-206.

[11] Ccooni C, Bernocchi P, Pepi P, Benigno M, Ferrari R: Metabolic disorders of skeletal muscle in congestive heart failure. *Basic Appl Myoi* 1996; 6 *(5):* 327-332.

[12] Charlicr C, Coppictcrs W, Farnir F, Grobet L. Leroy PL, Michaux C, Mni M, Schwers A, Vanmanshoven P, Hansel R: The mh gene causing double-muscling in eattle maps to bovine chromosome 2. *Mamm Genome* 1995; 6 (11): 788-792.

[13] Cockett NE, Jackson SP, Shay TL, Farnir F, Berghmans S, Snowder GD, Nielsen DM, Georges M: Polar overdominance at the ovine callipyge locus. *Science* 1996; 273: 236-238.

[14] Coekett NE, Jackson SP, Shay TL, Nielsen D, Moore SS, Steele MR, Barendse W, Green RD, Georges M: Chromosomal localization of the callipyge gene in sheep (Ovisaries) using bovine DNA markers. *Proc Nat Ac Sci USA* 1994; 91: 3019-3023.

[15] Cundiff LV, Bishop MD, Johnson RK: Challenges and opportunities for integrating genetically modified animals into traditional animal breeding plans. *J Anim Sci* 1993; 71 Suppl 3: 20-25.

[16] Dodson MV: personal communication.

[17] Doevcndans PA, Van Bilsen M: Transcription factors and the cardiac gene pro gramme. *IntJBiochem Cell Bio!* 1996; 28 (4): 387-403.

[18] Epstein HG, Bernstein Sf: Genetic approaches to understanding muscle development. *Dev Biol* 1992; 154 (2): 231-244.

[19] Fuller SJ, Chien KR: Genetic engineering of cardiac musele cells: In vitro and in vivo. *Genetic Engineering* 1994; 16: 17-31.

[20] Gatica R, Roland MP, Crosby TF, Gordon I: Micromanipulation of sheep morulae to produce monozygotic twins. *Theria* 1984; 21: 555-560.

[21] Gerrard DE, Judge MD: Induction of myoblast proliferation in L6 myoblast cultures by fetal serum of double-muscled and normal cattle. *J Anim Sci* 1993; 71: 1464-1470.

[22] Grobert L, Royo Martin LJ, Onelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Menissier F, Massabanda J, Fries R, Hanset R, Georges M: A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. *Nat Genet* 1997; 17(1): 71-74.

[23] Hammer RE, Purse! VG, Rexroad CE, Wall RJ, Boll DJ, Evert KM, Palmilcr RD. Brinstcr RL: Production of transgenic rabbits, sheep and pigs by microinjection. *Nature* i985; 315: 680.

[24] Hughs SH, Pclropoulos CJ, Fcdcrspicl MJ, Sutrave P: Vectors and genes for improvement of animal strains. *JReprod Fertti* 1990; 41 Suppl: 39-49.

[25] Illmensee K, Hoppe PC: Nuclear transplantation in Mus muscuius: developmental potential of nuclei from prcimplantation embryos. *Cell* 1981; 23: 9-18.

[26] Inui K, Okada S, Dickson G: Gene therapy in Duchenne muscular dystrophy. *Brain Dev* 1996; 18 (5): 357-361.

[27] Jackson SP. Green RD, Miller MF: Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: I. Inheritance of the condition and production characteristics. *J Anim Sci* 1997; 75: 14-18.

[28] Jackson SP, Miller MF, Green RD: Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: III. Muscle weights and musele weight distribution. *J Anim Sci* 1997; 75: 133-138.

[29] Kambadur R, Sharma M, Smith TPL, Bass JJ: Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Picdmontcse cattle. *PCR MethodsAppi* 1997; 7 (9): 910-915.

[30] Koohmaraie M, Shackelford SD, Wheeler TL, Lonergan Stvl, Doumit MR: A muscle hypertrophy condition in Iamb (callipyge): characterization of effects on muscle growth and meat quality traits. *J Anim Sci* 1995; 73: 3596-6307.

[3 I] Koohmaraie M, Shackelford SD, Wheeler TL: Effects of a p-adrcncrgic agonist (L-644, 969) and male sex condition on muscle growth and meat quality of callipyge lambs. *J Anim Sci* 1996; 74: 70-79.

[32] Lamberth VA, Looney CR, Voelkei SA, Jackson DA, Hill KG, Godke R.A: Microsurgery on bovine embryos at the morula stage to produce rnonozygotic twin calves. *Therio* 1983; 20: 85-95.

[33] Li Z, Colucci E, Babinet C, Paulin D: The human dcsmin gene: A specific regulatory programme in skeletal muscle both in vitro and in transgenic mice. *Neuromuscular Disor* 1993; 3 (5-6): 423-427.

[34] Mayer NT, Rubin SA: The molecular and cellular biology of heart failure. *Curr Opin Cardiol* 1995; 10 (3): 23 8-245.

[35] McGrane MM, Yun JS, Moorman AFM, Lamers WH, Hendrick GK, Arafah BM, Park EA, Wagner TE, Hanson Rw: Mealbolic effect of developmental, tissue-, and cell-specific expression of a chimeric phosphocnolpyruvatc carboxykinase (GTPJ/bovine growth hormone gene in transgenic mice. *J Bio! Chem* \ 990; 265: 22371 -22379.

[36] McGrath J, SolterD: Nuclear transplantation in the mouse embryos by microsurgery and cell fusion. *Science* 1983; 220: 1300-1302.

[37] McPherron AC, Lawler AM, Lee SJ: Regulation of skeletal muscle mass in mice by a new TGF-b superfamily member. *Nature* 1997; 387: 83-90.

[38] Ozil JP: Production of identical twins by bisection of blastocysts in the cow. *JReprodFertil* 1983; 69: 463-468.

[39] Poole-Wilson PA: Skeletal and cardiac abnormalities in chronic heart failure. *Basic Appl Myol* 1995; 5 (4): 316-370.

[40] Prather RS, Barnes FL, Sims MM, Robl JM, Eyestonc WH, First NL: Nuclear transplantation in the bovine embryo: assessment of donor nuclei and recipient oocyte. *Biol Repro* 1987; 37: 859-866.

[41] Purscl VG, Hammer RE, Bolt DJ, Palmiter RD, Brinster RL: Integration, expression and germ-line transmission of growth-related genes in pigs. J *Repro Pert* 1990; 41 Suppl: 77-87.

[42] Pursel VG, Rexroad CE: Status of research with transgenic farm animals. *JAnim Sci* 1993; 71 Suppl 3: 10-19.

[43] Seidei GE: Resource requirements for transgenic livestock research. *J Anim Sci* 1993; 71 Suppl 3: 26-33.

[44] Smith LC, Wilmut I: Influence of nuclear and cytoplasmic activity on the development in vivo of sheep embryos after nuclear transplantation. *Biol Reprod 1999;* 40: 1027-1035.

[45] Stephenson LW: Skeletal muscle-cardiac assist in the Ainericas. *Basic Appl Myol* 1993; 3 (4): 267-348.

[46] Swatland H J, Kieffer NM: Fetal development of the double muscled condition in cattle. *J Anim Sci* 1974; 38:7 52-757.

[47] Willadsen SM: Nuclear transplantation in sheep embryos. *Nature* 1986; 320: 63-65. [48]

Willadsen SM: The viability of early cleavage stages conlaining half the normal number of blastomeres in the sheep. *J Reprod Fertil* 1980; 59: 357-362.

[49] Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS: Viable offspring derived from fetal and adult mammalian cells. *Nature* 1997; 385: 810-813.