Potential Methods to Manipulate Cellular Control of Skeletal Muscle Hypertrophy

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Abstract

Skeletal muscle hypertrophy is a complex process that is regulated at multiple levels. The fundamental stimuli that promote hypertrophy are well known. However, only recently have advances in molecular biology techniques allowed us to evaluate alterations in gene expression and respective protein products at multiple levels of the hypertrophy cascade in response to certain stimuli. Of these stimuli, resistance exercise training is the safest and most effective method of eliciting muscle growth in humans, because it affects key signaling events in the hypertrophy cascade. Improved understanding of the key signaling events influenced by exercise has assisted in the development of novel interventions that may exert effects similar to those of exercise training to augment skeletal muscle hypertrophy. Genetic characterization, through the use of biotechnology to screen for candidate molecules associated with advantageous phenotypes, has also been used to identify individuals with superior physical ability, or in the selective breeding of livestock to obtain heavily muscled offspring. Recent advances in the discipline have identified specific and innovative methods of promoting skeletal muscle hypertrophy, including gene therapy and pharmacological and nutritional interventions. This review discusses the current status and potential of these novel interventions, as well as examples from the literature regarding their mechanism of action in relation to skeletal muscle hypertrophy. A better understanding of these interventions and their future potential is warranted if novel approaches are to be implemented in humans to attenuate disease and age-related muscle loss or to augment skeletal muscle hypertrophy. Although these interventions are targeted at humans, they show great potential for future use in livestock.

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Proceedings of the 61st American Meat Science Association ***Reciprocal Meat Conference*** *(pp. 1–8)*
June 22–25, 2008, Gainesville, Florida
www.meatscience.org

Introduction

Skeletal muscle is one of the most adaptable tissues in the body. Every structural aspect of skeletal muscle, including fiber diameter, fiber type distribution, mitochondrial density, myosin profile, and capillarity is modifiable, provided the proper stimulus is imposed (Lieber, 1992). Skeletal muscle hypertrophy is a change that occurs in muscles of animals and humans as a result of various forms of mechanical overload and stretch and of hormonal and molecular stimuli. It is generally accepted that in response to a hypertrophic stimulus, physical alterations include increases in fiber diameter, mitochondrial density, contractile protein content (actin and myosin), and capillarity. Despite the fact that these physical adaptations have been well characterized, the signaling pathways that mediate these adaptations are still under investigation.

Thus, the purpose of this review is to provide an overview of our current knowledge regarding the primary and secondary signaling cascades that regulate skeletal muscle hypertrophy. This paper will then explore innovative methods in both humans and animals to promote hypertrophy of skeletal muscle, including gene therapy, pharmacology, and nutritional intervention. Evidence regarding the current status and potential of these novel interventions, examples from the literature including the proposed mechanism of action, and future directions in research and therapeutic interventions are discussed. A better understanding of these interventions and their future potential is warranted if novel approaches are to be implemented to maximize skeletal muscle growth in humans and livestock.

Signaling Molecules Involved in Skeletal Muscle Hypertrophy

Converting a mechanical signal generated during a contraction into a molecular event that promotes skeletal muscle hypertrophy involves primary and secondary mes-

sengers. The role of these messengers is to initiate a series of cellular events that activate and repress specific signaling pathways that regulate muscle protein synthesis and degradation, respectively. Primary messengers include mechanical stretch, calcium (Ca^{2+}) signaling, redox potential, and phosphorylation potential (Spriet, 2002). Primary messengers induce the activation of secondary signaling cascades. Numerous secondary signaling cascades exist in skeletal muscle, and these pathways are highly regulated at multiple levels. Understanding and delineating specific roles of secondary messengers has been difficult because there is substantial interaction among pathways, resembling a highly complex network. To this end, an overview of these pathways would exceed the scope of this discussion. Thus, the discussion of secondary messengers in this paper is restricted to the phosphatidylinositol-3-kinase (PI3K) and AKT (protein kinase B) signaling pathway because of its significant role in maintaining the balance between skeletal muscle protein synthesis and degradation (Sacheck et al., 2004; Stitt et al., 2004).

PI3K/AKT Pathway

The PI3K/AKT signaling pathway is involved in the regulation of a variety of biological processes, including cell growth, glycogen metabolism, and cell signaling. There is strong evidence that the activity of the PI3K/AKT pathway is a regulator of the muscle hypertrophy program (Bodine et al., 2001; Glass, 2003; Sandri et al., 2004). Under normal circumstances, activation of PI3K by insulin or insulin-like growth factor (IGF) results in the suppression of phosphatase and tensin homolog, a negative regulator of muscle growth. Subsequently, phosphoinositide-dependent kinase-1 is phosphorylated or activated, which in turn phosphorylates AKT on 2 sites (Ser473 and Thr308), resulting in the suppression and inactivation of proteolytic molecules and the forkhead box O transcription factors (Sandri et al., 2004).

When AKT is phosphorylated, particularly in response to exercise or feeding, up-regulation of the mammalian target of rapamycin (mTOR) pathway occurs. Transcription factors downstream from mTOR are required for protein synthesis and are capable of sensing diverse signals, producing a multitude of anabolic responses, including mRNA translation and ribosomal biogenesis (Bassel-Duby and Olson, 2006). Much research has focused on the role of insulin and IGF-I signaling pathways in animal models because of the predominant role these hormones play in reducing protein degradation (Sacheck et al., 2004; Deldicque et al., 2005; Schakman et al., 2005). This work has confirmed the role of IGF-I as a key anabolic molecule that regulates protein synthesis pathways (Bodine et al., 2001; Sacheck et al., 2004; Chow et al., 2006).

Novel Interventions to Promote Muscle Protein Synthesis in Humans and Animals

The challenge in decoding skeletal muscle hypertrophy has been met with great enthusiasm from research scientists. Since the advent of the human genome project, a torrent of biological data has been generated at the cellular level, providing certain clues regarding the molecular basis of skeletal muscle hypertrophy. This information has allowed scientists to begin to explore the mechanism of action of individual genes and how manipulation through biotechnology, gene therapy, or pharmacological, nutritional, and physical methods might augment the activity of anabolic signaling pathways and subsequent gains in skeletal muscle growth. Indeed, multiple molecular targets and modes of intervention have been identified, yet the practicality of intervention is still a critical topic of investigation.

A schematic depicting the main regulators of skeletal muscle protein synthesis and the critical points at which novel interventions may influence cell signaling is presented in Figure 1. Gene therapy, pharmacology, nutrition, and exercise training are all feasible means of stimulating hypertrophic pathways in skeletal muscle. However, as the figure illustrates, the level at which each intervention exerts its effects is highly specific, playing a significant role in the overall efficacy of the intervention. The following section highlights these interventions, their mechanisms of action, and the current success and potential of implementing these treatments in humans and animals.

Use of Biotechnology to Exploit Desirable Traits

Biotechnology is the selective breeding of plants and animals to obtain desirable phenotypes. The first evidence of possible genetic influence on the development of muscle size and strength was the selective breeding of Belgian Blue and Piedmontese cattle to obtain the double-muscled trait. Although this and other selective-breeding practices have been occurring for hundreds of years based on phenotypical outcomes, the genetic basis of physical traits was not characterized until this past decade.

Since mid-1990, the genetic basis of skeletal muscle hypertrophy has been under thorough investigation in an effort to understand individual variations in athletic performance, overall health, and disease susceptibility. Scientists suggested that athletic prowess provided an advantage in human evolution, so it has been hypothesized that single nucleotide polymorphisms (SNP), also known as single gene mutations, were the targets of natural selection in humans. Several large-scale investigations have attempted to link SNP in candidate genes with individual differences in athletic phenotypes (Delmonico et al., 2007; Hand et al., 2007; Windelinckx et al., 2007).

The origin of the double-muscled phenotype in cattle has been explained by a mutation in the myostatin gene. Myostatin is also known as growth and differentiation factor 8, and it functions as a negative regulator of skeletal muscle growth (Kambadur et al., 1997). The action of myostatin was first described in a mouse model in which myostatin-null mutants had 200 to 300% more skeletal muscle mass, caused by an increase in skeletal muscle fiber size and number (McPherron et al., 1997). Subsequent

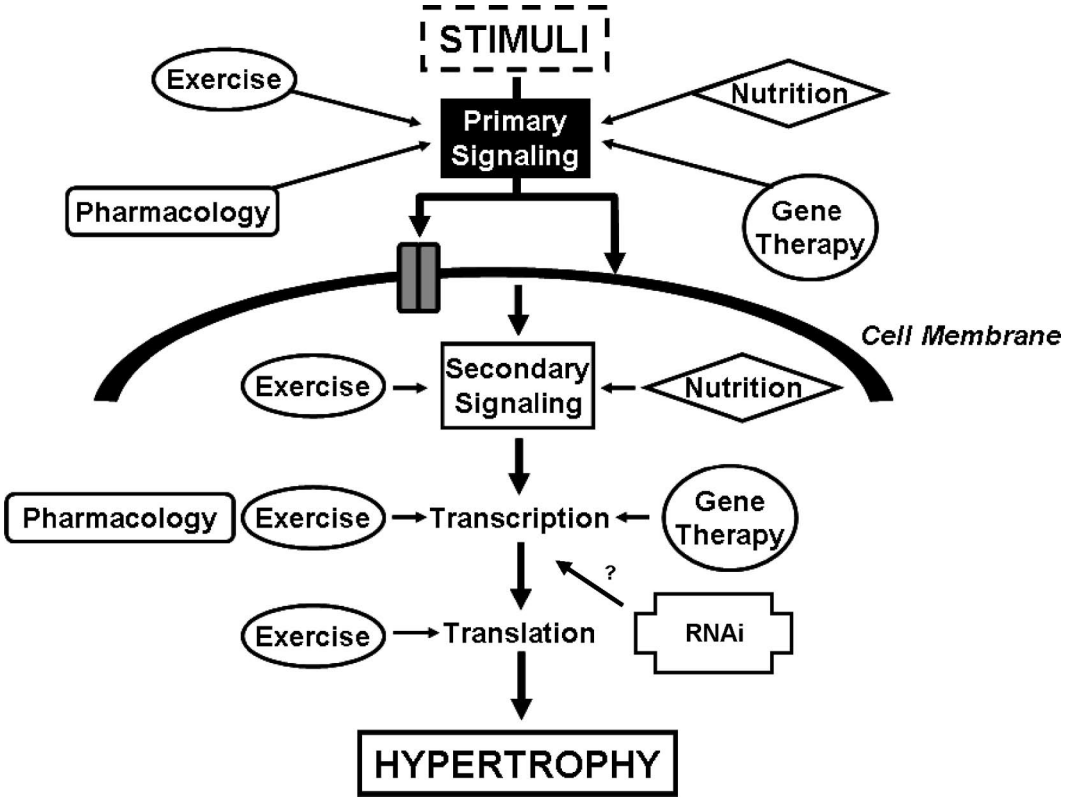

Figure 1. Potential mechanisms to induce skeletal muscle hypertrophy.

analysis of double-muscled cattle with similar phenotypes identified myostatin mutations, including deletions, that result in decreased expression or complete loss of function of the myostatin gene, or both (McPherron et al., 1997). In humans, despite numerous large-scale investigations to identify mutations in regulators of muscle growth (Jones et al., 2002; Clarkson et al., 2005; Delmonico et al., 2007; Hand et al., 2007; Windelinckx et al., 2007), only a single case study exists describing a loss-of-function mutation in the myostatin gene leading to gross muscle hypertrophy, and is therefore not a polymorphism driving normal human variation (Schuelke et al., 2004). Thus, although it is clear that the selective breeding of animals is based on a few genetic loci with strong effects, muscle quantitative trait loci (QTL) in humans appear to be more complex.

In regard to the current practice of selective breeding in livestock to exploit genetic aptitude and obtain greater amounts of lean muscle, certain negative side effects do exist (Gordon et al., 2005). Selective breeding in livestock is often associated with reduced stress tolerance, poor calf viability, and female infertility (Kambadur et al., 1997). In humans, investigators have only recently begun to identify possible genetic mutations that suggest athletic prowess. Although these associations are rare, it is not clear what possible negative consequences exist, and the most recent work has identified that more complex, polygenic, gene-gene interactions are responsible for physical variation.

In sum, biotechnology is now being used in livestock to enhance lean meat production, despite limitations that selective breeding may affect animal health. In humans, biotechnology is still a new area of investigation and conclusive data do not exist regarding an association between single gene mutations and athletic prowess. Ongoing research projects are focusing on the interaction among SNP on multiple genes and on various other QTL in both humans and animals. Additionally, methods to attenuate negative aspects of selective breeding are a topic of future investigation.

Gene Therapy

Experimental evidence exists to support the potential use of gene therapy to promote gains in skeletal muscle and attenuate muscle dysfunction and atrophy in several inherited disease conditions. Currently, the most promising form of gene therapy is the local administration of a single agent to maintain an optimal, elevated concentration of a particular transcript within the muscle for an extended period of time. Disadvantages, including the difficulty of developing agents of the superior quality necessary for human use, safety concerns, and limitations associated with gene regulation and specificity of action, have prevented immediate and widespread use in humans. However, the development and use of gene therapy in animals has assisted in the progression to clinical investigations focused on potential gene therapy in humans.

Although genetic medicines and treatments are a simple concept, the challenges associated with incorporating effective treatments are significant. For instance, biologi-

cal barriers exist for all genetic medicines, predominantly in the concept of delivering and maintaining new genetic information. In regard to gene transfer therapy, challenges exist in suppressing immune defenses that are raised against vectors hosting the new gene, transferring the gene to multiple cells to result in modification of a phenotype, and overall control of overexpression of the gene. Finally, for RNA-modification therapy, the most critical obstacle is maintaining RNA specificity.

Recent advances in molecular biology techniques have allowed us to gain a better understanding of potential targets, facilitating interventions based on the molecular basis of the disorder (or enhancement), genotype-phenotype relationships that result in the modified phenotype, and the way in which a particular phenotype is modulated by a single gene. This is one of the most significant challenges, because most of the successful therapies have focused on monogenic solutions because interventions designed to interfere with the activity of multiple genes or pathways have been less successful. Consequently, treatment strategies to enhance skeletal muscle quality and performance through the manipulation of a single gene are lacking. Candidate molecules that have received much attention include IGF-I (Schakman et al., 2005; Schakman and Thissen, 2006), growth hormone (GH; Kusano et al., 2007), and myostatin (Acosta et al., 2005; Bartoli et al., 2007).

Insulin-like growth factor-I has been investigated in regard to its anabolic effects. Intramuscular injection of adeno-associated viral (AAV) vectors increases muscle mass and strength in mice and rats (Schakman et al., 2005; Schakman and Thissen, 2006). However, the efficacy of these methods has not yet been tested in larger animals or nonhuman primates because of the complexity of delivery and possible negative effects when working with a larger animal. Future work may identify this class of IGF-I gene therapy as a promising avenue for enhancing muscle mass in livestock.

Gene therapy focused on GH treatments is being developed, because there is significant difficulty in maintaining elevated GH levels without multiple injections in compromised populations that rely heavily on GH treatment. Expression of AAV-mediated GH has been explored in the myocardium, exhibiting improved cardiac function and reduced pathology of postmyocardial infarction (Kusano et al., 2007). This research is at the forefront of GH research; thus, the efficacy and feasibility of treatments in skeletal muscle and livestock have not been confirmed.

With the discovery that targeted disruption of the myostatin gene in mice, selective breeding of cattle, and loss-of-function mutations in humans result in double muscling, strategies to inhibit myostatin signaling have been a leading topic of investigation. Promising avenues of gene therapy include AAV-mediated expression of mutated myostatin propeptide to promote increased muscle mass, and in some cases muscle force, in animal models (Bartoli et al., 2007). Similar to IGF-I and GH treatment regimens, certain challenges, including inherent ethical issues, are introduced with myostatin therapy, reducing its potential use in humans. However, the aforementioned paper provides convincing evidence to suggest that treatments in livestock to enhance skeletal muscle mass are viable.

Indeed, given the proven ability to promote gains in skeletal muscle size and quality, gene therapy targeted at IGF-I, GH, and myostatin is a promising area of research and future intervention. However, regarding humans and the remarkable potential of gene therapy to enhance performance, administration and use of gene therapy are likely to be topics of controversy in the coming years. In livestock, aside from the possible impact on meat quality and taste, research efforts focused on gene therapies designed to improve musculature are expected to take precedence.

Pharmacological

Pharmacological therapy focuses on the injection or oral administration of drugs that exert acute effects on signaling pathways. There is widespread use of pharmacology to treat various diseases and conditions affecting human health and function. With regard to pharmacological treatment to promote skeletal muscle growth, anabolic steroids have received attention over the years in both diseased (ethical treatment of wasting disorders) and healthy (unethical use to enhance performance) populations. Anabolic steroids enhance skeletal muscle hypertrophy beyond natural genetic limits via increased transcription of DNA for myofibrillar proteins, accelerated activation of satellite cells, and subsequent synthesis of nuclear and contractile proteins. Because of the systemic mechanism of action of anabolic steroids, negative side effects associated with steroid use have limited their use to individuals with critical wasting diseases.

Recent advances in our understanding of protein-protein interactions and cell-signaling networks have led to research investigating novel, yet safe, pharmacological approaches to increase protein synthesis and in skeletal muscle. These investigations have shown efficacy in individuals with wasting diseases as well as in the aged (Bhasin et al., 2005; Pupim et al., 2005; Balagopal et al., 2006; Johansen et al., 2006). The most recent evidence has provided mechanistic studies examining not only alterations in phenotype, but also alterations in the expression of genes regulating protein synthetic and degradation pathways (Deldicque et al., 2005; Balagopal et al., 2006; Burniston et al., 2007). This work has confirmed the efficacy at both the systems and molecular level of the most promising anabolic agents, including testosterone enanthate (Bhasin et al., 2005; Rogerson et al., 2007), Oxandrolone (Balagopal et al., 2006), recombinant human growth hormone (Pupim et al., 2005), insulin (Chow et al., 2006; Orellana et al., 2006; Pawlikowska et al., 2006), nandrolone decanoate (Johansen et al., 2006), and creatine (Deldicque et al., 2005).

Certain limitations do exist with pharmacological interventions designed to promote anabolic signaling in skel-

etal muscle. For instance, Clenbuterol is a β-adrenergic agonist administered to livestock to increase leanness and muscle mass (Lafontan et al., 1988), yet consumption of meat products from animals treated with Clenbuterol can poison humans (Martinez-Navarro, 1990). Although Clenbuterol is now illegal in the food industry, it is still marketed for competition purposes in livestock shows. As a result, since its ban there have been several incidences of acute poisoning in humans who have consumed meat from livestock treated with Clenbuterol. Although many pharmacological agents may achieve the intended results in livestock, the true measure of the intervention is whether the meat is safe for human consumption.

As mentioned previously, a second limitation of pharmacological intervention is the notion that anabolic pathways in skeletal muscle are affected by the interaction of several cell-signaling networks. Pharmacological treatments exert their effects on single specific molecules involved in the hypertrophy cascade, and thus may not initiate physiologically appropriate adaptations. Thus, the possibility of developing a single agent to promote all the physiological benefits that typically accompany exercise-induced gains in skeletal muscle mass and size is minimal. Although our knowledge of these systems is improving at a remarkable rate, many aspects of these complex signaling networks still remain elusive. In livestock, many of these interventions hold promise, but ethical and environmental effects must be considered, in particular the possible abuse of these agents by athletes, if readily available, and the possible long-term health effects of these drugs on humans ingesting meat products from livestock treated with pharmacological agents.

Nutritional

Understanding how nutrition and diet affect protein turnover in skeletal muscle has been an objective of investigators for the past half century. Significant gains have been made in the past decade as technology has enabled scientists to identify the acute effects of feeding on key protein-signaling pathways in skeletal muscle. This work has afforded us a new appreciation for the dynamic nature of protein metabolism and, subsequently, new techniques and strategies to manipulate these pathways. Because testing nutritional supplements is generally safe, there has been a much more rapid rate of discovery and implementation of nutritional interventions compared with gene therapy and pharmacology. The result is known methods to optimize cell signaling to stimulate protein synthesis and hypertrophy.

Recent work suggests that there is a synergistic effect between the timing and composition of meals and resistance exercise on overall gains in muscle strength and size (Blomstrand et al., 2006; Miller, 2007; Tipton et al., 2007). Because skeletal muscle mass accretion is dependent on a higher rate of protein synthesis than is degradation, insufficient dietary protein, or fasting, results in a negative nitrogen balance (Lemon et al., 1992). Thus, optimal nutrition is critical, particularly with exercise, in promoting a net positive nitrogen balance. Failure to maintain a caloric balance, especially during the hours immediately postexercise, results in a net negative protein balance favoring a catabolic state and an increase in the rate of protein degradation, offsetting the effects of the exercise designed to stimulate protein synthesis.

To this end, interventions designed to deliver specific branched-chain amino acids known to promote a positive nitrogen balance have been investigated in both humans and animals (Vary and Lynch, 2007). Of these interventions, essential amino acid cocktails with a high concentration of leucine, administered immediately pre- or postexercise or both or in neonates, have shown the greatest benefits in up-regulating protein synthesis pathways and maintaining a net protein balance (Escobar et al., 2005; Matthews, 2005; Moore et al., 2005; Paddon-Jones et al., 2005; Blomstrand et al., 2006; Fujita et al., 2007). With regard to livestock, although exercise does promote enhanced protein synthesis when amino acid supplementation is administered, scientific evidence supporting the effects of diets high in leucine and other essential amino acids (e.g., lysine) during development support the benefits of these diets in promoting increases in lean body mass (Escobar et al., 2005; Moore et al., 2005; Hevroy et al., 2007). This work identifies the critical role of enhanced feed in livestock, primarily in the developmental stages.

The beneficial effects of essential amino acid intake have also been shown to be augmented when administered before exercise and when combined with carbohydrate ingestion (Roy et al., 1997). Although not confirmed, this phenomenon has been attributed to the increased blood flow during exercise and subsequent increased availability of amino acids from circulation. Moreover, the increased carbohydrate is likely to stimulate insulin signaling, promoting the up-regulation of insulin-stimulated anabolic pathways (e.g., AKT/mTOR). Interestingly, whole-protein supplementation (e.g., whey) has not proven to be an effective intervention to stimulate protein synthesis following an exercise bout (Tipton et al., 2007). This information, which was gleaned from a recent paper, validates the argument that there is still much to be learned regarding effective nutritional interventions to stimulate and promote protein synthesis.

Physical

Physical exercise is a potent mechanism to stimulate skeletal muscle protein turnover. Considerable research in both humans and animals has been completed in the past decade elucidating the key molecules regulating skeletal muscle hypertrophy in response to resistance exercise. At this time, scientists have provided significant data regarding the transcriptional and posttranslational regulation of protein synthetic pathways in response to exercise. The primary signaling molecules that mediate the response to exercise through the up-regulation of protein synthesis in-

clude the anabolic hormones IGF-I and Mechano growth factor (MGF), and the negative regulator of skeletal muscle growth, myostatin. The impact of these molecules on signaling include the altered expression of thousands of transcription factors, up-regulation of the mTOR protein synthesis pathway, and stimulation of satellite cell proliferation and differentiation (Rennie et al., 2004). Thus, repeated sessions of resistance exercise are the most effective intervention currently being used in humans to promote hypertrophy through the induction of signaling pathways regulating protein synthesis.

The majority of studies assessing muscle protein turnover and signaling pathway activation and their relationship to resistance exercise have focused on adaptations postexercise, because these are the changes most likely to result in skeletal muscle growth. Indeed alterations within skeletal muscle that occur during exercise are of great interest, but currently, there are no data on this critical period because of limitations with tissue collection as well as the poor time resolution of the methods available for measurement of protein turnover. Of the limited previous research available, there is evidence implying that during prolonged aerobic exercise, protein synthesis is either unchanged or depressed (Carraro et al., 1990). Conversely, ample research has established that in the period after resistance exercise, protein synthesis rates are nearly doubled within 4 h, and an elevated rate of protein synthesis endures for the subsequent 24 h, with return to near-basal rates within 48 h of recovery (Chesley et al., 1992; Phillips et al., 1997). Although the rate of muscle protein synthesis increases dramatically as a result of a single exercise bout, the rate of net accretion of muscle protein follows a slower time course, with gains in muscle mass equaling approximately 1% per week of intense training (Seynnes et al., 2007). This is largely due to nutritional interactions, specifically the availability of exogenous essential amino acids to offset the increase in muscle protein degradation that also typically occurs in the hours postexercise (Fujita et al., 2007). Thus, with appropriate attention to the ingestion of amino acids before and after training, exercise- and nutrition-stimulated pathways that promote protein synthesis will act synergistically. Accordingly, this will optimize the net gain in skeletal muscle mass compared with exercise or nutrition alone.

Indeed, the idea of exercising livestock is unconventional. However, in certain countries traction and plowing are two of the most reliable sources for rural work power. In these countries, animals receive several weeks of training before harvest to get used to handling and progressively increasing workloads. Training studies have been completed examining the effects of this type of training on various aspects of muscle adaptation, including work output (VO_{2max}; Pearson et al., 1996; Lawrence and Dijkman, 1998; Veeneklaas et al., 2004). Thus, although exercising cattle may not be practical, it is reasonable to suggest that to optimize the anabolic effects of the aforementioned nutritional interventions, daily activity (e.g., movement of a herd from point A to point B) could be tailored to augment the synergistic effects of nutrition and exercise. Furthermore, an intervention such as this poses no outside harm to the animal or the quality of meat for human consumption.

Future Directions

The greatest challenge to date has been singling out the most critical genes and proteins involved in promoting skeletal muscle hypertrophy. It is critical that we continue to improve our understanding of the complex signaling networks involved in skeletal muscle protein turnover by using a basic research approach that explores the interconnecting biological mechanisms that regulate skeletal muscle growth. Identifying key genes whose expression is altered in response to muscle growth will permit future investigations of entire pathways in which the proteins encoded by these genes function. The results of this work will allow us to design the most practical, yet safe, intervention that will stimulate hypertrophy in a manner physiologically similar to exercise.

To accomplish this, some of the most recent work conducted by scientists involves the use of sophisticated techniques to attenuate the activity of single genes to better understand their roles in complex pathways. Ribonucleic acid interference (RNAi) is one such tool that allows the study of single genes in cell culture and in vivo experiments (Herndon and Fromm, 2007). This tool uses double-stranded RNA that is synthesized with a sequence complementary to the target gene and subsequently introduced into the cell or organism. Because this exogenous material is recognized as such by the cell, the RNAi pathway is activated, causing a significant decrease in the expression level of the target gene. The effects of this decrease identify the physiological role of the protein product. By not completely abolishing the expression of target genes, RNAi is superior to knockout experiments, resulting in a more physiologically accurate system. Regardless of the fact that additional, novel techniques are being used, coordinated efforts among laboratories are essential in decoding the most critical regulators of skeletal muscle hypertrophy in both humans and livestock.

In conclusion, great strides have been made in our understanding of the molecular basis of hypertrophy. However, with these advances, discoveries have been made that only add to the number of questions to be answered before we can fully understand the signaling processes in skeletal muscle that promote hypertrophy. Continued advances in technology that facilitate our understanding of this system will be critical in completing our quest to fully understand the complex interactions involved in promoting skeletal muscle growth. Until then, interventions based on biotechnology, gene therapy, pharmacology, nutrition, and physical exercise will continue to be explored. These interventions are important models for the meat industry as well as for biomedical research in human health and disease.

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