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# Effects of Distraction on Muscle Length: Mechanisms Involved in Sarcomerogenesis

*Vincent J. Caiozzo, PhD; Ali Utkan, MD; Richard Chou, BS; Afshin Khalafi, MD; Heena Chandra, BS; Michael Baker, MS; Bryan Rourke, PhD; Greg Adams, PhD; Ken Baldwin, PhD; and Stuart Green, MD*

**Although a great deal of interest has been given to understanding the mechanisms involved in regulating the radial growth that occurs because of resistance training, much less has been given to studying the longitudinal growth of skeletal muscle that occurs because of passive stretch. The current authors provide a brief overview of key issues relevant to the longitudinal growth of skeletal muscle that occurs during distraction osteogenesis. Specifically, five key issues are addressed: (1) the pattern of sarcomerogenesis during distraction; (2) sarcomerogenesis and altered expression of sarcomeric and nonsarcomeric genes; (3) the satellite cell hypothesis; (4) mitogenic factors; and (5) new approaches for studying the longitudinal growth of skeletal muscle. A discussion is provided that revolves around the concept of a negative feedback loop. One of the most interesting issues to be resolved in muscle biology is the role of satellite cells in regulating the growth of skeletal muscle. Currently, it is not known whether satellite cell activation is a prerequisite for the longitudinal growth of skeletal muscle. Gene chip analyses provide a paradoxical view, showing that distraction osteogenesis results in the upregulation of a gene, GADD45, involved with growth arrest and deoxyribonucleic acid destruction.**

**List of Abbreviations Used**

| | |
|---|---|
| BrdU | bromodeoxyuridine |
| CAK | CDK-activated kinase |
| DNA | deoxyribonucleic acid |
| E2F | transcription factor |
| GADD45 | growth arrest deoxyribonucleic acid destruction |
| IGF-1 | insulinlike growth factor-1 |
| MGF | mechanogrowth factor |
| MHC | myosin heavy chain |
| pRb | retinoblastoma protein |
| RT-PCR | reverse transcription-polymerase chain reaction |

Limb lengthening has been an attractive modality in the treatment of limb length inequality, and congenital and acquired limb de-

From the Departments of Orthopaedics and Physiology & Biophysics, College of Medicine, University of California, Irvine, CA.

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Reprint requests to Vincent J. Caiozzo, PhD, Department of Orthopaedics, Medical Sciences I B-152, College of Medicine, University of California, Irvine, CA 92697.

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ficiencies. As reported previously, limb lengthening first was described in 1905 by Codivilla of Bologna, Italy.[24,28,31] During the past 80 years, numerous limb lengthening devices have been described.[24,28,31] Currently, three different mechanisms have evolved for filling the distraction site (gap where new bone will be formed). The three methods are known as the Wagner technique, the Ilizarov technique, and the Wasserstein technique. The Wagner technique involves incremental lengthening of the bone. Once the desired length has been achieved, a second operation is done filling the distraction gap with cancellous bone grafts, and plating the distraction gap. Unlike the Wagner technique, the Ilizarov procedure, pioneered by Ilizarov, involves a coricotomy (cutting the cortex), making sure not to damage the medullary cavity that contains the nutrient artery and medullary circulation.[19–21] The bone then is lengthened in small daily increments. The Wasserstein method uses a circular fixator similar to that designed by Ilizarov. However, unlike the Ilizarov technique, which avoids damage to the medullary canal, the Wasserstein approach involves the placement of an intramedullary nail into the bone. The bone then is lengthened until the desired length is achieved. A second operation is done to insert allograft bone around the intramedullary nail, and the fixator is kept in place until the allograft has been incorporated. With respect to the three techniques described above, the Ilizarov procedure has gained acceptance in the Western literature.[18] The presumed attributes of the Ilizarov procedure compared with the Wagner and Wasserstein approaches are: (1) it does not inherently require a second operation whereas the approaches of Wagner and Wasserstein do require a second operation; and (2) the preservation of the nutrient medullary vessels and periosteum are thought to play a critical role in distraction osteogenesis.[24]

Although it is difficult to accurately assess the complication rate associated with limb lengthening procedures, it generally is thought that they have one of the highest complication rates among orthopaedic surgical procedures.[11] Complication rates range from 5% (Ilizarov) to 225% (Wagner).[11] Dahl et al[11] examined the relationship between surgical experience and complication rate. They found that their surgical complication rate initially was 72% and declined to 25%.

One of the major problems that interferes with limb lengthening is the resistance of the myofascial structures. Paley[25] reported that the permanent loss of joint motion was the most common complication of lengthening using the Ilizarov technique and the complication that is least reliably reported. Paley[25] concluded that the role of muscle and not bone is the most significant unsolved problem in limb lengthening today. Consistent with this perspective, Eldridge and Bell[15] stated that every patient who had limb lengthening had problems with joint stiffness because of the properties of the associated musculature. Velazquez et al[30] concluded that the most serious complication that occurred after tibial distraction was a loss of joint motion (range of motion [ROM]), and that this loss in joint motion persisted for as many as 2 years after surgery. Velazquez et al[30] concluded that the mechanisms underlying the loss in joint motion could be understood better by focusing on the involvement of skeletal muscle in this process.

To date, few studies have attempted to identify underlying mechanisms regulating the longitudinal growth of skeletal muscle during distraction-induced stretch. The majority of the data related to the effects of stretch on muscle fiber length have been derived principally from the cast immobilization model. There are several classic studies that examined the effects of chronic stretch on muscle fiber length and sarcomere number.[16,23,29,32,33] In these studies, the investigators used the cast technique to fix the plantar and dorsiflexors of the ankle in a lengthened or shortened position. Interestingly, it was found that static chronic stretch did not affect the relationship between passive tension and angular displacement. This finding suggested that the muscle fibers of the plantar flexors increased the number of sarcomeres in series to maintain a normal passive length-tension relationship. Additional analyses showed that

there was a large increase in the number of sarcomeres in series.

The data of Williams and Goldspink[33] suggested that, during static chronic stretch, the addition of sarcomeres occurs at the ends of the muscle fibers. They used $^3$H-adenosine to identify the new sarcomeres that were produced by the static chronic stretch. Although the data produced by Williams and Goldspink[33] were equivocal, they concluded that sarcomeres usually are added to the ends of the fibers. More recently, Dix and Eisenberg[14] examined the effects of static chronic stretch imposed on the tibialis anterior muscle of rabbits. Stretch of the tibialis anterior muscle was created by applying a cast to the ankle in complete plantar flexion. Consistent with the conclusion of Williams and Goldspink,[33] Dix and Eisenberg[14] reported that the myotendinous junction was a key site involved in the regulation of myofibril assembly under these conditions, and that this form of stretch produced an increase in the slow MHC mRNA isoform in this region of the muscle fiber.

In addition to the cast model, several different avian species have been used to examine the influence of static chronic stretch on various issues related to skeletal muscle.[7,8] Progressive stretch overload is induced by attaching a weight to one of the wings of a bird. This model principally has been used to examine issues related to hyperplasia and hypertrophy. This model has not been used to examine factors regulating muscle fiber length.

## What is the Pattern of Sarcomerogenesis During Distraction?

Currently, there are numerous fundamental issues that remain unresolved regarding the response of skeletal muscle to distraction-induced stretch. Muscle fibers can accommodate stretch by increasing sarcomere length, longitudinal growth via an increase in the number of sarcomeres in series, or both. As shown in Figure 1A, sarcomere length during distraction might be regulated in a negative feedback fashion. According to this scheme, sarcomerogenesis would not occur until sarcomere length exceeded a set point. Once beyond the set point, some undefined cellular and/or molecular response(s) (inverted amplifier) would be initiated to produce an increase in the number of sarcomeres in a series, thereby returning sarcomere length to its proper set point.

Some hypothetical responses to stretch induced by distraction are shown in Figure 1B. Model 1 shows a scenario where the set point is the resting length of the sarcomere (for example, 2.3 μm) and sarcomerogenesis begins immediately after the onset of stretch induced by distraction. In this model, however, the rate of sarcomerogenesis cannot match the rate of distraction and there is a concomitant increase in sarcomere length and sarcomere number. Model 2 shows a response that is characterized by a set point that corresponds to approximately 2.7 μm, and once sarcomere length reaches this set point sarcomerogenesis begins such that sarcomere length is clamped at the set point and the length of the fiber increases simply by the addition of sarcomeres in series. Finally, Model 3 describes a situation whereby there is a delay in sarcomerogenesis such that sarcomere length substantially increases during the initial phase of distraction. In this model, the delay in sarcomerogenesis is followed by an accelerated response where sarcomere length returns to its set point.

Within this context, the time courses of change in sarcomere length and sarcomere number were examined in rat soleus muscles where muscles were distracted at either 0.25 or 0.5 mm per day. Consistent with the set point concept as described in Model 2, sarcomere number in the group that had distraction at 0.25 mm per day did not increase significantly until sarcomere length was between 2.6 and 2.7 μm (Fig 2). Four days of distraction produced a sarcomere length of approximately 2.6 μm in the group that had distraction at 0.5 mm per day; however, sarcomere number remained unchanged. In contrast, after 8 days of distraction, the sarcomere length in this group leveled off at a length of approximately 2.7 μm, which was accompanied by a concomitant increase in sarcomere number.

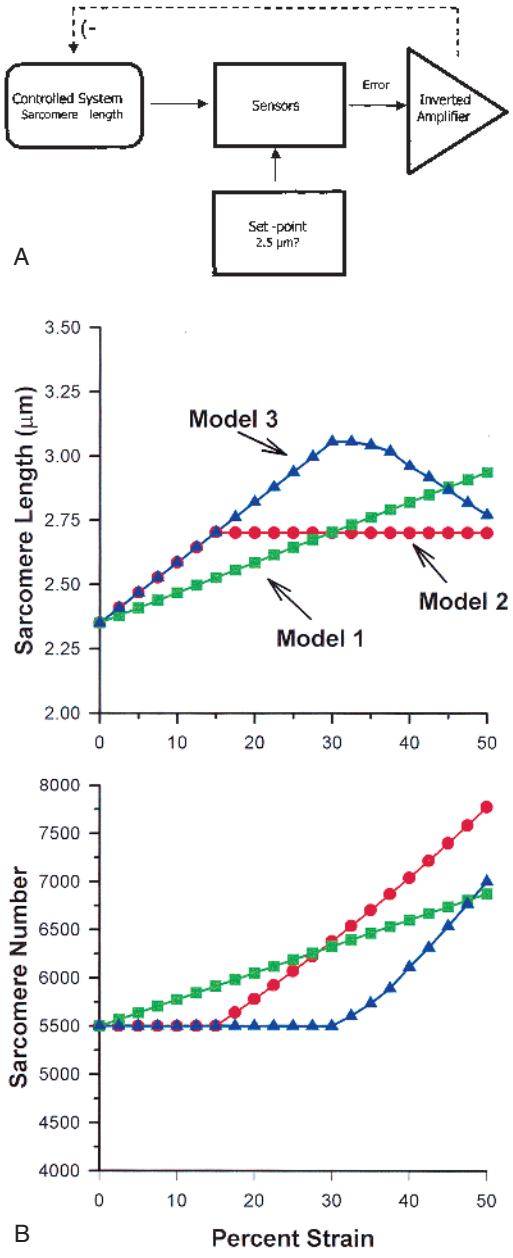


**Fig 1A–B.** (A) As a first approximation, it might be envisioned that sarcomere length is regulated according to a negative feedback loop. From a fundamental biologic perspective, it is not clear where the sensors are located, what the set point might be, or what cellular and/or molecular mechanisms represent the inverted amplifier. Therefore, much is still to be learned about the longitudinal growth of skeletal muscle. (B) The various patterns of sarcomere behavior that might be observed during distraction are shown.

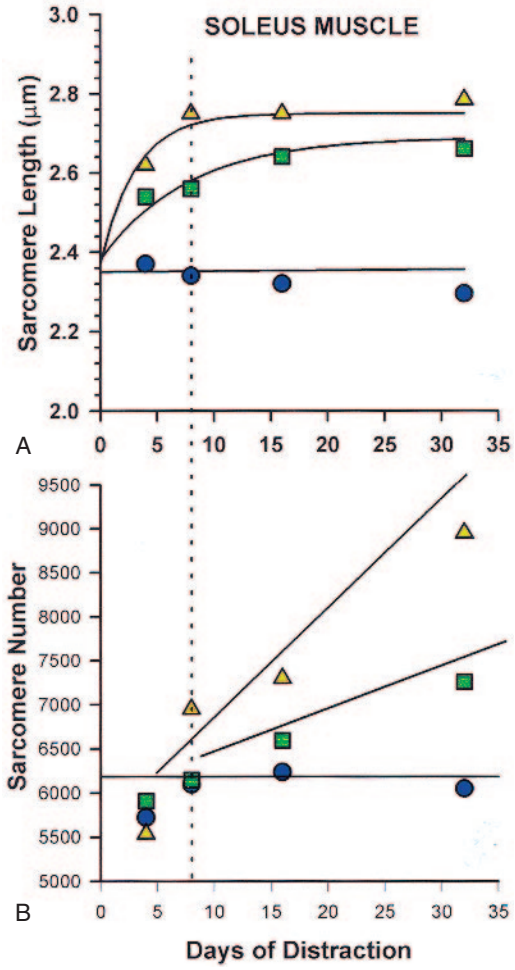


**Fig 2A–B.** The responses of the rat soleus muscle to distraction of the tibia at rates of 0.5 and 0.25 mm per day are shown. (A) Sarcomere length data are shown. (B) Sarcomere number is shown. During the initial phases of distraction (Days 4 and 8), sarcomere length progressively increases. Beyond this point, however, sarcomere length remains constant and there is a progressive increase in the number of sarcomeres in series. This data set suggests that the set point for sarcomere length might be approximately 2.7 μm. Circles = control data; squares = muscles distracted at 0.25 mm per day; triangles = muscles distracted at 0.5 mm per day

These findings suggest that the rodent soleus muscle seems to have a high capacity for longitudinal growth as exemplified by the fact that approximately 2700 sarcomeres were added in series during a 4-week period. This translates to approximately 4 sarcomeres per hour per myofibril. Assuming that each myofibril is approximately 1 μm in diameter and that the cross-sectional area of a fiber in the soleus muscle is approximately 2000 $\mu m^2$, then this equates to approximately 8000 sarcomeres per hour per fiber. If the longitudinal growth of muscle fibers occurs exclusively at the myotendonous junction as suggested,[14,33] then it is clear that the myotendonous junction has a remarkable synthetic capacity for producing sarcomeres. The second key finding of the data shown in Figure 2 is that the mean sarcomere lengths of the two distraction groups were similar to one another. This finding might suggest the presence of a length sensor (length sensor hypothesis) that has a set point of approximately 2.7 μm in the rodent soleus muscle.

### Sarcomerogenesis and Altered Expression of Sarcomeric and Nonsarcomeric Genes

Currently, there is no clear consensus as to where longitudinal growth occurs in response to stretch induced by cast immobilization or distraction. As noted previously, some investigators[14,33] have suggested that sarcomerogenesis occurs at the myotendonous junction in response to stretch, although it also is possible that sarcomerogenesis occurs throughout the length of the fiber. If longitudinal growth in skeletal muscle is restricted to the myotendinous junction, then skeletal muscle behaves in a fashion somewhat analogous to that of bone where growth occurs at the epiphysis. In a sense, the myotendinous junction would act like an epiphysis, and could be referred to as a myophysis.

Many sarcomeric genes are known to have varying numbers of isoforms. For instance, it is known that there are two developmental (embryonic, neonatal) and four adult MHC isoforms (slow Type I, fast Type IIA, fast Type IIX, and fast Type IIB) in rat skeletal muscle. As shown in Figure 3, stretch seems to produce phenotypic alterations in MHC isoform gene expression such that, in fast muscles, the Type IIB MHC isoform gene is downregulated whereas the slower isoforms are upregulated. Similar phenomenon have been observed by DeDeyne et al,[13] and the reader is referred to the work of DeDeyne included in this workshop.[12] Also, stretch induced by distraction leads to a significant increase in the expression of the developmental isoforms. This phenomenon is shown in Figure 3 where the upregulation of the embryonic MHC mRNA isoform clearly is visible in the rat tibialis anterior muscle. In contrast to the observations shown in Figure 3, DeDeyne et al[13] reported that distraction of the rabbit tibia leads to an increase in the neonatal MHC isoform. Currently, it is not clear why distraction seems to affect the developmental isoforms. With respect to the embryonic MHC gene, its upregulation might reflect: (1) a growth stimulus that activates satellite cells and leads to an ontologic recapitulation of MHC isoform expression; (2) an injury response that activates satellite cells and leads to an ontologic recapitulation of MHC isoform expression; and/or (3) the appearance of immature nascent sarcomeres that are reflective of sarcomerogenesis.

As discussed by Dabiri et al,[10] it is thought that premyofibrils are the initial scaffold on which mature sarcomeres develop. Such premyofibrils are characterized by the presence of nonmuscle myosin IIB, and this nonmuscle form of myosin is thought to associate with short sarcomeric units of alpha-actinin and actin filaments.[10] Subsequently, mature myofibrils develop as a result of Z bodies fusing to form Z bands and the incorporation of muscle specific forms of myosin and the loss of the nonmuscle IIB myosin. Although such models of myofibrillogenesis have been developed for cultured cardiac myocytes, it still is uncertain whether such a model applies to the type of longitudinal growth that occurs in response to stretch of adult muscle fibers. If nonmuscle IIB myosin expression is upregulated in response to stretch, then it might prove to be a valuable marker for identifying regions in the fiber that are involved in

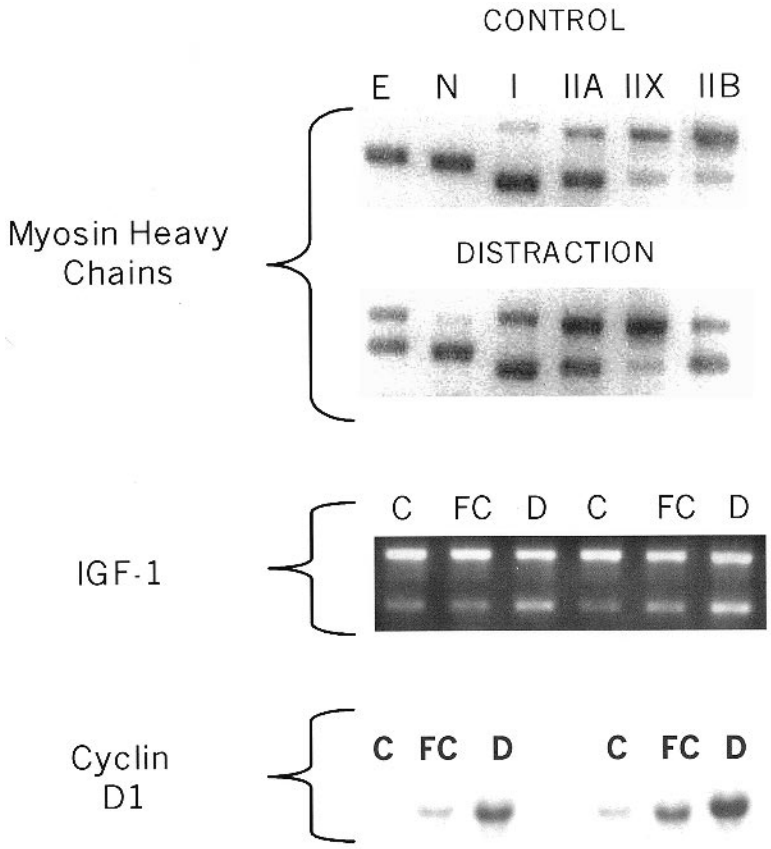


**Fig 3.** This figure shows the effects of distraction on MHC isoform mRNA, IGF-1, and cyclin D1 levels of rat tibialis anterior muscle after 4 weeks of distraction at 0.5 mm per day. Distraction results in a marked upregulation of the embryonic MHC isoform mRNA. This may be suggestive of satellite cell proliferation, muscle injury, or an initial or intermediate step in sarcomerogenesis. It has been postulated that stretch leads to an activation of satellite cells that is mediated by an upregulation of IGF-1. Distraction produced an increase in IGF-1. Consistent with the satellite cell hypothesis, distraction significantly increased the expression of cyclin D1, a key factor mediating the progression of cell division. C-control, FC-frame control; D-distraction

sarcomerogenesis. The reader is encouraged to examine the works of Sanger et al[26] and Bloch et al[9] for a more detailed discussion about issues related to myofibrillogenesis and the organization of proteins at the sarcolemmal level.

### The Satellite Cell Hypothesis and the Control of Cell Cycle

The concept of a nuclear domain implies that a given myonucleus controls the protein content of a specific cell volume, and dictates that skeletal muscle fibers must maintain a constant cell volume/myonuclei ratio during growth. If this is true, then growth only can occur by activation of satellite cells (Fig 4). Consistent with this concept, Allen et al[4–6] showed that the cell volume/myonuclei ratio remained constant in slow and fast fibers that hypertrophied in response to overload.

The role of satellite cells in mediating the longitudinal growth of muscle fibers during chronic stretch remains poorly characterized. To the authors' knowledge, Williams and Goldspink[33] were the first investigators to explore

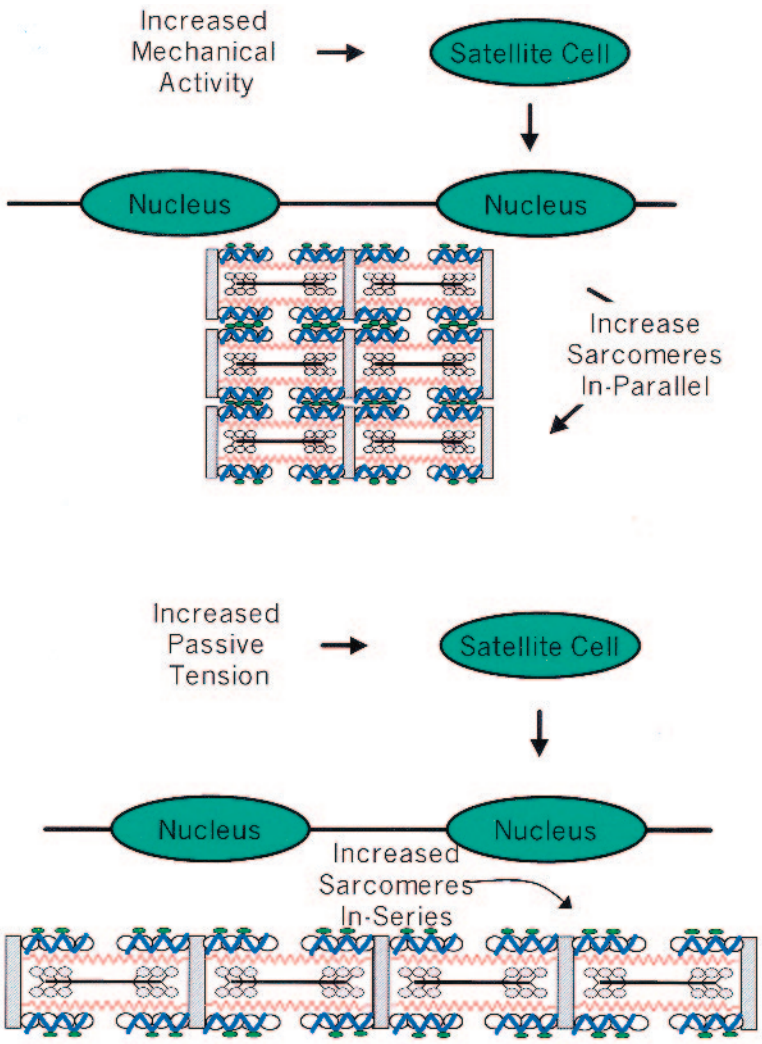


**Fig 4A–B.** This figure shows a hypothetical relationship between muscle fiber volume and nuclei. Specifically, it has been hypothesized that skeletal muscle growth must be accompanied by activation and proliferation of satellite cells that results in daughter myonuclei and maintains a constant cell volume to myonuclei ratio. If true, then this means that (A) increased mechanical loading as occurs with resistance training and (B) passive stretch that occurs via distraction must activate satellite cell proliferation. Currently, it is not known whether activation of satellite cells always is obligatory for growth.

the effects of static chronic stretch on myonuclei number. They found that static chronic stretch imposed by cast immobilization produced a significant increase in the number of myonuclei. More recently, Schumacher et al[27] used BrdU to label myonuclei derived from satellite cells, and reported that 10% distraction of the rabbit tibia produced a significant increase in labeled myonuclei. Interestingly, Schumacher et al[27] reported that this increase occurred late in the distraction process (28 days of distraction).

If satellite cells are activated by distraction-induced stretch, then factors involved in pro-

moting cell division should be upregulated. In this context, examining the response of genes associated with controlling the cell cycle should prove very informative. For instance, cyclin D1 levels are increased as the cell progresses from quiescence ($G_0$) to the $G_1$ interval, and are thought to be induced via mitogenic stimulation. Cyclin D1 assembles with cyclin dependent kinase-4, and then this complex can be activated via CAK-mediated phosphorylation. The D-type cyclin-dependent kinases phosphorylate pRb, and this leads to a disassociation between transcription factors such as E2F and pRb. These steps then lead to entry into the S-phase of mitosis.

The response of cyclin D1 mRNA levels to 4 weeks of distraction using a distraction rate of 0.5 mm per day are shown in Figure 3. This figure clearly shows that distraction produces a marked increase in the expression of cyclin D1, and is consistent with the concept that longitudinal growth is associated and possibly dependent on satellite cell activation.

## Mitogenic Factors: IGF-1 and MGF

How do skeletal muscle fibers know that the appropriate response to increased weightbearing is the addition of sarcomeres in parallel, whereas the appropriate response to stretch is that of adding sarcomeres in series? Currently, the mechanotransduction pathways mediating the response of skeletal muscle to various mechanical stimuli remain poorly understood. During the past 5 to 10 years, there has been an increasing interest in the role of the growth hormone-IGF-1 axis, and evidence is accumulating to suggest that IGF-1 may play an important role in mediating growth.[1–4,16,22,23] Additionally, there is an evolving recognition that the source of IGF-1 is not the liver as once thought, but that mechanically induced elevations in IGF-1 occur because of an autocrine/paracrine response of skeletal muscle. There are numerous IGF-1 splice variants, and McKoy and colleagues[23] identified a muscle-specific variant that seemed to be expressed in skeletal muscle only when active. They defined this variant as MGF. McKoy and colleagues[23] examined the expression of MGF after stretch, stimulation, and a combination of these two interventions. They found that stretch and stretch plus stimulation produced significant elevations in MGF mRNA expression. The reader should refer to the study by Goldspink[16] for additional discussions related to these issues.

As mentioned previously, the upregulation of cyclin D1 is thought to be mediated by mitogenic factors, and in some instances it has been reported that IGF-1 can play a role in regulating cyclin D1 levels. With respect to this point and the aforementioned discussion regarding IGF-1, the effects of 4 weeks of distraction on IGF-1 mRNA levels were examined in the rat tibialis anterior muscle. Figure 5 shows that this produced a significant increase in IGF-1 levels. Subsequently, the response of MGF mRNA levels in the rat soleus muscle to distraction-induced stretch was examined at early points of 4 and 8 days of dis-

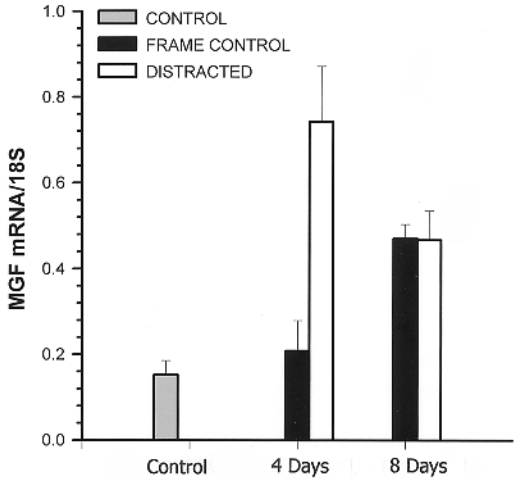


**Fig 5.** Goldspink[16] reported that stretch leads to the upregulation of an IGF-1 splice variant, MGF. The data shown in this figure are from rat soleus muscles distracted at a rate of 0.5 mm for 4 and 8 days. Distraction produces a significant increase in MGF mRNA levels. However, there also was a significant increase for the frame control muscles that were not exposed to stretch. Therefore, these data suggest that the increase in MGF that occurs during distraction might be related to an immobilization phenomenon and not growth.

traction. These results are shown in Figure 5, and it is obvious that there was a rapid and large increase in MGF. Interestingly, however, the time course analyses also show that there was a significant increase in the MGF mRNA levels of soleus muscles taken from control animals. Based on these results, it seems as though distraction does not mediate the expression of MGF via passive stretch, but does so possibly in response to immobilization of the muscle, unloading of the muscle, or both. These data suggest that the expression of MGF must be examined in much more detail.

## New Approaches for Understanding the Longitudinal Growth of Skeletal Muscles: Gene Chip Analyses and Interesting Paradoxes

During the past 10 years, it has become increasingly apparent that the pathways involved with mediating growth in skeletal muscle are very complex. Although the reductionist approach has been one of the primary paradigms used to develop an understanding of growth in skeletal muscle, it might be argued that the approach of studying individual molecules is inadequate for exploring such complex events. It also could be argued that a full understanding of such complex pathways only will be achieved via a more integrative approach whereby the response of the entire genome to altered loading conditions can be studied.

In this context, DNA microarrays may prove to be a very powerful tool. There are two primary types of DNA microarrays that can be classified as cDNA arrays and oligonucleotide arrays. An example of a high density oligonucleotide array (also referred to as a gene chip) is shown in Figure 6. The expression of each gene is determined using 16 to 20 different probe pairs, and each probe pair consists of a perfect match that consists of a 25-base sequence specific for that gene. Additionally, each probe pair consists of an internal control where the thirteenth base is designed to be a mismatch, controlling for nonspecific hybridization. The difference in signal intensity for the mismatch is subtracted from the perfect match and summed across all 16 to 20 different probe pairs to yield one value that represents the expression level for that gene. Currently, approximately 8500 genes can be analyzed for the rat on a given chip. Therefore, the gene chips (or high density oligonucleotide arrays) are powerful tools because: (1) they provide insight regarding gene regulation across a large proportion of the genome; (2) the use of 16 to 20 different probe pairs to identify the expression level provides, in a sense, a high degree of statistical power given the large amount of redundancy; (3) the use of a mismatch probe allows for the control of nonspecific hybridization; and (4) in some instances there actually may be more than one probe set for a given gene on a chip.

There are numerous potentially important applications and, within the context of the current work, some of these include: (1) examining global alterations in gene expression in response to a given perturbation; (2) identifying common promoter sequences; (3) discovering networks of genes based on various types of contrasts; (4) identifying genes that are sensitive to high loading conditions as occurs with resistance training; (5) identifying genes that are regulated by distraction; (6) identifying growth genes that are specific to one form of growth and identifying others that are common to all forms of growth; and (7) determining the effect of overexpression or knockout of a given gene on global gene expression.

An example of the approach that might be used to identify genes specific to one form of growth and others that are common to all types of growth is shown in Figure 7. In one example, a muscle undergoes radial growth in response to resistance training (hypertrophy), and genes A, B, and C are upregulated. In the other example, a muscle undergoes longitudinal growth that is induced by distraction, and genes A, B, and D are upregulated. In this simple example, genes A and B would be identified as common growth genes whereas genes C and D would be unique to each form of growth. Using this paradigm, it then would follow that genes C and D would be of particular interest.

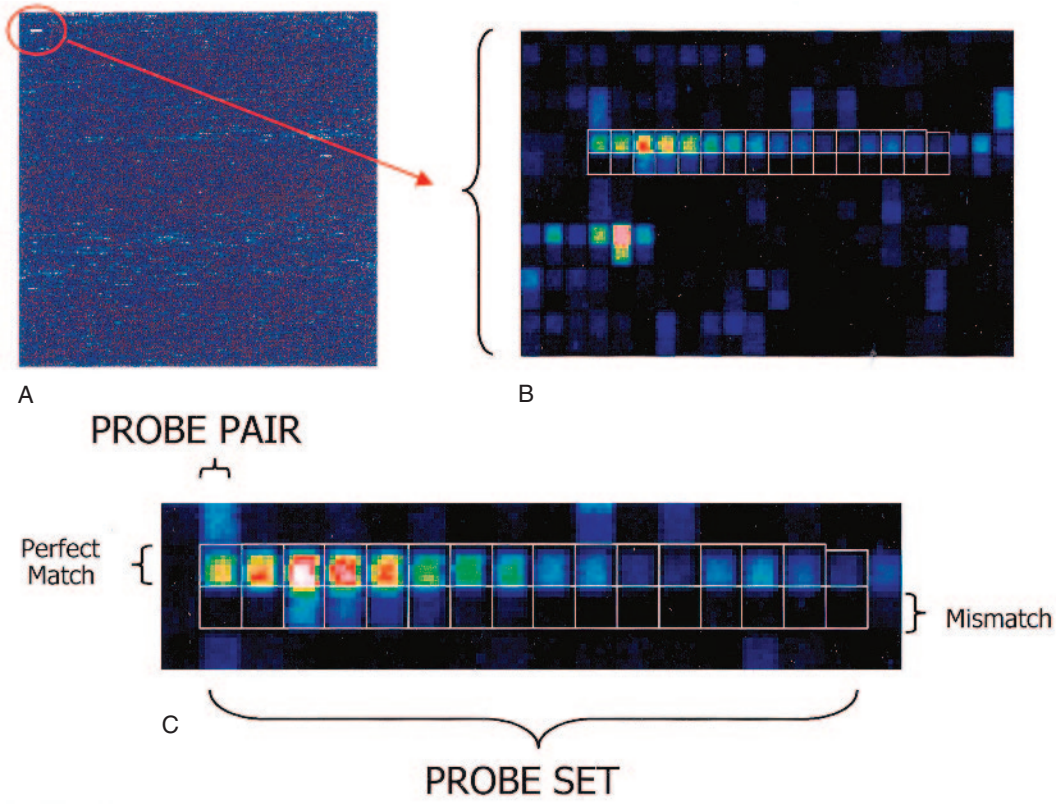


**Fig 6A–C.** (A) High density oligonucleotide arrays (gene chips) represent potentially one of the most significant technologic advancements in the past 5 years. These chips allow investigators to examine the effects of various perturbations on large numbers of genes (thousands to tens of thousands), and, in some cases, it is possible to examine the entire known genome. This will allow investigators to develop a more comprehensive understanding of various pathways and systems involved in mediating various adaptive responses. (A) An image from the Affymetrix U34A rat gene chip (Affymetrix, Santa Clara, CA) that contains approximately 8500 genes is shown. The red circle identifies a key gene, GADD45, which seems to be upregulated with distraction. (B) This region of the gene chip is shown. (C) An overview of the anatomy of a probe set is shown. The level of expression for a gene is defined by the probe set. The probe set is composed of 16 different 25-base sequences. These are known as the perfect match. Below each perfect match is an internal control (mismatch) that has a mismatch at the thirteenth base of the 25-base sequence. This controls for nonspecific hybridization. The expression level for the gene then is determined by subtracting the value for the mismatch from the perfect match. These values then are summed for all 16 probe pairs and the total value represents the expression level for that gene.

Microarrays also provide the potential for gene discovery. They provide the possibility of quickly identifying genes that may be very important but outside the realm of an investigator's current interests. Alternatively, the microarrays also might provide the opportunity for discovering unknown genes such as occurred with the discovery of atrogin by Gomes and colleagues.[17]

As mentioned previously, it has been proposed that skeletal muscle is responsible for some of the complications that occur during distraction osteogenesis. More specifically, it

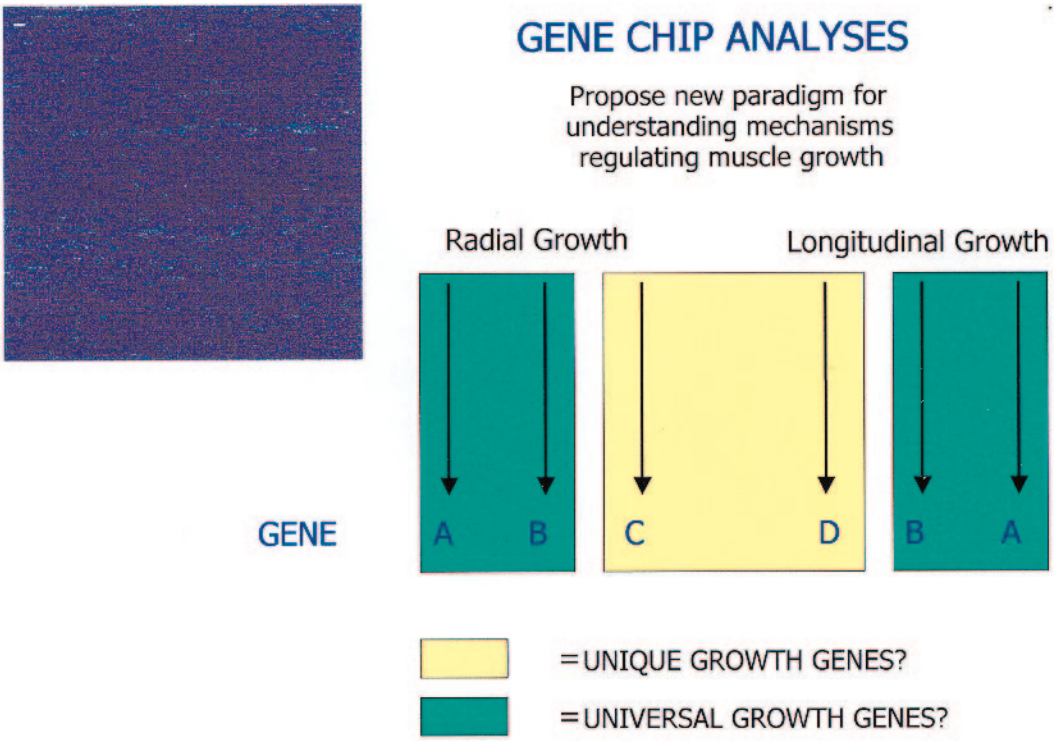


**Fig 7.** One of the potentially powerful approaches that might be developed to explore global gene expression during different forms of growth is shown. For example, it is not clear whether the expression of IGF-1 is specific to radial growth or common to both forms of growth.

has been proposed that during distraction of the tibia the longitudinal growth of the plantar flexor muscles lags behind the lengthening of bone and gives rise to the equinus contracture. As cited previously, stretch induced by distraction upregulates the expression of some genes involved with promoting activation of satellite cells and DNA replication. In this context, however, gene chip analyses revealed a potentially important but paradoxic event; the upregulation of genes involved with growth arrest. Specifically, Figure 8 shows that distraction (or the immobilization associated with distraction) results in the dramatic upregulation of a gene, GADD45, known to associated with growth arrest and DNA destruction. It also has been observed that distraction concomitantly upregulates p21, a known inhibitor of the cell cycle. The paradox is that distraction seems to upregulate genes (and presumably their protein products) that are responsible for promoting mitosis (cyclin D1, IGF-1) while concomitantly upregulating genes involved with growth arrest. Is it possible that these latter genes might retard the longitudinal growth of skeletal muscle during distraction, producing complications such as the equinus contracture?

To date, a large number of studies have been devoted to understanding the underlying mechanisms regulating the radial growth of skeletal muscle in response to increased loading. In contrast, far fewer studies have focused on the longitudinal growth of skeletal muscle. This is puzzling from several perspectives. First, as noted previously, the longitudinal growth of skeletal muscle may be responsible for numerous complications that occur during distraction osteogenesis. Second, contrasting

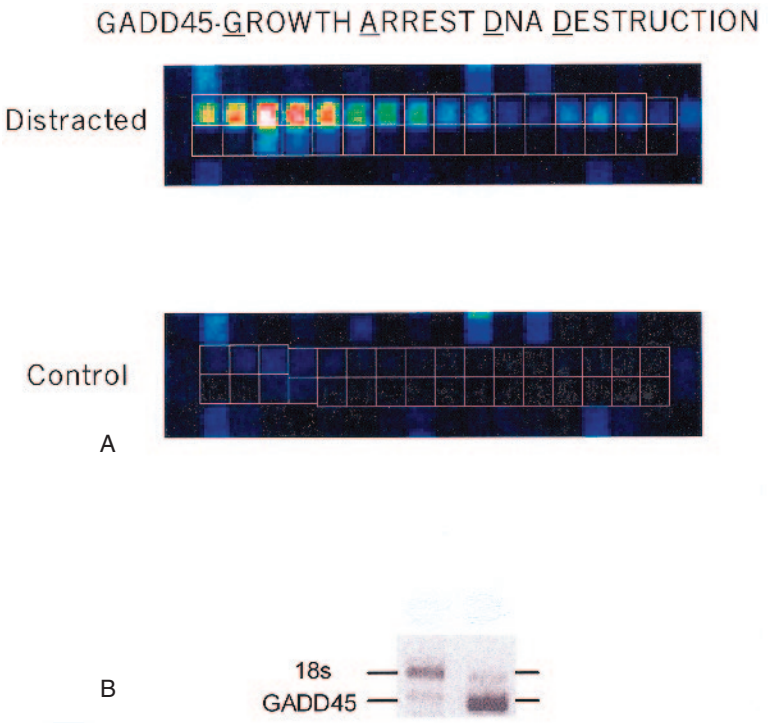


**Fig 8A–B.** (A) The probe set for GADD45 taken from a pool of three soleus muscles subjected to distraction for 8 days at a rate of 0.5 mm per day is shown. The dramatic increase in GADD45 expression that accompanies distraction can be seen. GADD45 is a key gene involved with inhibition of growth and DNA repair. Therefore, its expression might reflect a paradoxic event that acts against promoting growth during stretch induced by distraction. (B) Confirmation of the elevation in GADD45 as detected by the gene chip was done using RT-PCR. Lane 1 is from a control muscle. Lane 2 is from a muscle stretched by distraction.

radial and longitudinal growth of skeletal muscle may represent a unique paradigm for identifying pathways that are essential for one form of growth and those that are common to both forms of growth. Finally, the upregulation of GADD45 might suggest that distraction osteogenesis activates pathways that prevent rather than promote the longitudinal growth of skeletal muscle.

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