Investigation into bone and soft tissue reaction during limb lengthening in the animal model

PhD thesis

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Introduction

Callus distraction is a useful and reliable method in the treatment of bone length discrepancy. The numerous complications which had arisen during limb lengthening in the past have been reduced or eliminated as a result of research. At the present time limb lengthening is a procedure supported by numerous research.

Limb length discrepancy may be the result of numerous etiologies such as fractures, infection, congenital or traumatic growth plate lesions, tumours. These etiologies result in limb length discrepancy. The most important goal of treatment is a balanced pelvis which can undergo balanced loading and equalisation of limb length. Even if ideal callus formation is achieved, the procedure is strictly limited by the reaction of the soft tissues, in particular by the striated muscles. It appears that the behaviour of the striated muscles is not merely a passive procedure but an active reaction to limb lengthening which is called " histogenesis."

Striated muscles are under normal tone when the limb is resting. During limb lengthening this tone is significantly increased. Muscle contracture is a response to limb lengthening. It is caused by increased tension.

It has been demonstrated both in clinical practice as well as in animal experiments that dynamisation results in enhanced callus formation. Unfortunately, the definitions associated with this are not at all clear. There are even examples in the literature where it is concluded that early dynamisation does not provide any advantage. Precise definition of dynamisation and its measurability is an important aspect because it can reduce the time of treatment which involves the inconvenience of the patient having to wear an external fixator which can interfere with a comfortable daily life.

Objectives

The present investigation has set as its objective to define the complications associated with striated muscle reaction and examines the effect of controlled axial dynamisation on callus formation. Following numerous surgical experimentation, I first established a reproducible and safe operative technique.

During our research we examined the striated muscle characteristics of animals of different age and the ability of these muscles to adapt to limb lengthening and we tried to establish the upper limits of their regenerative capacity. The important significance of this research is that it may help in establishing improved protocols of lengthening and it can forecast possible expected complications.

It was a most important objective of our study to examine possible methods which could accelerate the lengthening procedure using a specially designed new type of external fixator. A reduction of time during which the patient has to wear an external fixator is an important consideration in order to reduce inconvenience and interruptions to his or her daily life.

At the outset of this study I had set out the following questions to which we sought answers

1. Is it possible to conceive a limb lengthening procedure which could achieve reproducible and safe limb lengthening in the rabbit?
2. What is the reaction to the lengthening of striated muscles adjacent to bone segments ?

3. What is the correlation between complications and the age of the experimental model ?
4. Is there a correlation between the daily lengthening rate and the development of complications?
5. Is there an upper limit to compensatory mechanisms during the limb lengthening procedure which is a response to degenerative complications?
6. Our goal was to conceive a lengthening device specially and specifically designed for manual controlled axial dynamisation.
7. Is it possible to reduce the total time necessary to achieve the desired length using a manually dynamised device?

Methods

We used 69 New Zealand White Rabbits. We used the first 20 Rabbits to establish the ideal method of applying the external fixator. Histopathologic changes were started on 39 Rabbits. The surgery was immediately followed by seven days of continuous compression. The study was divided into two parts. In part 1 we divided the animals into six groups and in part 2 we had three groups. In the first group (G1) we applied 0.8mm daily lengthening to 4 adult animals. In the second group (G 2) we applied lengthening of 1.6 mm (2 x 0.8mm) to 5 adult rabbits. In the third group (G3) there were five young animals the tibia of which we lengthened at the daily rate of 0.8 mm. In group 4 (G4) there were four young rabbits whose tibia were lengthened 1.6 mm (2 x 0.8mm) daily. In all cases lengthening was continued until the limb achieved 120% of this total length.

The control groups consisted of G5 with two adult rabbits and G6 with three young rabbits. In the control groups we applied the same type of osteotomy and the same external fixator as in all the other groups but no lengthening whatsoever took place. All young animals were 9 weeks old and all adult animals were 28 weeks old.
In the second phase of the hystopathologic investigations there were only young rabbits. These Rabbits were divided into three groups: F1 were lengthened at 0.8 mm per day, F2 were lengthened at 1.6 mm per day (2×0.8 mm), F3 at 3.2 mm per day (4×0.8 mm per day). We finally examined the quality of callus in 10 animals. We divided the rabbits into NDG (five adult animals) and dynamised DDG (five adult animals). In the DDG group we applied 1mm controlled axial dynamisation three times per day (3 x 10 minutes).

We used rabbits in our study because the external fixator suitable for rabbits' tibiae is the same type of fixator used in human metacarpals.

We used the original Orthofix M 100 fixator as well as a modified M 100 and (ITEC) models.

Operative technique

Appropriate preparation included shaving, thorough disinfection of the skin surface. The animal was placed and fixed in a supine position. The skin incision is applied on the medial side of the leg.
Blunt dissection is used to divide the tibial cranialis muscle and the extensor digitorum muscle to expose the tibia. The periosteum is incised longitudinally for the application of the external fixator. We divided the rabbits into three groups. In group A a regular electric drill

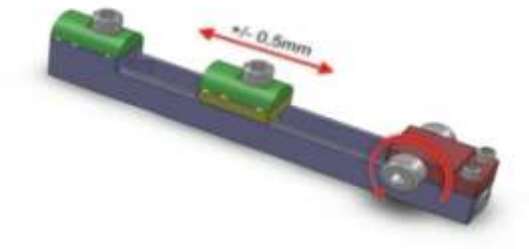
was used. In group B slow speed drilling was used and the pins were inserted in a careful right and left rotating movement taking special care during penetrating of the second cortex. In the first series we placed the fixators in an antero-medial position. However, this resulted in the distal end of the fixator grazing / irritating the metatarsal surface. In order to avoid this we placed the fixators in the second and subsequent series in the frontal plane. In group C the bone was drilled manually. We used protection sleeves placed inside the fixator clamps in order to ensure that drilling of the bone is exactly at right angles to the axis of the fixator and that the bone pins were parallel. We locked the bone pins in the fixator clamps and we checked for fixator stability prior to performing the osteotomy. We then performed the osteotomy. We paid particular attention to protecting the periosteum and the soft tissues. Finally we compressed the osteotomy in order to assist callus formation and to reduce post-operative pain. We applied relaxing incisions along the line of the fixator bone screws and covered the periosteum and the osteotomy site.

Lengthening protocol

Following compression of the osteotomy during seven days we lengthened at 0.8 mm per day. Our objective was to achieve a 20% lengthening which we monitored using x-ray measurements measuring the distance between the pin groups. We determined the number of lengthening days by measuring the original length of the tibia and based on x-ray. We assessed results on a daily basis during lengthening. The rabbits were under x-ray control and continuous physical control during the post operative period. We carefully noted every complication, pain, infection, any information and any abnormal change in the position of the bone screws or any type of instability. We also carefully noted how the rabbits walked and body stance.

Suitable devices for axial dynamisation

At the outset of our investigation we modified the Orthofix M 100 fixator. We added a manual actuator to perform controlled axial dynamisation. The eccentric mechanism inside the actuator results in 1 millimetre of axial movement between the two pin groups of the fixator when the actuator is turned 360°. Compared to their initial position, the pin clamps move 0 .5 – 0 .5 mm along the long axis of the fixator. We paid special attention to the group that was dynamised manually for 10 minutes 3 times a day, to ensure that the initial position and the position after dynamisation is according to the above-mentioned guidelines. In this way we could avoid length discrepancy to develop between the normal lengthened group and the dynamised lengthened group.



Device for controlled axial dynimazation (modified M100 Orthofix external fixator

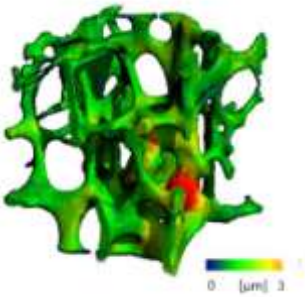
Preparation of the animals for the harvesting of histological samples

The animals were prepared using formalin fixation. The animals were perfused because this speeds up considerably the penetration of the formalin into the tissue. The rabbits were anaesthetised according to usual protocol and they received 300 ML NaCl infusion transcardially and 2000 ml 4% formalin (pH 7.4) solution infusion.

We removed the flexor digitorum longus from the legs of all animals. We took samples from the distal and proximal ends of muscles and from the myotendinosus –junctions (MTJ) . We placed these in paraffin and prepared 5 um thick slices. We dyed some of them with haematoxylin-eosin, Wiegart Van Gieson trichrome and Masson trichrome and we marked some of them with immunhystochemicals.

Micro-CT investigations

Bone formation monitoring was performed using micro-CT. The isotropic voxel measurement was 17 um. We used Sky 1172 Skyscan (Skyscan, Kontich, Belgium} equipment. 100 kv and 100 micro A with 0.5 mm aluminium filter. We filmed the samples in 180° in 7/10 steps. We used NRecon software (Skyscan, Kontich, Belgium} to achieve 3D reconstruction and we use 50% radiation amplification and 20% arteficial fact filter. We analysed the pictures using CT Analyser software (1.7.0.5, Skyscan, Kontich, Belgium) which provided bone volume, total volume, bone surface information as well as providing us with the bone lengthening zone information. This was the source from which we later calculated the proportional bone dimentions.



microCT of the bone tissue, 3 D reconstruction

Analysis of histological changes

The slices were analysed using Zeiss ICM 405 microscope and the pictures were produced using a Zeiss M 35 camera. We used a speciallens to examine the relevant areas. We analysed the histopathologic changes using Lee semi quantitative point system. We expanded the 5 parameters used by Lee to 9 parameters. The size of the squares agreed with those recommended by Lee. We counted 20 views from each section.

We applied 0-3 points to the changes. Zero meant normal. The source of normal value was samples taken from intact muscles.

We used Kruskal-Wallis and Mann-Whitney U tests to analyse our results.

First we analysed the normality of the samples analysed using micro-CT. BV, BS and be CS passed the normality tests, therefore, we subsequently used to the T test. The TV test did not show normal division, therefore, we applied the Mann-Whitney test. We used Sigma STAT 2.0 software with statistical significance level of 0.05.

Results

At the outset of our study the most important step was to design a safe fixator application technique with low complications.

We used pre-drilling with an electric drill in the case of the first eight rabbits. Micro fractures occurred frequently in the first cortical around the bone **pins** which resulted in fracture of the tibia either post operatively or during lengthening. For this reason we first changed the technique of pin introduction. In group B we carefully inserted the pins using a left- right rotational movement. Although this reduced the incidence of fractures, the success rate was only 40% (no fractures) was still short of the desired results.

We then used a hand drill with protection sleeve in group C containing the 12 remaining rabbits. We continued with the left- right rotational pin insertion technique. With the exception of two fatal infections, using this technique we achieved the desired success rate.

The main post-operative complication was micro fracture around the inserted bone pins. There were no other serious complications during the healing period and/or any other simple complications were treated and resolved successfully.

In the control group the m flexor digitorum longus (FDL) were of equal length both on the control and on the operated groups. According to our expectations the length of the FDL increased significantly on the lengthened limbs compared to the control side.

Results of the histopathologic investigations

Our histopathologic investigations consisted of two parts. In the first group we analysed the reactions of the striated muscles of adult rabbits. In the second group we looked at young rabbits exclusively including excessively long lengthenings. Here follow the lengthening parameters used in respect of the groups.

Histopathologic Analysis of young and adult animals- Part 1 (G series)

Those animals that were lengthened at a rate of 1.6 mm per day, (G2) showed significantly larger muscle mass diameter changes (suggesting muscle atrophy) than in those groups where only 0.8mm lengthening took place (G1) (p/0,005). We found no statistically significant difference between the two groups of young animals (G3, G4) although the changes became more significant under excessive limb lengthening.

Where we compared the results according to the age of the animals, we saw a significant rise in the change of muscle mass diameter in adult animals (0.8mm per day and 1.6 mm per day lengthening rate), compared to the young animals. (Average values G1: 1.5; G3 : 1.2, G2: 2.35; G4: 1.375). The values also increased in the distal part of the muscles, however, the differences were not significant. (G1 prox: 1.375; G1 dist: 1.625; G 2 prox: 2.1; G2 dist: 2.6; G3 prox: 1; G3 dist: 1.4; G4 prox: 1.25; G4 dist: 1.4)

The rate of muscle degeneration increased significantly in the adult groups G1, and G2 (p/0.05) compared to the younger groups. There was no difference in the degeneration between proximal and distal muscle samples. (Average values: G1: 1.1875; G2: 1.95; G3: 0.85; G4: 1.25).

We observed that the regeneration capacity of muscle was significantly greater in younger rabbits.

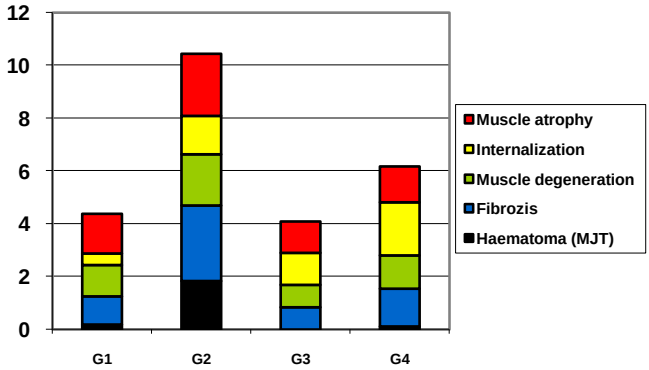
When we compared groups G1 and G2, we found that the regeneration values in the G2 group was more than double that of group G1 (p/0,001). (Average values : G1: 0.375; G: 0.957 G3: 0.8; G4: .75). The rate of muscle regeneration in the younger groups is similar but the absolute values of regeneration are higher. There were no differences between the distal and proximal samples. (Average values: G1 proximal: 0.375; G1 distal: 0.375; G2 proximal 0.9; G2 distal G3 proximal : 0.8; G3 prox: 0.8; G4 proximal: 1.625; G 4 prox:: 1.75).

The peri and endomysialis fibrosis in adult animals was much stronger than in the younger ones. It would appear that longer lengthenings result in greater fibrous tissue. (G1 – a G2: p/ 0,001; G3 – G4: p/ 0,01). There were no differences between the distal and proximal muscle regions. (Average values: G1 proximal: 1; G1 distal: 1.125; G2 proximal: 2.8; G2 distal: 2.9; G3 proximal: 0.8; G3 distal 0.9; G4 proximal: 1.75; G 4 distal: 1.5).

In the animals where the daily rate of lengthening was 1.6 mm (G2, G4) there was a significant increase in the internalization of nuclei in the area of the MTJ, compared to the 0 .8 mm (G1, G3) groups (G1 – G2: P / 0.001; G3 – G4: P/0.001). In addition, the younger animals demonstrated increased internalization of nuclei compared to the adult groups.

Quicker rate of lengthening resulted in a raised cell count in MTJ region compared to a slower rate of lengthening. (G1 – G2: p/0.001; G3 – G4: p– 0.01).

We detected a raised capillary value in group G4 compared to G3. A considerably larger quantity of heamatoma developed in the adult groups where the lengthening rate was 1.6 mm per day in MTJ region, compared to the 0. 8mm per day lengthened group (G1 – G2: P/0.001). Younger subjects are less affected by this phenomenon. (D1: 0.1875; G2: 1.85; G3: 0; G4: 0 .1).



Summary of the muscle degenertion parameters

Part 2 – Histopathologic analysis of young animals. (F series)

A lengthening a rate of 3.2 mm per day demonstrated a significantly higher rate of atrophy compared to the 0.8 mm per day (F1 group) or the 1.6 mm per day (F2 group) (p/0.05) (table 8). There was no such difference between groups F1 and F2. (F1: 1.2; F2: 1.3 757; F: 2.56).

We obtained similar results for the internalization of nuclei investigations. There was no difference between groups F1 and F2. However, the values were significantly higher (p/0.05) than in group F1 or in group F2 (F1: 0.8; F2: 0.94; S3: 2.19).

When we analysed the results for internalization of nuclei at the myotendynosus junction, we found significant differences between the 0.8mm per day and 1.6 mm per day groups as well as between the 0. 8mm per day and the 3.2 mm per day lengthening rate groups. The values for the F3 group are a little higher than that of the F2 group but the difference was not statistically significant. (F1: 1.2; F2: 2; F3: 2.16).

Looking at the results of signs of degeneration of muscle mass, the values for group F3 was significantly higher than for F1 and compared to group F2, (p/0.001). There was no difference between the groups which underwent lengthening at a low daily rate. (F1: 0.85; F2: 1.25; F3: 1.69).

The values of muscle regeneration for the F2 group were double of group F1 (P/0.05). At a daily lengthening rate of 1.6 mm, we found more muscle mass than in the group that underwent 3.2 mm per day lengthening rate but this result was not significant. (F1: 0.8; F2: 1.69; F3: 1.3) (figure 23).

The histological samples taken from animals who underwent lengthening at at 3.2 mm per day contained significantly more histological fibrous than those that underwent lengthening at the rate of 0.8 and 1.6 mm bay respectively (p/0.05). We found no difference between groups F1 and F2. The extent of fibrosis was almost 3 times as big in the F3 group than in the F1 Group. (F1: 0.857: F2: 1.44; F3: 2.8).

We found larger cell proliferation in the MTJ region in the group F3 (P/0.05) than in F1 group. There were no differences between group F2 and F1 or between groups F2 and F3. (Table 23) (F1: 0.95; F2: 1.625; F3: 2.375).

When we looked at capillary values, we found significant differences between groups F1 and F2 and also between F1 and F3 groups. We found no differences within this parameter between groups F2 and F3. (F1: 0; F2: 1.375; F3: 1.31).
The values for haematoma increased in line with the daily lengthening rate. Haematomas occurred most often in the 3.2 mm per day group, however, we did not find significant differences between the groups (F1: 0; F2: 0.125; F3: 0.25)

Analysis of the modified operative technique.

The rabbits tolerated the fixator well. We noted full weight-bearing in animals which suffered no complications. The device did not really limit mobility of the animals; however, where we applied the fixators medially, this tended to force the rabbit to place its leg sideways.
We performed lengthening on a total of 20 rabbits; in the case of 10 of these we noted no complications whatsoever.

Following a successful lengthening procedure, healing at the osteotomy site is usually good with strong callus. Ilizarov (1989) recommended corticotomy rather than osteotomy in order to preserve endosteal blood supply. However, performing corticotomy can be

challenging and preservation of endosteal blood flow cannot be guaranteed. We have observed good callus formation in our lengthened cases. According to Green (1994) the oscillating saw can result in delayed new bone formation, however, we have noted early callus formation on x-ray and we saw no evidence of delayed union in our cases.

Apart from a few minor complications such as suture failure and soft tissue reaction or inflammation around the bone pins, our results were excellent.

The most frequent complication that we saw was fractures of the tibia either post operatively or during the lengthening. In most cases the fracture occurred a few days post operatively. We saw 7 cases of tibial fractures out of a total of 20 cases. 3 such fractures occurred in group A where we had a pre-drilled using an electric drill (100% fracture incidence). We assume that these fractures had occurred as a result of micro fractures. We, therefore, modified our pin insertion technique rotating the pins left and right prior to penetration of the second cortex. However, despite this, the fracture incidence was still 40%. We then decided to use a hand drill in group C. In group C we achieved a success rate of 80% if we discount systemic infection or animals which died as a result of the anaesthetic, while our success rate in group B was 60%.

We consider 7 fractures out of a total of 20 animals to be too high. In the light of this, we modified our pre-drilling technique by using a protective sleeve and this had reduced the incidence of fractures.

In our opinion the incidence of complications was low in this investigation. In the cases where we had successfully lengthened we witnessed excellent new bone formation which, we believe, indicates that our method is good. We used a hand drill as only in this way could we guarantee to control the drilling speed. In our opinion the drilling speed is a most important factor in this procedure because rabbit bone is rather fragile. Similar results may be achieved using an electric drill provided that its speed can be reliably controlled by dialling in the precise speed of the machine.

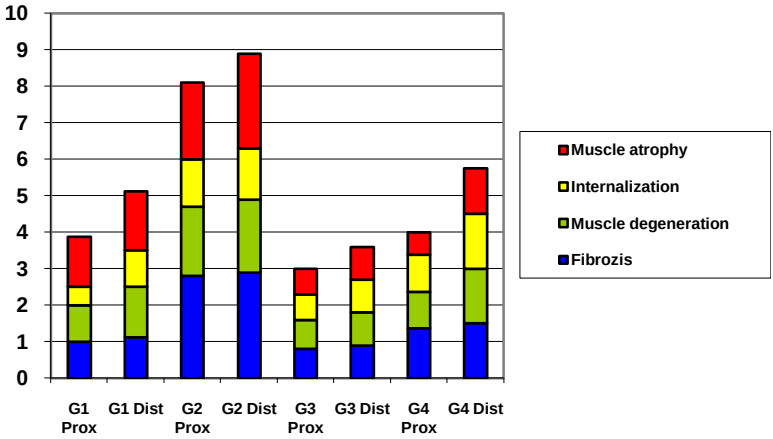
Analysis of histopathologic changes

This investigation has led us to the conclusion that increasing the daily lengthening rate proportionately increases the occurance of muscle damage. Although we have no statistical proof, we observed the tendency that bone lengthening disproportionately affects the distal region of the muscle. It should be noted, however, that striated muscles in younger subject adapt significantly easier to lengthening than in older subjects.

Haematoma developed mostly in adult rabbits whose legs were lengthened at a rate of 1.6 mm per day. It tends to localise in the MTJ region between muscle strands and their morphologic characteristic is that mono nuclear infiltration is visible in them which excludes the possibility of artifactal origin. The occurrence of haematoma in the 0.8mm per day group was minimal. This proves that haematoma is formed as a result of lengthening and its occurrence is determined by the daily lengthening rate.

Signs of degeneration occurred much more frequently in adult groups. First signs of degeneration are usually seen at the MTJ region. It would appear that limb lengthening leads to muscle damage which manifests in muscle strand necrosis and disturbed cell membrane function. It was interesting to note that we found less signs of muscle degeneration in young

animals at a daily lengthening rate of 3.2 mm than at the daily rate of 1.6 m in adult subjects.



Degenerative alterations of FDL muscle, proximal and distal parts

In younger animals a faster daily rate increases internalization of nuclei in the MTJ region. The raised value demonstrated a significant increase at the daily lengthening rate of 1.6 mm and 3.2 mm compared to the 0.8mm daily rate. Caiozzo described in 2002 that the mini satelite cells which are situated primarily in the MTJ region, play a key role in sarcomerogenesis. This type of reaction of MTJ is probably an active and adaptive response to lengthening (Cooper 1999,Tatsumi and Mtsai 2001). It is due to this response that the striated muscles regenerate, and that the developing muscle cells fuse with the existing muscle syntitium. (Hawk 2001, Schultz 1994). For this reason, if the MTJ reacts more sensitively to the lengthening, the regeneration background which could reduce the damaging effect of lengthening, may be reduced.

The quantity of peri and endomyseal fibrosis increases in line with the amount of lengthening and with the age of the subject. Williams (1999) found that connecting tissue and collagen content of muscles increased significantly in the group which underwent a medium amount of lengthening (1.6 mm per day) compared to the group whose rate of lengthening was 0. 8mm per day. Even in young animals, when the daily lengthening rate was 3.2 mm, there was an extreme increase in connective tissue. The increase of connective tissue can affect the muscle strands because according to Lee (1993) such connective tissue can be found within the damaged muscle strands. Moreover, the increased volume of peri and endomyseal fibrosis reduces the range of movement of joints moved by these muscles (Williams). It may be that this morphologic change is the main cause of complications in limb lengthening (because of the reduction of the range of movement of the adjacent joint).

We found signs of hyper vascularisation in our samples which is one of the signs of muscle regeneration. In young animals increasing the daily lengthening rate increased the

capillary values but when the lengthening rate exceeded 3.2 mm per day, we saw a reduction in capillary values.

A sure sign of regeneration processes is an increase in muscle cell values. Tsujimura at al found that stem cells found in striated muscle are activated as a result of lengthening of sacromers. According to Caiozzo, muscle mass works similarly to a switch. If the sacromer reaches a certain length, the satelite cells are activated. The activated satelite cells divide, and then they fuse with existing muscle mass and then begin the creation of new muscle mass. Satelite cells are not activated in up to 5-7% muscle lengthenings (0.5 mm per day). Schumacher (1994) noted cell growth only after 8-10% muscle lengthening. It would appear that increasing the daily lengthening rate increases satelite cell division. The number of satelite cells continually decrease during lifetime. This may be behind the greater extent of cell activation in older animals. The tendency towards growing satelite cell proliferation as the daily lengthening rate is increased, continues only up to a limit. In our investigations, it increased up to 1.6 mm per day lengthening rate. As soon as the daily lengthening rate reached 3.2 mm, the regeneration parameters fell even in younger subjects.

It is important to note that we saw an increase in cell proliferation in the MTJ region in all groups that underwent lengthening. This was a stronger in younger animals. We found the greatest proliferation at a daily rate of 3.2 mm lengthening. It is probable that this is the response of this particular region to lengthening. It was noted frequently that signs of muscle regeneration appear already before the excision of necrotic tissue and for this reason these two processes can be seen concurrently.

It would appear that satelite cell activation is the greatest in the MJ region. For this reason this region has the greatest potential for new muscle formation. This region is often referred to as the "engine" of regeneration. Signs of regeneration occurred much more frequently in young animals. One of the explanations for this may be that on average a larger number of satelite cells can be activated and can be found in young animals then in older animals.

Using the points system of Lee, we concluded that striated muscles of young animals adapt more easily to limb lengthening. In our opinion, the distal third of muscles (and the MTJ region) participate more prominently in the responses associated with lengthening, although, we were not successful in demonstrating this statistically. The damage caused as a result of lengthening may start the muscle regeneration process, while the necrotic tissue which develops during lengthening is in time replaced by connective – fat tissue and newly formed muscle mass. The ratio of this newly formed muscle mass is strongly influenced by the daily lengthening rate (Pap 2008).

Muscles of young animals possess stronger self healing capacity than those of older animals. Moreover, we saw that muscle regeneration mechanisms are finite. We reached this at 3.2 mm per day lengthening rate. Moreover, following excessive lengthenings the degenerative signs increased. This part of my investigation sought to emphasise that it is important to preserve the fragile balance which exists between the degenerative and regenerative mechanisms in terms of muscles since if this fragile balance is upset, this will negatively affect the function of the lengthened limb.

Results regarding controlled axial dynamisation

It has been known for some time that the effect of mechanical stimulation is fracture healing. This fact formed the starting point of our investigation. According to Martinez the beginning of stimulation should be coordinated with evolving callus formation. According to our lengthening protocol, lengthening was preceded by one week of compression. During

this period callus formation had begun, and this was then followed by a daily rate of 1.6 mm of distraction which marked the beginning of dynamisation. To date there is still no consensus in the literature as regards the ideal length and frequency of dynamisation. Although dynamisation is desirable from the point of view of new bone formation, and can be stated to be a biologic motivator of bone formation, this phenomenon is not yet supported by precise definitions or clarification. In our investigations we applied daily dynamisation of 1.0 mm length. Since the external fixator designed by myself and its ability to apply manual, controlled, axial dynamisation is a new method of callotasis, no ground rules (protocol) have been established associated with the above mentioned two factors. For this reason, and as a first step, we wanted to test one of the initial end points of dynamisation. Based on our results, it can be stated that such large amount of dynamisation has not reduced the volume of callus formation, on the contrary, it's quality was a little better than in rabbits which belonged to the NDG group. The objective of our subsequent investigation was to find the ideal dynamisation length and ideal daily lengthening rate.

We investigated the effect of conventional (NDG) and dynamised (DDG) on callus formation. We analysed callus formation using rtg and micro CT. On the two -directional pictures we did not see any bone screw mal position, no pseudoarthrosis, and we found no micro fractures around the Schanz screws using rtg pictures. There were also no bone screw breakages. 3D reconstruction views showed intact callus.

In the rabbits belonging to the DDG group, the micro CT showed 20% larger new bone dimentions (BV) but we found no statistically significant differences. ($P = 0.27$). At the same time, the total lengthening zone (TV) was the same in the two groups. The surface of the callus formation in the DDG group was larger than in the normal lengthened group, however, the difference was not statistically significant ($p= 0.139$). We can calculate the quality of the callus formation by dividing the lengthened zone (TV) by the new bone (BV). The result thus obtained in the DDG group was 24% higher but showed no difference in significance analysis ($p=0 .233$).

Conclusions

1. At the outset of our investigations we succeeded in formulating a safe and reproducible technique. Serious complications have been avoided by carefully pre-drilling in a parallel fashion, using low-temperature drilling and tissue protecting sleeves.

2. I found during this investigation signs of cell proliferation in the proximal as well as in the distal part of striated muscles. Similarly, signs of degenerative changes could be seen along the whole length of the muscle. Degenerative signs could be found more frequently in the distal muscle samples , however, this was not statistically significant. Most damaged cells are replaced by fresh myoblast.

3. There is an obvious correlation between complications that occur during lengthening and the age of the subject. Our investigations show clearly that the striated muscles of younger subjects have a significantly greater capacity to lengthen and to regenerate. It is very important to keep this in mind in clinical practice.

4. We found obvious correlation between increasing the daily rate of lengthening and development of degenerative signs. The number of complications increase in line with the increase in the daily lengthening rate. In excessive lengthenings these signs increase significantly within the striated muscles.

5. It appears that there is an upper limit to the compensatory regeneration which is a response to degenerative complications. At daily lengthening rates of 0.8mm and 1.6 mm, the regeneration signs increased, however, at a daily lengthening rate of 3.2 mm we saw a considerable reduction of these signs. In the light of the above I can state with conviction that choice of the daily lengthening rate is critical in clinical practice because beyond a certain limit the balance between regeneration and degeneration is upset and this can increase considerably the number of complications.

6. During this investigation I managed to conceive and design a safe limb lengthening apparatus dedicated to manually controlled axial dynamisation.

7. Based on the results of our investigations, it was not possible to shorten the time of limb lengthening through manual controlled axial dynamisation using an Orthofix type unilateral external fixator. Using a protocol of axial dynamisation of 1.0 mm length, daily 3×10 minutes, the stretched callus quality did not deteriorate, in fact, it improved somewhat. However, the difference was not significant. We were successful in determining the dynamisation end point. Following on from this a reduction in the length of dynamisation and the ideal frequency may be determined.

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