ACTA
ORTHOPAEDICA
et
TRAUMATOLOGICA
TURCICA

Author's translation
Acta Orthop Traumatol Turc 2005;39(1):64-69

Histopathologic and morphometric changes in rat nerve and blood vessels associated with femoral lengthening

Femoral uzatmanın sinir ve damarlara etkisi: Sıçanlarda histopatolojik ve morfometrik değişiklikler

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Amaç: Femoral uzatma sonucu sıçanların femoral arter, ven ve sinirlerinde ortaya çıkan histopatolojik ve morfometrik değişiklikler araştırıldı.

Çalışma planı: Çalışmada 42 adet Wistar-albino erkek sıçan kullanıldı. Genel anestezi altında sıçanlara sol femoral osteotomi yapıldı ve eksternal fiksatör uygulandı. Kontrol grubu olarak ayrılan yedi sıçan dışındaki denekler beş gruba ayrılarak, her bir gruba osteotomi sonrası birinci günden itibaren günde dört kez (4 x 0.35 mm) femoral uzatma uygulandı. Grup 1'de üç gün (%10), diğer gruplarda yedi gün (%30) uzatma yapıldı. Denekler grup sırasına göre 3, 7, 14, 21 ve 31. günlerde genel anestezi altında öldürülmeden önce uzatma bölgesinden femoral arter, ven ve sinir paketi biyopsi olarak alındı. Örneklerde histopatolojik ve histomorfometrik incelemeler yapıldı.

Sonuçlar: Uzatma yapılan sıçanların arterlerinde, ilk dört grupta, internal ve eksternal elastik tabakalarda düzleşme ve/veya fokal kayıplar; tunika media düz kas hücrelerinde hidropik dejenerasyon ve sitoplazmik vakuolizasyon görüldü. Grup 5'te fibrozis dışında normal arteriyel görünüm vardı. Ven değişiklikleri açısından, ilk üç grupta sitoplazmik vakuolizasyon ve dejenerasyon, 4 ve 5. gruplarda ise elastik liflerde düzensizlik ve bağ dokuda fibrozis görüldü. Grup 5'te femoral vende %27.9 oranında incelme saptandı. Sinir yapısında histolojik olarak belirgin bir değişiklik saptanmadı. Grup 2'de perinörium kalınlığında %164.6, miyelinize sinir liflerinin çapında %58.4 artış saptandı.

Çıkarımlar: Bulgularımız, femoral uzatmada, kan damarlarının sinirlere göre daha fazla etkilendiğini gösterdi.

Anahtar sözcükler: Arter; kan damarı; kemik uzatma/yöntem; femur/cerrahi; kas, iskelet/patoloji; sinir iletimi; periferik sinirler; sıçan; ven.

Objectives: We investigated histopathologic and morphometric changes in rat femoral arteries, veins, and nerves associated with femoral lengthening.

Methods: The study included 42 male Wistar-albino rats. All the rats underwent left femoral osteotomy which was stabilized with an external fixator device. The rats were divided into five groups except for seven control rats which were left untreated. Femoral lengthening was performed with a distraction of 0.35 mm four times a day, which was continued for three days in group 1 (10%), and seven days in the other groups (30%). Before sacrification of each group of rats under general anesthesia after 3, 7, 14, 21, and 31 days of osteotomy, respectively, biopsy samples were obtained from distraction sites involving femoral artery, vein, and nerves for histopathologic and histomorphometric studies.

Results: Arterial changes observed in the first four groups were flattening and/or focal absence of the lamina elastica interna and externa, and hydropic degeneration and cytoplasmic vacuolization of the smooth muscle cells of the tunica media. Group 5 exhibited a normal arterial appearance except for fibrosis. Femoral vein changes were characterized by smooth muscle cell degeneration and cytoplasmic vacuolization in the first three groups, and by irregularities in elastic fibres and fibrosis in the remaining two. In group 5, the diameter of the femoral vein decreased by 27.9%. No histologic changes were noted in nerve morphology. In group 2, perineurium thickness and the diameter of myelinated nerve fibers notably increased by 164.6% and 58.4%, respectively.

Conclusion: Our results show that the effect of femoral lengthening is heavily on blood vessels rather than nerves.

Key words: Arteries; blood vessels; bone lengthening/methods; femur/surgery; muscle, skeletal/pathology; neural conduction; peripheral nerves; rats; veins.

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Received: 01.03.2004 **Accepted:** 12.02.2005

Nowadays lengthening procedure is being done frequently whole around the world. So many changes occur at bone, muscle and soft tissue after these procedures. Even though numerous experimental studies have done there is no conception between researchers. While some of them proposes that vascular damage constitutes more than neurological damage the others advocates just the opposite.

In this experimental study we examined the histological changes at femoral arteries veins and nerves which can be significant contribution to clinical applications. We determined these changes at the femur's of rats which we lengthened 10 and 30 % histologically and morphometrically at the end of procedure and at the following days. Also we looked for the course, the patterns of changes.

Material and method

Forty-two Wistar-Albino male rats with an average of 225 ± 25 gr. were used in the study.

Under general anesthesia, specifically designed external fixators (Hipokrat Medical Devices Manufacturing Co., Istanbul, Turkey) were applied to the left femora, and subperiosteal corticotomy was performed. Postoperatively, a single dose of

Ceftriaxone (50mg/kg) was injected by intraperitoneal route. Throughout the experimental period, the rats were free to ambulate in their cases. The rats were randomly divided into six groups (seven rats in each group). The first group control group and no distraction were performed. In the other groups femoral lengthening 0.35mm was performed for 4 times a day starting from first postoperative day. In the second group lengthening was continued for three days (10% lengthening) and the rats were sacrificed at the end the distraction. In groups 3, 4, 5, 6, lengthening was continued for seven days (30% lengthening), and the rats were sacrificed at 7, 14, 21 and days postoperatively and 1.5-2 cm biopsy specimens were taken from femoral arteria, vein and nerve bundle.

Table 1: Lengthening groups

	n	distraction rate	time (day)	sacrification (day)
Control	7	–	–	7
Group 1	7	0.35 mm x 4 (%10)	3	3
Group 2	7	0.35 mm x 4 (%30)	7	7
Group 3	7	0.35 mm x 4 (%30)	7	14
Group 4	7	0.35 mm x 4 (%30)	7	21
Group 5	7	0.35 mm x 4 (%30)	7	31

After taking serial sections from the femoral neurovascular bundle we made three different preparates from each. While staining one of the samples with hematoxylin-eosine (H-E),the others were stained with Verhoeff and Mason special stains. Femoral nerve blocks also stained with Kluver-Barrera luxol fast blue. After than samples were examined with light microscope (Olympus CH2) two times.

For the first time histopathological changes were noted between control and the other groups which applied femoral lengthening procedure. Then by mounting a special lens to the same microscope we measured thickness of tunica intima and tunica media and the thickness of whole wall for femoral arteria and vein and thickness of perineurium and mean radius of nerve fibers of myelinized nerve fibers of femoral nerve.

Results

Femoral arterial alterations:

Control group: Mean arterial wall thickness was measured 113.7 micron. Mean tunica media thickness was52.5 micron and formed with loose elastic and thin collagen fibrils which were dispersed between smooth muscle cells. Tunica media was separated from intima by lamina elastica (fig 1a). Thickness of tunica intima was 18.2 micron. Tunica adventisia was made up of fibro connective tissue (Table 2).

Group 1: A significant thinning recognized at the intima layer of arteria. Mean thickness was measured as 11.6 micron. Thickness tunica media which were formed of smooth muscle cells with vacuolizated cytoplasms increased insignificantly (mean tunica media thickness was 55.2 micron). Flattening and focal losses was recognized at internal layer of lamina elastica (fig 1b). The thickness of whole arterial wall was increased (tb.2)

Group 2: Reducing at the thickness of intima layer of arteria persisted (11.4 micron). There was reduction also at the thickness of tunica media where

Table 2: Histomorphometric measurements in femoral nerves, veins and arters (micron)

	Arter			Vein	Nerves	
	T. Int.	T. Med.	Art.	T.Int + T.Med.	T. Perin.	D.Mye.
Control	18.2	52.5	113.7	21.8	9.6	8.1
Group 1	11.6	55.2	128.3	21.9	8.0	6.7
Group 2	11.4	48.9	96.4	27.1	25.3	12.8
Group 3	9.3	55.4	114.3	31.4	8.9	7.9
Group 4	10.6	57.0	118.8	18.3	6.0	8.1
Group 5	12.0	59.8	121.3	15.7	10.0	6.7

T. Int.: Thickness of intima, T.Med.: Thickness of media
Art.: Total arterial wall thickness
T.Perin.: Thickness of perineurium
D.Mye.: Diameter of myelinizated nevre fibres

cytoplasm vacuolization exits (mean 48.9 micron). There was fragmentation and confusion at external and internal layers of lamina elastica. The reduction at the thickness of the entire arteriel wall has been determined (tbl 2).

Group 3: The slimming of the tunica intima is more pronounced (mean thickness 9.3). In lamina elastica interna and externa is flattened and also regional focal losses observed. Smooth muscle cells have continued to have cytoplasmic vacuolization; mean tunica media thickness found to be 55.4 micron. The increase in tunica media thickness has not reach the levels of control and group 1 levels. All the arterial wall thicknesses increased (mean 114.3 um) (table 2)

Group 4: The tunica intima layer's thickness (mean 10.6 um) was thinner than the control group, but it was increased compared to group 3. Both in two examples, arterial lumen in thromboses' organization and re-canalization is attention catching. Lamina elastica interna has regionally flattened and breakings. Tunica media mean thickness was 57 um

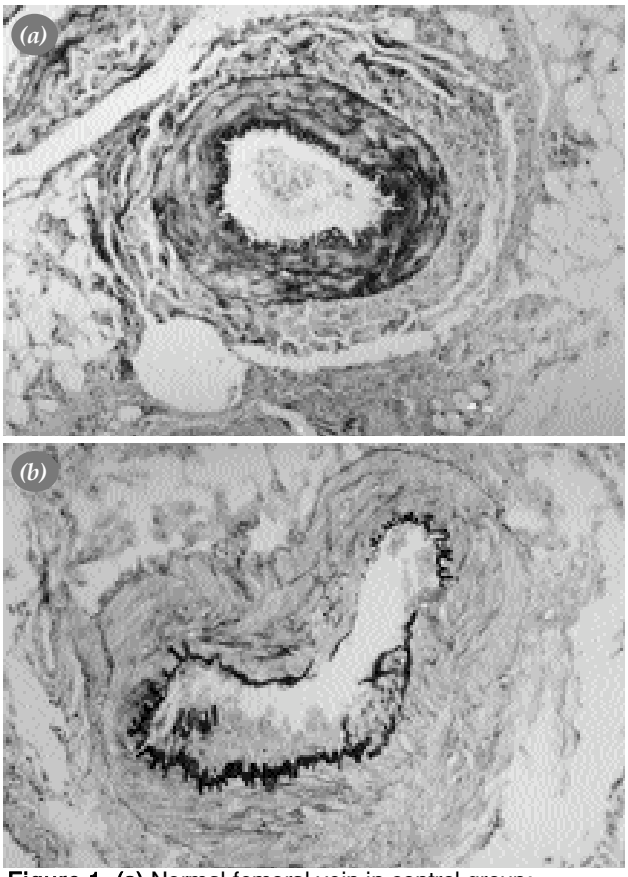

Figure 1. (a) Normal femoral vein in control group; **(b)** Flattening and focal losses at lamina elastica interna in group 1(Verhoeff x 10)

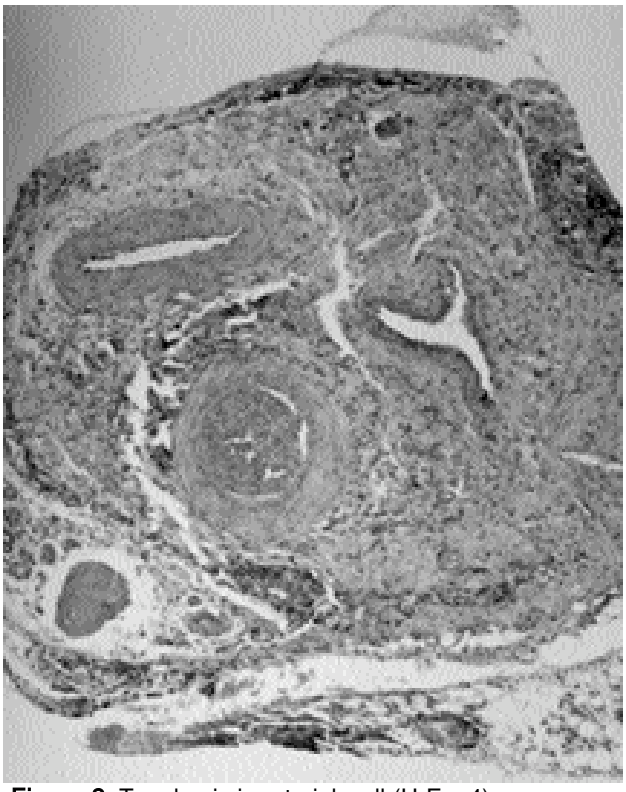

Figure 2. Trombosis in arterial wall (H-E x 4)

and smooth muscle hydropic degeneration and vacuolization continued. All artery wall thickness mean found to be 118.8 um found (table 2). Around connective tissue has increased inflammation and fibrosis observed. The continued tunica adventitia borders could not be determined.

Group 5: The increment of tunica intima (mean 12.0 micron) and tunica media (mean 59.8 micron) has been determined. In one of the samples thrombosis has been determined in the arterial lumen (Fig 2). The whole arterial wall thickness (121.3 micron) has been increased paralel to the growing of tunica intima and media tbl.2 .

Femoral vein alterations

Control group: Tunica intima and media has been measured as mean 21.8 micron. Adventitia layer has been constructed of fibrose bands with elastic fibrils. There was a thin elastic layer between tunica media and intima fig.3a)

Lengthening group: Degeneration and citoplasmic vacuolization of smooth muscle cells seen at groups 1,2 and 3. Irregularities at elastic fibrils, fibrosis at peripheric connective tissue, and lengthening and fissures at veins has been determined at groups 4 and 5 (fig 3b). As the thickness of tunica intima and media in group 1,2and 3 increases however they decreases in group 4and 5 morphometrically (tbl 2).

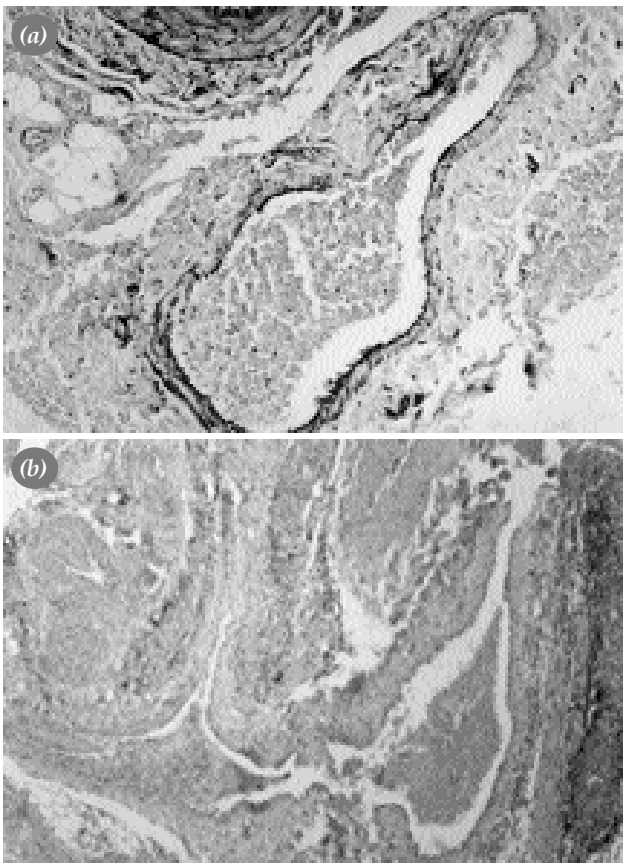

Figure 3. (a) Normal femoral vein in control group (Verhoeff x 10); **(b)** Fissures at veins in group 4 (H-E x 4)

Femoral nerve alterations

At lengthening groups we haven't seen any histological alterations; normal nerve histology has been seen (fig. 4). But in group 2 thickness of perineurium increased mean 164.6% and the thickness of myelinized nerve increased mean 58.4% (fig 2).

Discussion

Although there are so many clinical and experimental researches about bone and soft tissue alterations after lengthening procedures in the literature, there isn't any conception between them.[1-10] In this study the patterns of histological and histomorphometric alterations which happened after 10 and 30 % lengthening have been evaluated. In all of the lengthening groups similar findings has been seen with histopathologic evaluations of femoral arteries. The first findings of lengthening procedure were flattening of lamina elastica intima regional missing. Flattening and fragmentation has been seen at elastica externa layer of 30% lengthening group additionally. As the intensity of distraction increases the percentage of fragmentation at elastic fibrils also increases. But we haven't seen any degenerative alterations in the layers of tunica intima, lamina elastica interna and externa except fibrosis at the rats which we have followed 31 days. The results of our study supports the results of İppolito and et al. who sad that they haven't seen any alterations after 2

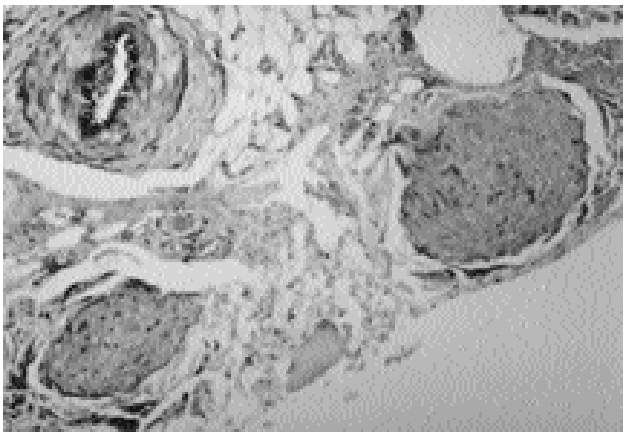

Figure 4. Normal femoral nerve in control group (Verhoeff x 10)

month at the palmar arteries of the calf which they have lengthened 33 %.[2] Gil-Albarova and et al[1] reported that they haven't seen any histological difference at proximal femoral arteria after lengthening procedure.

Although in our study; all groups other than fifth group which proceeded 31 days, we observed that hydrophic degeneration and cytoplasmic vacuolization of the smooth muscle cells of tunica media of femoral artery. Furthermore, there were seen cellular infiltration in both tunica adventitia and peripheral fibro-collagenous tissue from the beginning. In the course of time, increased peripheral collagenous tissue and fibrosis encircles the artery like rubber leather. Even though clinical experiences were not reported ischemic failure frequently, vascular degeneration in fibro-collagenous tissue occurs after all.

Morphometrically, the most affected layer of femoral artery wall is tunica intima beginning from %10 lengthening group. The thickness of tunica intima decreases at the most in %30 lengthening, the second group and increases in other groups gradually. Even femoral artery wall thickness decreases in the second group maximally, thereafter starts to increase to become control group level at the following lengthening days (group 3) and increases later (group 4-5).

In femoral vein analysis, disturbance of elastic fibers, focal loss of tunica intima cells, absolute swelling of smooth muscle cell nucleuses, degeneration and widespread cytoplasmic vacuolization were seen in all lengthening groups excluding group 5.These findings are according to distraction. Ippolito et al. enounced that normal palmar vein in %8 lengthening, %70 thinning of tunica media in %20 lengthening but vein was becoming original structure and thickness in two months after lengthening procedure finished. Gil-Albarova et al. noticed that thrombosis phenomenon and endothelial loss areas were seen in lengthened posterior femoral vein which became normal after distraction stopped but in some experimental animal's endothelial disturbances still established in small areas.

In femoral vein histomorphometrical analysis, thickness of intima and media layers gradually increases as against control group in first week in group 1,2,3 but decreases suddenly in group 4,5. Femoral vein thickness of fifth group decreased about %27.9 compared with control group. The pronounced decrement of diameter was toughed to related with excessive fibrosis of tunica adventitia and peripheral fibro-collagenous tissue becoming in advanced lengthening periods. Our study reveals different results as against Ippolito et al. We don't think that decrement or totally disappearance of fibrosis once it's formed. After limb lengthening procedures, persistence of edema and cyanotic appearance of distal part of lengthened limb and regression of these signs with elevation may be related with progression of the venous incompliance according to structural changes.

We couldn't get any significant different results from histologic researches of nerves.

We measured the thickness of perineurium and diameter of myelinized nerve fiber for histomorphometric investigation of femoral nerve. We determined the only significant results at the rats which we have lengthened the femur 30%. In this group thickness of perineurium

Have increased 164.6% and diameter of nerve fiber increased 58.4%. Beside these there were similarities between the results of other groups and the control ones. Gil- Abarova et al. reported that they haven't seen any histological differences at researches of sciatic nerve of similar lengthening procedures. But Ippolito et al reported that peripheric nerves injured more than blood vessels.

They had seen changes at myelinized neuron fibers even at 8% lengthened extremities but they returned normal structure quickly after ceasing the procedure. It has been reported that iskemia of neurons developed at 15% lengthened extremities but histologic changes formed after stretching 4-50% of original length.[10-12] Wall and at al [8-10] reported that no histological changes formed after applying 6-12% of stretching force to tibial nerve of 24 rabbits. The mechanism of stretch injury of nerves has not been understood clearly. The development of intraneuronal arterial occlusion after 15% of lengthening procedure has been reported by two separate research group.[11-12]

Nevertheless Ippolito and et al[2] showed that capillaries of epineurium and perineurium mostly nor-

mal with results of electron microscopic studies, and they said that they didn't accept the idea being the etiologic result of isquemia was the stretch of vaso nervosum.

As a result we haven't seen any significant histologic and morphometric changes at femoral nerve after lengthening procedure. Contrary to this we determined that vessels been effected more than nerves after this procedure. Histologically we determined that vessels have returned to normal structure except fibrosis ,but morphologically 6.5% increase at femoral arterial diameter and 28% decrease at femoral vein.

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