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Tourniquet-induced neuromuscular injury

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The clinical problem

The pneumatic tourniquet was introduced by Harvey Cushing (1904) as an adjunct for extremity surgery (Klenerman 1962). Severe neurologic injury may be caused by tourniquet application (Eckhoff 1931, Moldaver 1954, Bolton and McFarlane 1978, Aho et al. 1983, Rorabeck and Kennedy 1980, Larsen and Hommelgaard 1987, Crandall and Weeks 1988, Lundborg 1989, Kurihara and Goto 1990). The incidence of severe nerve palsy following tourniquet application was 0.13% and 0.01% in retrospective studies by Flatt (1972) and Middleton and Varian (1974), respectively. Histologic changes following skeletal muscle ischemia have been described by many authors (Table I).

However, other forms of neuromuscular injury may be caused by routine tourniquet use (Love 1978). The "post-tourniquet syndrome" (Bruner 1951) is comprised of weakness, stiffness, edema, dysaesthesia, and pain following operation in a bloodless field. Clinicians may attribute such symptoms and signs to surgical trauma or to poor patient motivation if they do not suspect injury related to tourniquet application (Love 1978).

Electromyographic (EMG) abnormalities occurred in 72% (Weingarden et al. 1979) and 63% (Saunders et al. 1979) of patients following routine surgery using pneumatic tourniquets. Subsequent randomized, prospective studies demonstrated EMG abnormalities in 70% of lower extremity surgeries (Dobner and Nitz 1982) and in 77% of upper extremity surgeries (Nitz and Dobner 1989) performed with a tourniquet, compared to incidences of 0.0% and 3.4% following similar cases performed without a tourniquet. Such abnormalities are associated with decreased post-operative function, and longer clinical recovery time (Weingarden et al. 1979, Saunders et al. 1979, Krebs 1989). EMG changes persist up to six months after tourniquet application (Weingarden et al. 1979, Saunders et al. 1979, Dobner and Nitz 1982, Nitz and Dobner 1989). Elevation of serum myoglobin (Jorgensen 1987, Laurence and Norris 1988) and rhabdomyolysis (Shenton et al. 1990) may be associated with tourniquet application in humans. Sherman et al. (1986), found that a tourniquet time greater than sixty minutes correlated with post-operative complications after knee arthroscopy.

The "safe" duration of tourniquet ischemia is controversial, and time limits of one hour (Bruner 1951, Bruner 1970, Chiu et al. 1976, Sanders 1973), one and a quarter hours (Rorabeck 1980), one and a

Table 1. Previous reports on histologic changes following skeletal muscle ischemia

Ischemic duration (hours)	Interval to biopsy	Observations	
0.5	0–6 hours	Inflammatory reaction, mild edema, minimal degeneration	Dahlbäck and Rais 1966
0.5	2 hours	Mast cell degranulation, areas of no-reflow	Strock and Majno 1969b
1	–24 hours	Granular degeneration	Heppenstall et al. 1979
1	–5 days	Edema, fiber degeneration	Sjöström et al. 1982
1.5	10 min	Endothelial swelling and ultrastructural degeneration	Gidlöf et al. 1988
2	4 hours	Dilatation of sarcoplasmic reticulum and T tubules	Harris et al. 1986
2	–21 days	Acute inflammation, edema	Sanderson et al. 1975
2	–226 days	Mitochondrial swelling, glycogen depletion	Mäkitie and Teräväinen 1977
2.5	0.1–5 hours	Generalized no-reflow, scattered necrosis	Strock and Majno 1969b
2.5	1–21 days	Fiber degeneration, inflammation	Jennische 1986
3	16–18 hours	Mitochondrial swelling and internal disorganization	Heppenstall et al. 1986
3	1–7 days	Mitochondrial degeneration	Patterson and Klenerman 1979
3	7 days	Mitochondrial swelling, dilatation of sarcoplasmic reticulum	Tountas and Bergmann 1977
3	–226 days	Capillary degeneration, motor end-plate degeneration	Mäkitie 1977, Mäkitie and Teräväinen 1977
4	4 hours	Mitochondrial swelling and degeneration, calcium deposition	Labbe et al. 1988
4	5 hours	Calcification, no-reflow	Jennische and Hansson 1986
4	–30 days	Necrosis	Harman 1948
6	4 hours	Myofibrillar edema, mitochondrial swelling, sarcolemmal disruption	Blebea et al. 1987

half hours (Heppenstall et al. 1979, Sapega et al. 1985), two hours (Wilgis 1971), and three hours (Enger 1977, Klenerman 1980, Klenerman et al. 1982, Patterson and Klenerman 1979, Patterson et al. 1981, Tountas and Bergman 1977) have been suggested. Reperfusion (ie., rebreathing) intervals are recommended to extend the duration of tourniquet hemostasis (Bruner 1951, Bunnell 1956, Wilgis 1971, Chiu et al. 1976, Heppenstall et al. 1979, Nakahara 1984, Newman 1984, Sapega et al. 1985). Such recommendations are generally based upon studies of ischemic muscle injury distal to a tourniquet. To date, there are no experimental studies of effects of reperfusion intervals upon muscle injury beneath the pneumatic tourniquet.

Nerve injury tends to be greater beneath the tourniquet than distal to it, due to the combined effects of ischemia and direct mechanical deformation (Denny-Brown and Brenner 1944, Moldaver 1954, Lundborg 1970, Fowler et al. 1972, Ochoa et al. 1972, Rudge 1974, Bolton and McFarlane 1978, Rorabeck 1980, Rorabeck and Kennedy 1980, Hurst et al. 1981, Yates et al. 1981, Nitz 1982, Nitz et al. 1989). Relatively little information is available regarding critical pressure and time limits for muscle injury induced by pneumatic tourniquet compression (Patterson and Klenerman 1979, Patterson et al. 1981, Gersoff et al. 1989).

Various authors recommend use of lower tourniquet inflation pressures. Cuff pressure may be based upon the patient's systolic blood pressure (Klenerman and Hulands 1979, Klenerman 1980, McLaren and Rorabeck 1985), limb circumference (Van Roeckel and Thurston 1985, Moore et al. 1987, Crenshaw et al. 1988), or by direct measurement of the minimum necessary inflation pressure to occlude distal pulsatile flow (Reid et al. 1983). Wider cuffs decrease the minimum necessary inflation pressure compared to the narrower tourniquets (Muirhead and Newman 1987, Hargens et al. 1987, Moore et al. 1987, Crenshaw et al. 1988). A double tourniquet technique, with continuous ischemia induced by alternate inflation of adjacent cuffs at hourly intervals, may decrease tourniquet compression injury (Neimkin and Smith 1983, Dreyfuss and Smith 1988). Extremity cooling may decrease metabolic demand during prolonged tourniquet hemostasis (Paletta et al. 1960, Bruner 1970, Nakahara 1984, Ikemoto et al. 1988).

AIMS OF THE INVESTIGATION

The experiments reviewed here were designed to study, in the rabbit, effects of tourniquet pressure and duration on neuromuscular morphology, metabolism, and function. To this end a tourniquet model was designed to permit studies of tissues beneath and distal to a cuff inflated continuously or with reperfusion intervals. With this background, clinical experiments were performed in volunteers and in patients undergoing limb surgery. Cuffs of different shapes and widths were studied with respect to effects on arterial occlusion pressure.

Rabbit experiments

Materials and general methods

New Zealand White rabbits (n = 170), unselected for sex, weighing 2.4 to 4.2 kg were used. Study protocols were approved by the Animal Use Committee of the Veterans Administration Medical Center, San Diego. Animals were anesthetized prior to and during all experimental procedures, including shaving, tourniquet compression, intravenous injection, systemic circulation periods, and neurophysiologic testing. Anesthesia consisted of subcutaneous injection of ketamine (50 mg/kg body weight [bw]), rompum (5 mg/kg bw), and acepromazine (1 mg/kg bw), with supplemental cocktail given as needed. Both hindlimbs were shaved prior to tourniquet application in all studies. Animals were killed by an overdose of intravenous barbiturate.

The tourniquet model

Optimal cuff fit was achieved with a curved tourniquet (ie., banana-shaped, radius of curvature 19 cm) based upon a plaster of paris replica of a rabbit hindlimb. Straight (ie., standard) and curved tourniquets were heat-pressed from pre-fabricated cuffs (Pedisphyg, Upper Montclair, New Jersey, USA). Durable vinyl cuffs (Zimmer Aspen Labs, Aspen, Colorado, USA) were used in later studies. To minimize transverse deformation during cuff inflation, a stiff, two piece shell was designed to fit around the tourniquet without causing direct limb compression. The posterior half of the shell fit inside the anterior half, with overlap of the two surfaces, and the pieces were stabilized with tape. This shell also limited distal displacement during cuff inflation (Figure 1).

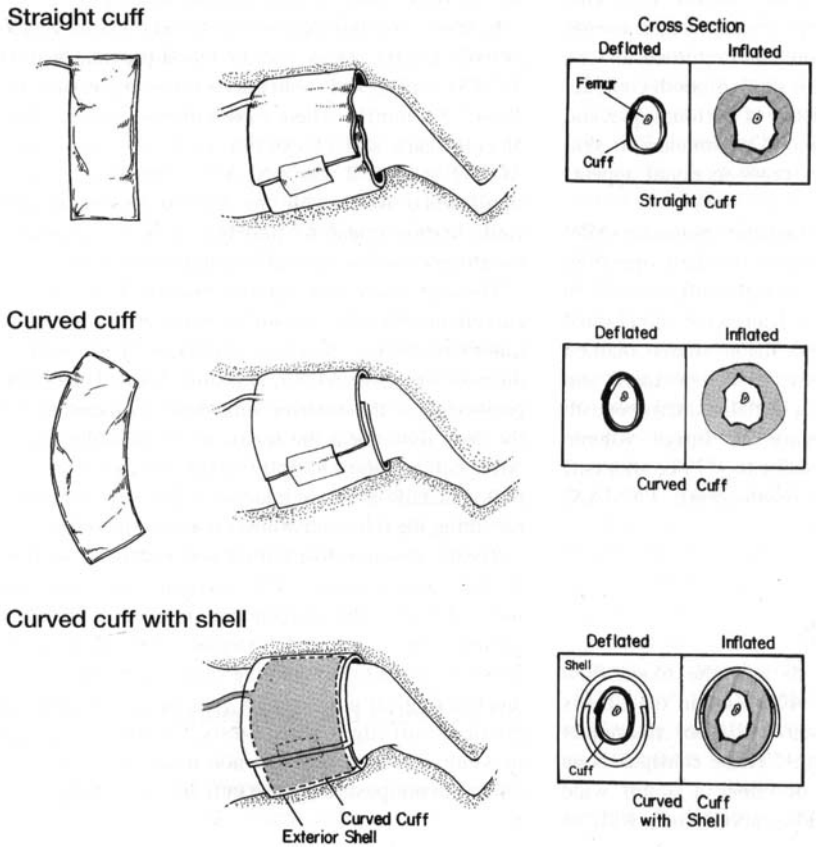
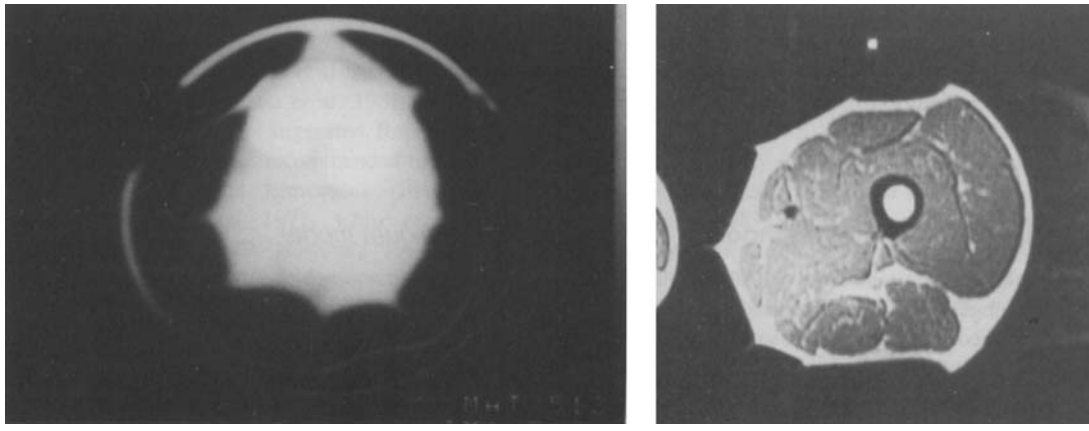


Figure 1. Tourniquet cuffs *used on the rabbit thigh.* [Reproduced with permission, Pedowitz et al. 1990].



A. CT image of a rabbit [Reproduced with permission, Pedowitz et al. 1990]. B. MR image of a human subject.

Figure 2. The thigh during tourniquet inflation.

Transverse, computerized tomographic (CT) images were made prior to and during curved cuff inflation to 300 mmHg, with and without the stiff exterior shell. Prior to tourniquet inflation, the ratio of the maximum antero-posterior (AP) to medial-lateral (ML) dimensions under the mid-position of the cuff was 1.64. This ratio decreased to 1.17 (a more circular shape) during inflation of a curved cuff alone, and returned to 1.61 after addition of a stiff exterior shell. Smooth contours were not observed at the interface of the tourniquet and the skin during cuff inflation; asymmetrical skin ridging produced a "stellate" cross-sectional appearance (Figure 2).

For correlative purposes, magnetic resonance (MR) imaging was performed using a standard operating room tourniquet (8 cm wide, straight cuff) inflated to 200 mmHg on a human thigh. Transverse and coronal MR images of a human thigh demonstrated marked tissue deformation, particularly at the proximal and distal edges of the cuff, with a stellate pattern beneath the inflated tourniquet (Figure 2). Image volume decreased from 1472 cc at baseline to 1179 cc after cuff inflation (3-dimensional reconstructions, CEMAX, San Mateo, California, USA).

Pressure measurements

The cuff pressure required to stop the blood flow (arterial occlusion pressure, AOP) within the tibialis posterior artery was assessed with four tourniquet configurations on rabbit thighs. These configurations consisted of combinations of either a 3 cm wide straight cuff or a 3 cm wide curved cuff, with or without a stiff exterior shell. The cuff was slowly inflated while recording simultaneously tourniquet inflation pressure and arterial pressure on a chart recorder. The AOP was defined as the minimum tourniquet inflation pressure which eliminated pulsatile arterial flow (mean of three measurements).

In seven rabbits, mean arterial blood pressure was 55 (46–62) mmHg, mean systolic blood pressure was 76 (62–88) mmHg, and mean diastolic blood pressure was 49 (42–57) mmHg. There were substantial effects both of cuff shape and of exterior shell application upon AOP ($P < 0.05$, 2-way ANOVA, interaction term not significant). AOP with the curved tourniquet/shell configuration (mean 67 mmHg) was lower than with the straight cuff/no shell configuration ($P = 0.002$).

Tissue pressure was recorded beneath the 3 cm wide curved tourniquet with a stiff exterior shell. Calibrated, transducer-tipped pressure catheters 1.37 mm in diameter (PC-340, Millar, Houston, Texas, USA) were positioned in the anterior subcutaneous tissue and in the deep tissue near the sciatic nerve of rabbit thighs. After cuff inflation to 350 mmHg, the catheters were pulled distally at 5 mm intervals while simultaneously recording the tissue pressures on a chart recorder.

Tissue pressure distribution was relatively uniform in the sagittal plane, and maximum pressure was induced under the longitudinal mid-position of the curved tourniquet and exterior shell (Figure 3). Pressures were lower near the sciatic nerve than in the anterior thigh at positions 15, 20, and 25 mm from the proximal cuff edge (paired t-tests, $P < 0.05$). The mean pressure recorded with the non-infusion slit catheter under the midposition of the cuff was 80 mmHg.

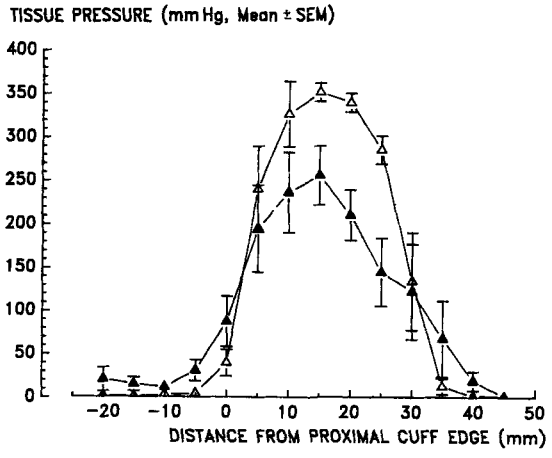


Figure 3. Tissue pressure distribution beneath the tourniquet in rabbits (proximal thigh at -30, distal leg at +50 on x axis). [Reproduced with permission, Pedowitz et al. 1990]

Tourniquet application protocol

Rabbits were supine with hips, knees, and ankles in neutral positions. The feet were restrained gently in clamps, and a curved tourniquet and a stiff exterior shell were positioned on the thigh and secured with tape. Venous exsanguination by limb elevation was allowed for at least five minutes prior to cuff inflation. Elastic wraps were not used due to the risk of uncontrolled tissue trauma.

Tourniquets were inflated using either a manual cuff inflator (Automatic low pressure tourniquet, Stille-Werner, Stockholm, Sweden) or a digital tourniquet inflator (ATS 1000, Zimmer Aspen Labs, Aspen, CO, USA). Compression at 1000 mmHg was controlled manually using a calibrated, compressed air source. Cuff inflation was completed in one to five seconds.

Cuff inflation at 125 mmHg (2- or 3-cm curved cuffs with shell), induced surgical hemostasis, defined as lack of punctate bleeding with skin incision at the ankle (pilot studies). Arterial occlusion was reflected by absence of palpable pulsation and coolness of the distal extremity. Distal pulsation was palpated within two minutes after tourniquet deflation in all cases. Animals were returned to routine cage activity after recovery from anesthesia.

Effects of cuff pressure and duration on muscle

Protocol: 63 rabbits were assigned to one of nine compression protocols (3-cm cuff), defined by a combination of one of three cuff inflation pressures (125, 200, or 350 mmHg) and one of three tourniquet times (1, 2, or 4 hours). Approximately forty-five hours after tourniquet application, animals were injected intravenously with a combination of 2.0 cc of ^{99m}technetium pyrophosphate (Tc-99) solution (1.0 millicurie/1 cc normal saline) and 4.0 cc of Evans blue solution (1 gm/100 cc normal saline). Rabbits were suspended in slings for three hours to ensure uniform distribution of the injectate and then killed.

Muscle samples (0.2 to 1.2 gm) were taken from the medial thigh (semimembranosus/semitendinosus, 3 samples), lateral thigh (biceps femoris, 3 samples), and anterior leg (tibialis anterior/extensor digitorum, 4 samples) of both limbs. Separate muscle samples were fixed in formaldehyde, embedded in paraffin, cut into ten micron cross sections, and stained with hematoxylin and eosin. Thigh muscle samples were taken from the mid-position of the zone of tourniquet compression, and leg muscle samples were distal to this zone. Samples were weighed, and gamma radiation for each muscle sample was counted. The percent injected dose of Tc-99 per gram of tissue sample (Tc-99 uptake) for each sample was determined and mean Tc-99 uptake for the thigh and leg regions was calculated (regional uptake). Regional uptake in the experimental limb was divided by regional uptake in the control limb (uptake ratio).

Data transformation, analysis, and presentation were performed according to recommendations for ratio type data (Sokal and Rohlf 1981). One-way *t*-tests were used to assess increased regional uptake compared to control. The Bonferroni approximation adjusted the critical p value when a priori multiple *t*-tests (Dixon 1988) were performed after analysis of variance (ANOVA). Statistical significance was considered when $P < 0.05$.

Observations: Evans blue discoloration localized the region of tourniquet application in thigh muscles after two and four hours of continuous compression, but extravasation was not observed after one hour of compression. Discoloration was not uniform within the thigh in some limbs following four hours of compression. Cross section of these muscles demonstrated a central whitish area surrounded by an intensely blue rim of tissue. In contrast, minimal blue discoloration was noted in ischemic leg muscles of any experimental group, and extravasation was not observed in control limbs.

The Tc-99 uptake ratios were increased in the thigh and leg regions of all experimental groups. There were no differences in Tc-99 uptake between the thigh and leg regions following one hour of tourniquet application, or after two hours of 125 mmHg tourniquet

Table 2. Mean Tc-99 uptake ratios after continuous tourniquet application in rabbits (n = 7 per group)

	Tourniquet pressure (mmHg)					
	125		200		350	
Duration	Thigh	Leg	Thigh	Leg	Thigh	Leg
One hour						
Mean	1.6	1.3	1.5	1.3	1.6	1.6
95% upper CI[a]	1.9	1.8	2.0	1.5	2.8	1.8
95% lower CI	1.3	1.1	1.3	1.2	1.2	1.4
Two hours						
Mean	1.8	1.8	5.3*	2.0	8.7**	1.4
95% upper CI	2.7	2.5	27.4	2.9	83.1	1.9
95% lower CI	1.4	1.4	2.4	1.5	3.3	1.1
Four hours						
Mean	30.2**	1.4	62.3**	2.8	42.0**	1.7
95% upper CI	38.2	2.1	100.0	8.5	70.7	2.3
95% lower CI	24.5	1.1	42.6	1.5	27.8	1.4

[a]CI confidence interval
*$p < 0.05$, **$p < 0.01$, paired *t*-tests, thigh versus leg.

compression. Thigh uptake was greater than leg uptake following two hours of continuous compression with either 200 or 350 mmHg cuff inflation pressure (paired *t*-tests). Regional uptake was greater in thighs than in legs of all groups subjected to four hours of continuous tourniquet inflation (Table 2). Tc-99 uptake in the thigh region was effected both by tourniquet time and cuff inflation pressure, with interaction of the variables (2-way ANOVA). Tc-99 uptake in the leg region was effected by tourniquet time, but was not effected by cuff inflation pressure (Table 2).

Histologic abnormalities were more severe in compressed than in ischemic muscles. Few pathologic findings were observed in either thigh or leg muscles following one hour of tourniquet application. Relatively few abnormalities were observed in thigh muscles subjected to a two hour, 125 mmHg tourniquet, compared to the marked changes caused by a two hour, 350 mmHg tourniquet (p. 8).

In thigh muscles compressed for four hours, common findings were altered fiber staining intensity, fiber size variation, increased extracellular space, fiber splitting, hyaline degeneration, cellular infiltration, and focal and regional necrosis. A consistent finding was that necrosis predominated in the central portion of the fascicles. Some thigh muscles had a general loss of fascicular architecture associated with an intense inflammatory response. There was striking variability of focal and regional necrosis within the same muscle and within a given fascicle, with necrotic fibers adjacent to fibers of generally normal appearance (Figure 4).

Leg muscles had a different injury pattern following four hours of tourniquet ischemia. Scattered focal fiber necrosis with local cellular infiltration was observed in some of these specimens; regional necrosis was rarely observed. The most common findings were variable fiber staining intensity, splitted or pycnotic fibers, internal nuclei, and a widened interfascicular space with increased numbers of inflammatory cells (Figure 5). In some leg muscles subjected to four hours of ischemia, few histologic abnormalities were observed.

Effects of reperfusion intervals

Protocol: Fifty-eight rabbits were subjected to a total of either two or four hours of compression with a 2 cm wide cuff. In the four-hour studies, the rabbits were assigned to either 125 or 350 mmHg pressure, with (A) tourniquets inflated continuously, (B) a ten minute reperfusion interval after two hours, or (C) ten minute

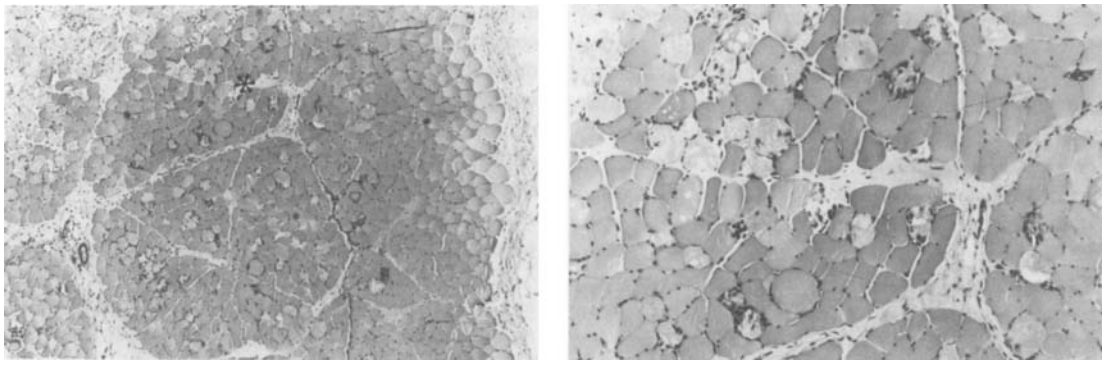


Figure 4. Rabbit thigh muscle after 4 hours of continuous, 350 mmHg cuff pressure. Asterisk in (A) indicates enlarged area (B). Magnification 4x and 10x. [Reproduced with permission, Pedowitz et al. 1991a]

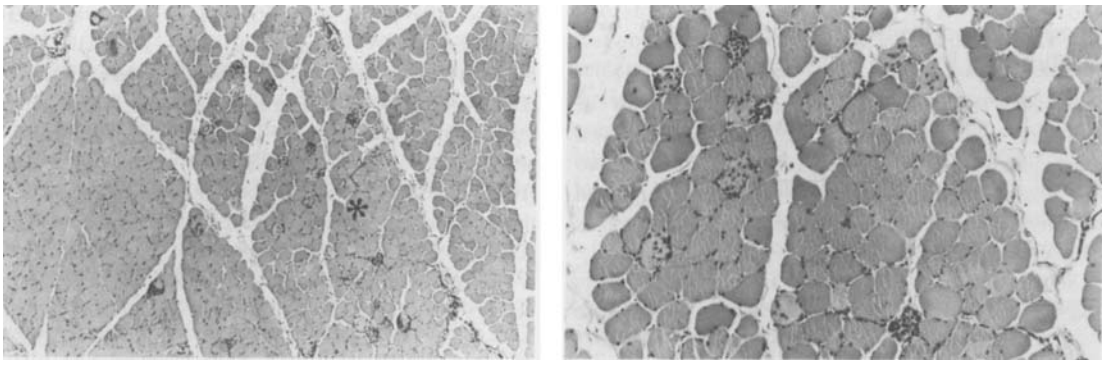


Figure 5. An extreme example of ischemic rabbit leg muscle following four hours of continuous ischemia induced by 350 mmHg tourniquet. Asterisk in (A) indicates enlarged area (B). Magnification 4x and 10x. [Reproduced with permission, Pedowitz et al. 1991a]

reperfusion intervals after each hour of cuff inflation. In the two-hour studies, 350 mmHg cuff pressure was applied with (D) tourniquets inflated continuously, or (E) a ten minute reperfusion interval after one hour. Tc-99 uptake was assessed 2 days after tourniquet application.

Preliminary analysis demonstrated variability (ie., bimodal distribution) of Tc-99 uptake ratios in the thigh region of the four hour, 125 mmHg, one reperfusion interval group. A similar phenomenon, with nearly identical mean Tc-99 uptake, was observed when this group was repeated. All twelve animals were included in the study because these data suggested a physiologic threshold effect, ie., either mild or severe injury with the same compression protocol.

Observations: Regional uptake ratios were increased in the thigh and leg of all experimental groups (Table 3). Regional uptake was greater in the thigh than in the leg of all groups following a total of four hours of tourniquet inflation. Continuous cuff inflation for two hours at 350 mmHg induced greater Tc-99 uptake in the thigh than in the leg. There was no difference between thigh and leg uptake when a ten minute reperfusion interval was allowed during two hours of cuff inflation (Table 4).

Regional uptake in the thigh was effected by cuff pressure and by reperfusion protocol, with interaction of the variables. In these studies, uptake in the leg was effected by proximal tourniquet pressure, but not by reperfusion protocol. With a two hour tourniquet duration, there were differences in uptake between the two reperfusion protocol groups in both the thigh and leg regions.

Table 3. Mean Tc-99 uptake ratios after four hours tourniquet application in rabbits, with and without ten minutes reperfusion intervals

	Continuous compression		Reperfusion after 2 h		Reperfusion after each hour	
Pressure	Thigh	Leg	Thigh	Leg	Thigh	Leg
125 mmHg						
Mean	40.0	1.5	6.6[b]	1.4[b]	3.9	1.5
95% upper CI[a]	63.3	2.4	16.7	1.7	6.4	1.7
95% lower CI	27.5	1.2	3.7	1.2	2.7	1.3
350 mmHg						
Mean	40.0	2.7	72.2	3.4	17.6	1.6
95% upper CI	63.3	10.8	91.0	11.7	55.1	5.0
95% lower CI	24.9	1.4	58.6	1.8	8.7	1.1

[a]CI confidence interval
[b]n = 12 in this group; n = 6 in all other groups

Table 4. Mean Tc-99 uptake ratios after two hours tourniquet application in rabbits, with and without ten minutes reperfusion intervals

	Continuous compression		Reperfusion after one hour	
Pressure	Thigh	Leg	Thigh	Leg
350 mmHg				
Mean	3.7	1.2	1.7	1.4
95% upper CI[a]	11.7	1.3	2.5	1.6
95% lower CI	2.0	1.1	1.3	1.3

[a]CI confidence interval

Enzyme- and immuno-histochemical analysis of injured muscle

Protocol: Ten animals underwent two hours of unilateral tourniquet application (3 cm wide curved tourniquet with shell) at either 125 or 350 mmHg. Two days later bilateral muscle samples from the thigh and leg were frozen in melting isopentane, and stored at –70 °C.

Serial (7–10μ) sections were stained with alizarin red, for NADH activity, and for myofibrillar ATPase activity after preincubation at pH 10.3, 4.6, and 4.3 (Dubowitz 1985). Specimens with regional fiber necrosis were stained for phosphorylase activity (Dubowitz 1985), and with antibodies against neonatal myosin heavy chain (Butler-Browne and Whalen 1984) and against the intermediate filament vimentin (Lazarides 1982). Antibody binding was visualized by the indirect peroxidase-antiperoxidase technique (Dakopatts, Copenhagen, Denmark).

Areas of regional fiber necrosis (absent ATPase 10.3 activity or intense non- specific neonatal myosin

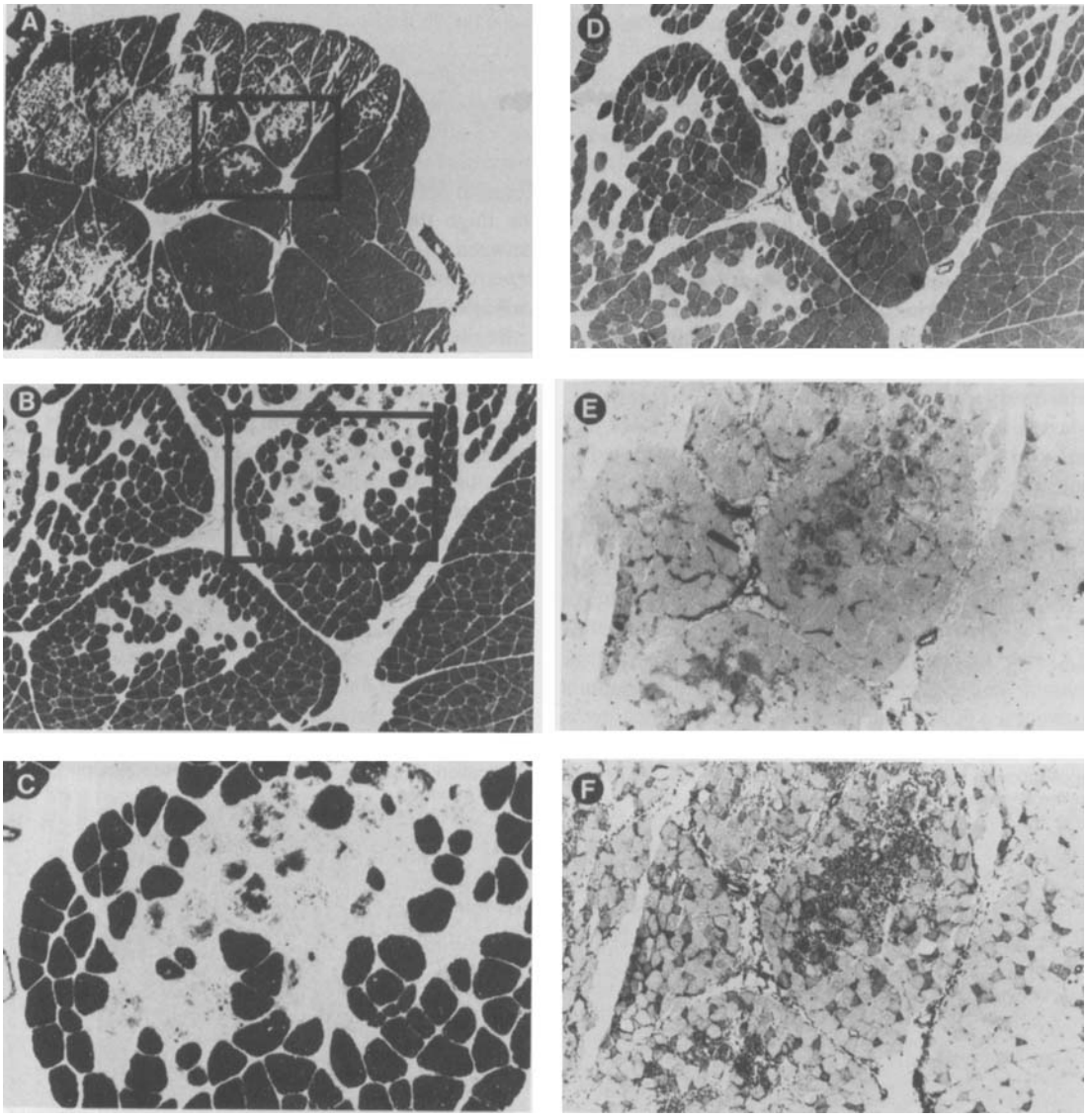


Figure 6. Topographic injury pattern, with necrosis at the center of fascicles or at the intersection of several fascicles, in semimembranosus muscle after two hour, 350 mmHg tourniquet. (A,B,C) ATPase 10.3; (D) ATPase 4.6; (E) ATPase 4.3; (F) NADH stain. Framed areas in (A) and (B) are enlarged areas in (B) and (C), respectively. Magnification: (A) 1x, (B,D-F) 4x, (C) 10x. [Reproduced with permission, Pedowitz et al. 1992a]

staining) were determined using an image analysis system (VIDAS, Kohtron, Eching Munich, Germany), and were expressed as a percentage of the total cross sectional area (CSA). Muscle fibers were typed using a modification of the classification described by Staron and Pette (1986). Quantitative morphometric parameters for approximately 13,000 fibers will be presented separately (Fridén et al. 1991).

Observations: Four of ten thigh muscle samples from the two hour, 350 mmHg tourniquet group demonstrated regional necrosis. Measurements of regional necrosis were similar using the ATPase 10.3 stain and neonatal myosin stain (mean 38 and 37 percent of CSA, respectively). None of the thigh muscles from the two hour, 125 mmHg tourniquet group demonstrated regional necrosis.

A striking topographic pattern of regional necrosis was observed; necrotic areas tended to occur within the center of fascicles or at the intersection of several fascicles. (Figure 6). Relatively normal fibers were noted at the perimeters of these regions, and some adjacent fascicles had a relatively normal appearance. Various degrees of hyaline degeneration and loss of fiber architecture with an intense inflammatory response were observed within the necrotic regions. Cellular infiltration and tissue calcification tended to be uniform from the center to the periphery of these necrotic regions (Figure 7).

Scattered focal fiber necrosis was noted in some thigh muscles without regional necrosis. Mild increases in the intra- and inter-fascicular space were associated with focal and perifascicular cellular

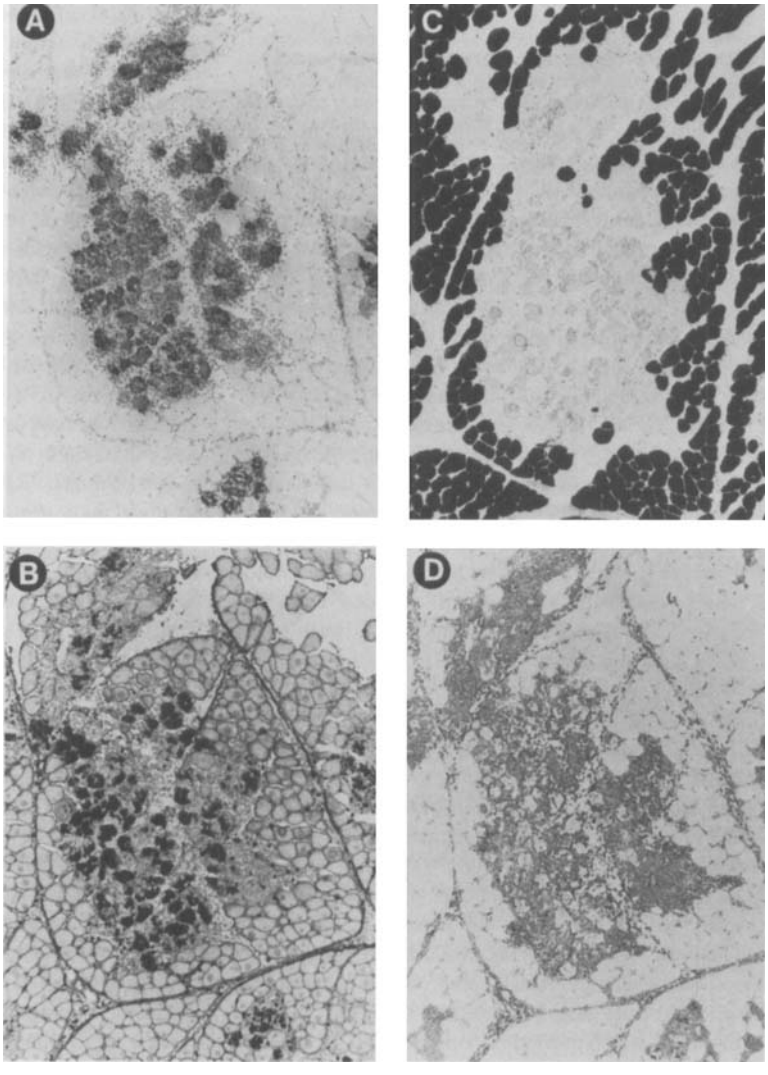


Figure 7. Necrotic region of semimembranosus muscle after two hour, 350 mmHg tourniquet demonstrating (A) tissue calcification (alizarin red) and increased number of nuclei (toluidine blue counterstain), (B) hyaline degeneration associated with non-specific NN5 antibody binding, (C) absence of phosphorylase activity, and (D) an intense inflammatory response associated with specific vimentin antibody binding. Magnification 4x. [Reproduced with permission, Pedowitz et al. 1992a]

infiltration. Very large and rounded fibers, with altered staining intensities and whorled intracellular patterns, were noted in several of the compressed biceps muscles. These swollen fibers were uniformly classified as Type IIB. Minor histologic abnormalities were observed in some thigh muscles after two hours of tourniquet compression, particularly with 125 mmHg pressure.

Regional necrosis was not observed in the leg muscles following two hours of ischemia. These muscles had a slight increase in the number of cells in the perifascicular space, although occasional focal fiber necrosis was observed. There were no obvious differences in the severity of findings between the two tourniquet pressure groups or between the tibialis anterior and soleus muscles.

Pressure effects on nerve function and structure

Protocol: A 3-cm-wide curved tourniquet with shell was inflated for two hours at either 350 or 1000 mmHg on one hindlimb of 24 rabbits. Nerve function was assessed either one hour, one day, or two days after 350 mmHg tourniquet compression, and two days after 1000 mmHg compression. The toe-spreading reflex was evaluated by holding the loose skin of the neck and suddenly lowering the animal toward the ground (Lundborg 1970). The reflex was not assessed one hour after compression due to continuous anesthesia.

Nerve conduction was assessed first in the experimental limb in animals tested one hour after tourniquet application; the testing order was randomized in the other animals. The sciatic and tibial nerves were exposed in continuity using loupes, and the peroneal nerve was divided. Compound motor action potentials (CMAP) were recorded from the abductor hallucis muscle with a Cadwell 5200 electrodiagnostic device (Cadwell Labs, Kennewick, WA, USA) which triggered a Grass SD9 stimulator. Supramaximal stimuli were administered to the nerve surface with a platinum electrode. CMAP amplitudes and latencies were measured using the computerized oscilloscope markers. Nerve conduction velocities (NCV) were determined for segments of sciatic and tibial nerve.

Four specific stimulation sites were chosen with respect to the presumptive region of tourniquet application. The distal thigh stimulation site was identified where the tibial nerve passed between the two heads of the gastrocnemius muscle. The nerve was stimulated at the proximal, middle, and distal cuff zones, and distal to the tourniquet. Two sets of serial CMAP recordings were made with nerve stimulation at approximately 5 mm intervals from the proximal thigh to the distal leg.

CMAP amplitudes at each stimulation site were expressed as a percentage of CMAP amplitude with distal tibial nerve stimulation to allow comparisons between groups (Gilliatt 1980). CMAP amplitude in the control nerves decreased gradually with more proximal stimulation. Rough estimates of CMAP area suggested effects of temporal dispersion with longer conduction distance. Therefore, CMAP amplitude in the experimental limb was expressed as a percentage of the mean normalized CMAP amplitude for the corresponding stimulation site in the control limbs.

The sciatic and tibial nerves were fixed at in-situ length in Karnovsky's solution, and 3 to 6 mm segments were cut from tissue beneath the tourniquet, at the distal cuff zone, and distal to the tourniquet. Non-compressed, sciatic nerve were taken as controls. Specimens were rinsed in cacodylate buffer, post-fixed in 1% osmium tetroxide, dehydrated in ethanol solutions, rinsed with propylene oxide, and infiltrated with Epon. One micron thick sections were stained with Richardsson's solution. Nerve fiber disruption was rated by a single unbiased observer, and was defined as myelin disruption and/or the presence of myelin ovoids. Light microscopic abnormalities were assessed for the entire area of tissue sample, and the degree of damage was rated as either: none; mild—1 to 3 fibers; moderate—up to 25% fibers; or severe—greater than 25% fibers. Ultrathin sections were collected on copper grids, stained with uranyl acetate, and examined in an electron microscope.

Observations: Ratings of the toe-spread reflex were identical in the groups examined one and two days after two hours of 350 mmHg inflation: two-thirds of the compressed limbs had decreased toe-spread, and one-third had absence of the reflex. Toe-spread was absent in all of the limbs subjected to 1000 mmHg pressure. Lack of ankle dorsiflexion was observed in most limbs with absent toe-spreading. The toe- spread reflex was normal in all of the control limbs.

Neurophysiology. Nerve conduction velocity (NCV) was decreased in the compressed thigh region in all groups tested after 350 mmHg compression. Complete conduction block at the thigh was observed in all animals after 1000 mmHg compression. NCV was decreased in the leg region one hour after a 350 mmHg tourniquet, but not after one or two days. NCV was markedly decreased in the leg two days after 1000 mmHg application (Table 5).

CMAP amplitudes elicited at the proximal and middle thigh stimulation sites were decreased one hour

Table 5. Nerve conduction velocity after tourniquet compression for two hours, m/s, mean *SEM*

Pressure	Thigh		Leg	
350 mmHg				
1 Hour	35	*4.8***	41	*4.2**
1 Day	50	*5.3**	51	*3.1*
2 Days	52	*3.3**	51	*4.7*
1000 mm Hg				
2 Days	0	*0*	42	2.0**
Controls	59	*1.3*	52	*1.5*

*$p < 0.05$ versus control
**$p < 0.005$ versus control

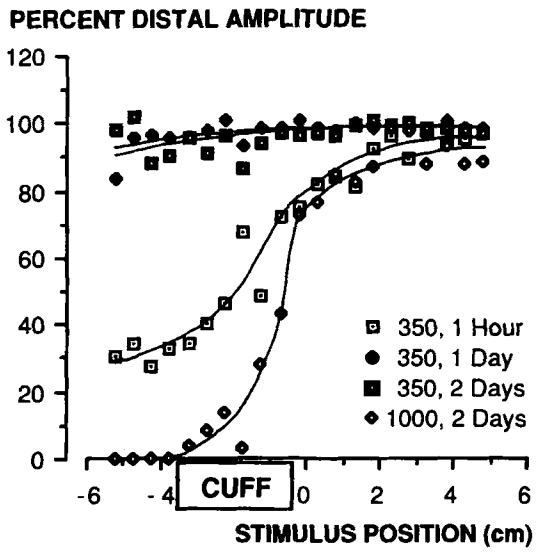


Figure 8. Normalized CMAP amplitudes recorded from abductor hallucis muscle with sequential stimulation along the sciatic nerve (proximal towards the left) and tibial nerve (distal towards the right). Marked changes in CMAP amplitude were noted at the distal cuff margin (position 0) one hour after 350 mmHg compression and two days after 1000 mmHg compression. [Reproduced with permission, Pedowitz et al. 1991b]

after 350 mmHg tourniquet compression, but returned to greater than 90% of control values one day later. Two days after 1000 mmHg, CMAPs could not be elicited from the proximal thigh. CMAP amplitude with distal thigh stimulation was lower than in animals tested two days after a 350 mmHg tourniquet.

Focal transition from no conduction to measurable conduction was observed in all nerves subjected to 1000 mmHg cuff pressure. Marked changes in CMAP amplitude were also noted between adjacent stimulation sites in some nerves following 350 mmHg compression. This transition was located at a transversely-oriented neurovascular bundle in three animals, and at the bifurcation of the gastrocnemius fascia in two animals. Sigmoid-shaped CMAP amplitude curves were observed for the groups tested one hour after 350 mmHg compression and two days after 1000 mmHg compression; the greatest changes were noted in the region of the distal cuff margin (Figure 8).

Light microscopy. Few light microscopic abnormalities were observed beneath or distal to the cuff two days after 350 mmHg tourniquet application. Moderate fiber damage was observed in one specimen from beneath the tourniquet; abnormalities in the other specimens were graded as either absent or mild. The nodes of Ranvier were easy to discern in longitudinal sections, and were generally normal in appearance. Myelin ovoids were occasionally observed in the non-compressed, control limbs.

Marked histologic abnormalities were observed after 1000 mmHg tourniquet application, and these changes were most severe in the distal cuff zone. Tearing of myelin sheaths was the dominant histologic finding, and few myelin ovoids were observed. The nodes of Ranvier were extremely difficult to identify, and myelin invagination (Ochoa et al. 1972) was not observed (Figure 9). In the tibial nerves, fiber damage tended to be more severe in the 1000 mmHg group than in the 350 mmHg group.

Epineurial infiltration of fibroblasts, granulocytes, and macrophages was noted in nerves compressed at 1000 mmHg. These changes tended to be more pronounced beneath than distal to the cuff. An endoneurial inflammatory response was not observed in these sections. The epineurial inflammatory response was less severe in nerves compressed at 350 mmHg.

Electron microscopy. Occasional, scattered degenerated axons, with floccular axoplasm devoid of organelles and intra-axonal membranous profiles, were observed beneath and distal to the 350 mmHg tourniquet. Some of these fibers had disintegrating myelin, while others had normal appearing myelin sheaths. Myelin tearing or deformation was not observed in nerve fibers with intact axons. Degeneration predominated in larger myelinated fibers and tended to be located in the periphery of the fascicles. Unmyelinated nerve fibers appeared undamaged. One single degenerating myelinated fiber was observed in the contralateral control sections.

Dramatic splitting, tearing, and displacement of myelin sheaths was observed frequently after 1000 mmHg tourniquet compression (Figure 10). The axons were well preserved in the majority of these fibers,

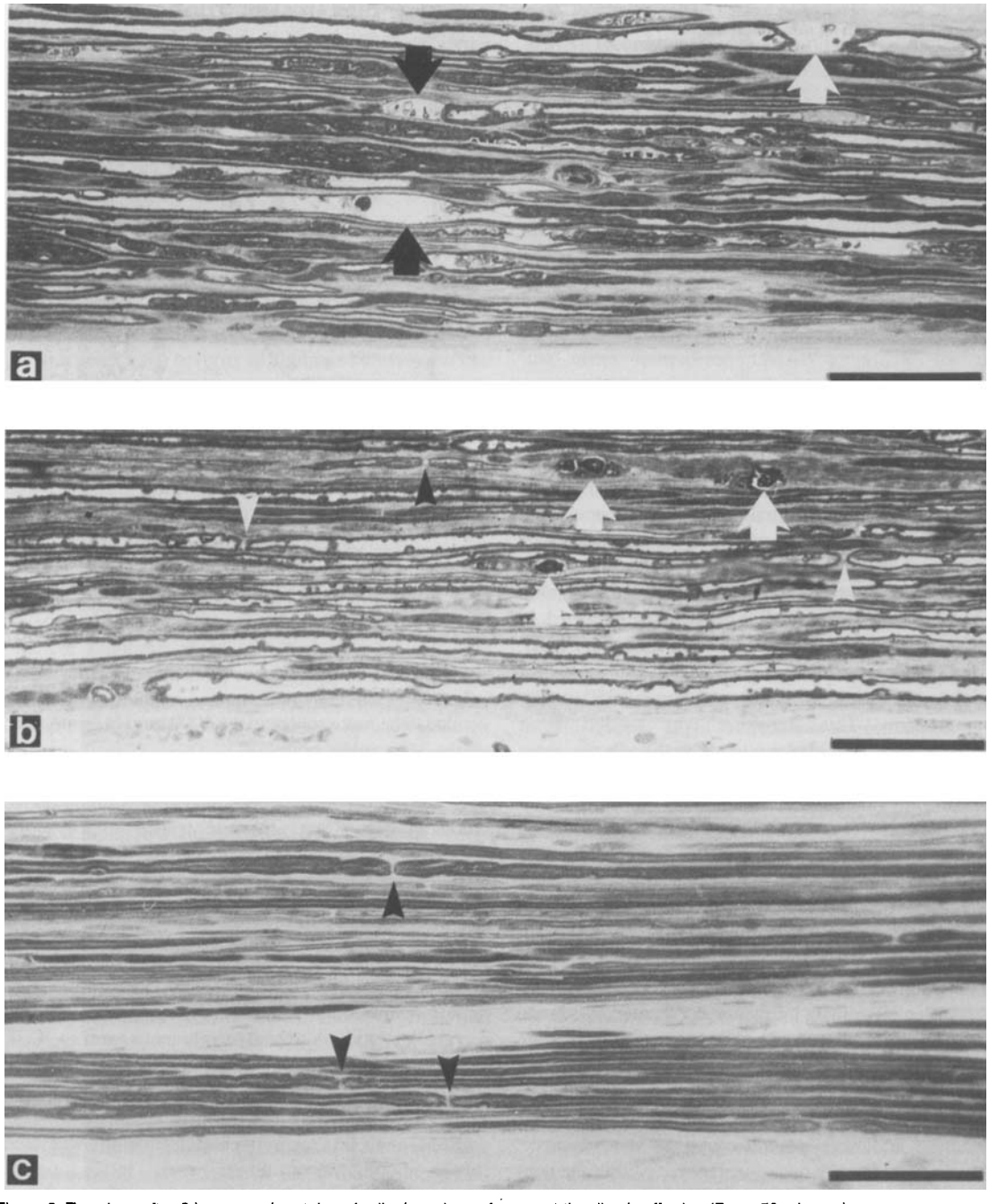


Figure 9. Two days after 2 hour tourniquet. Longitudinal sections of nerve at the distal cuff edge (Bar = 50 microns).
a. 1000 mm Hg cuff pressure—Tearing and displacement of myelin sheaths reflected by irregular thickness and discontinuities in myelinated fibers (arrows). Normal nodes of Ranvier are not seen.
b. 350 mm Hg cuff pressure —Scattered myelin ovoids (arrows) indicate nerve damage, but myelin tearing is not present. Several nodes of Ranvier are seen (arrow-heads).
c. Contralateral control—Notice normal nodes of Ranvier (arrow-heads). [Reproduced with permission, Pedowitz et al. 1991b]

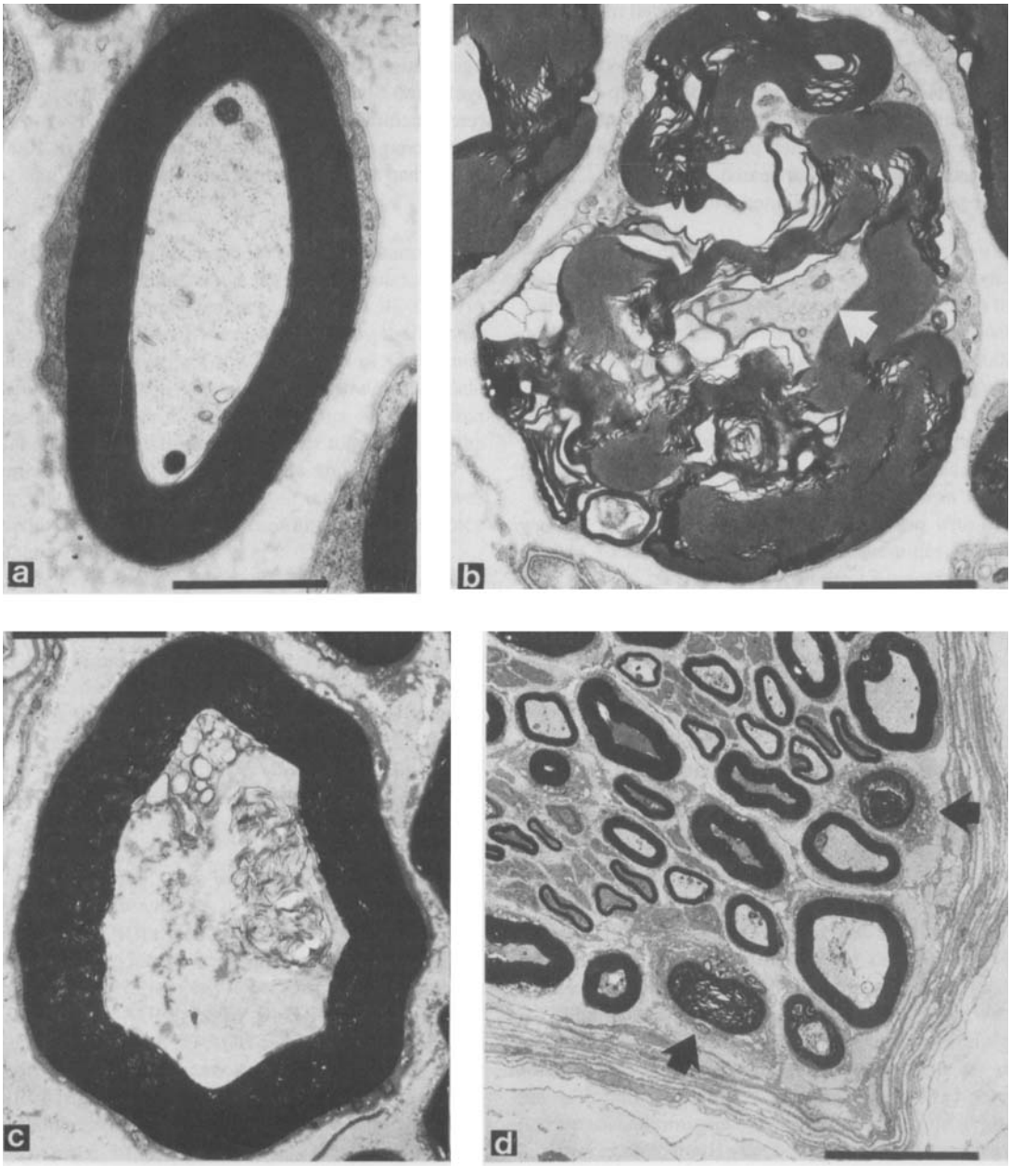


Figure 10. Two days after 2 hour, 1000 mmHg tourniquet.
a. Cross section distal to cuff. The structure of small and medium-sized myelinated fibers was generally well preserved in both the compressed and ischemic zones (Bar = 1.2 microns).
b. Cross section under the cuff. Torn and deformed myelin sheaths were observed under the cuff and at the distal cuff edge. The axons occasionally seemed compressed, however they appeared intact (arrow) in the majority of cases (Bar = 2.1 microns).
c. Cross section distal to cuff. Myelinated fiber with floccular axoplasm which is devoid of organelles but has some membranous profiles. The myelin sheath is loose, but intact (Bar = 2.9 microns).
d. Cross section distal to cuff. Marginal myelinated fiber (arrow) undergoing Wallerian degeneration (Bar = 10.3 microns). [Reproduced with permission, Pedowitz et al. 1991b]

however axonal degeneration was observed in some of these myelin-damaged fibers. Axonal degeneration was also noted within several fibers having undamaged myelin. The nodes of Ranvier were difficult to identify, and distinct myelin invagination was not observed. Degeneration predominated in the larger fibers, while unmyelinated fibers appeared unaffected. Fiber damage was evenly distributed within the fascicles.

Myelin tearing or displacement was not observed in nerves distal to the 1000 mmHg cuff. Some axonal degeneration was observed predominantly in the larger myelinated fibers, however most of these nerve fibers were structurally intact. Distal nerve fiber damage was more common and of greater severity after 1000 mmHg than after 350 mmHg tourniquet application.

Clinical studies of cuff design

The human study included 26 normal volunteers (10 male, 16 female), mean age 28 (15–43) years, and 60 patients (57 male, 3 female), mean age 48 (23–79) years. Study protocols were approved by the Human Subjects Committee of the University of California, San Diego and the Veterans Administration Medical Center, San Diego. Limb circumference was measured at the longitudinal midposition of the tourniquets in the supine position. Tourniquets were applied directly to the limb in the volunteer studies; cotton padding was used in the patient studies, according to the preference of the surgeon.

Volunteer studies—comparison of straight and curved cuffs on thighs: An 8-cm-wide straight and an 8-cm-wide curved tourniquet (radius of curvature of 132 cm, Zimmer Aspen Labs) were applied in random order to the right thigh of 14 subjects. An audible Doppler flow meter was positioned over the posterior tibial artery. A pulse oximeter sensor (Biox 3700, Ohmeda, Boulder, CO, USA) was placed on the pad of the middle digit of the foot. Tourniquets were slowly inflated, and the cuff pressure and oximeter tracing were recorded.

Observations: AOP was lower with the curved tourniquet than with the straight tourniquet on thighs. The mean AOP difference between the two tourniquets was 25 mmHg measured by the Doppler technique (D-AOP) and 22 mmHg lower using the oximeter technique (O-AOP). Mean D-AOP was 5.9 mmHg higher than mean O-AOP, with close correlation between the two measurement techniques using either cuff design. AOP correlated positively with thigh circumference using both the curved and straight tourniquets. In these volunteers, the mean systolic blood pressure was 126 (108–158) mmHg, and mean thigh circumference was 50 (44–61) cm.

Volunteer studies—comparison of straight and curved cuffs on arms, and of 8 and 12 cm wide curved cuffs on thighs: In twelve subjects, 8-cm-wide straight and curved cuffs were applied to the left upper arm, and 8-cm-wide curved and 12-cm-wide curved cuffs (radius of curvature 132 cm) were applied to the left thigh in random order. A photoplethysmograph (PPG) sensor (PPG-13, Vasculab, Mountain View, CA, USA) was applied to the pad of the left middle finger or toe, and the PPG output was displayed on an oscilloscope. Tourniquets were inflated to a pressure high enough to occlude pulsatile flow using a calibrated tourniquet inflator with a digital pressure display (ATS 1000, Zimmer Aspen Labs, Aspen CO, USA). Cuff pressure was slowly decreased until pulsatile flow resumed, and the AOP (mean of three determinations) was defined as the cuff pressure at which pulsatile flow resumed.

Observations: In the upper extremities, mean AOP was 4.3 mmHg lower with the curved than with the straight tourniquet. In the lower extremities, mean AOP was 8.6 mmHg lower with the 12-cm-wide than with the 8-cm-wide cuff. In these subjects, mean systolic blood pressure was 126 (100–154) mmHg, mean upper arm circumference was 28 (23–33) cm, and mean thigh circumference was 53 (45–59) cm.

Patient studies: Patients scheduled to undergo extremity procedures at the Veterans Administration Medical Center, San Diego volunteered for the study. Eight cm wide straight cuffs (15 cases) or 8-cm-wide curved cuffs (14 cases) were used in upper extremity cases. Eight cm wide straight cuffs (11 cases), 8-cm-wide curved cuffs (10 cases), or 12-cm-wide curved cuffs (10 cases) were used in lower extremity cases. A tourniquet was chosen at random, and after induction of anesthesia, AOP was determined using the PPG technique described above. The cuff was deflated and the PPG sensor removed. Limb exsanguination was performed according to the preference of the operating surgeon. The tourniquet inflation pressure was set at the AOP plus 50 mmHg. If the surgeon reported poor hemostasis, tourniquet pressure was increased in increments of 25 mmHg until adequate hemostasis was achieved. Surgeons were asked to subjectively rate the quality of hemostasis.

Observations: The mean tourniquet pressure used to induce surgical hemostasis was 184 (145–270) mmHg in the upper extremity cases, and 208 (160–280) mmHg in the lower extremities (Table 6). Half of the patients with fair or poor hemostasis had increased systolic blood pressure after the initial determination of the AOP.

Table 6. Clinical tourniquet studies, mean, *SD*, (range)

	Upper extremities		Lower extremities		
	8-cm-wide straight cuff	8-cm-wide curved cuff	8-cm-wide straight cuff	8-cm-wide curved cuff	12-cm-wide curved cuff
No of patients	15	14	11	10	10
Limb Right	7	12	5	8	5
Left	8	2	6	2	5
Age (years)	53 *18* (23–77)	55 *18* (28–74)	39 *17* (23–74)	45 *13* (29–65)	46 *19* (28–79)
Limb circumference (cm)	29 *3* (22–34)	31 *3* (25–35)	54 *4* (44–60)	51 *6* (42–62)	56 *7* (47–70)
Systolic blood pressure (mmHg)	128 *22* (100–180)	129 *14* (113–165)	129 *28* (90–170)	121 *20* (100–165)	118 *14* (95–140)
Arterial occlusion pressure (mmHg)	132 *32* (95–220)	130 *24* (90–175)	149 *38* (100–200)	142 *22* (116–175)	142 *36* (90–200)
Cuff pressure used for case (mmHg)	182 *32* (145–270)	186 *20* (155–225)	218 *38* (166–280)	208 *33* (175–275)	197 *37* (160–275)
Tourniquet time (minutes)	110 *63* (4–251)	73 *37* (24–120)	58 *31* (13–114)	86 *38* (26–135)	58 *29* (24–105)
Rating					
Excellent	12	8	6	8	7
Good	1	1	2	0	1
Fair	1	2	1	2	1
Poor	1	1	1	0	0
Not Reported	0	2	1	0	1
Anesthesia					
General	4	3	8	4	3
Spinal	0	0	2	4	6
Epidural	0	0	0	1	0
Regional	8	6	0	0	0
Local	2	3	1	0	0
Unknown	1	2	0	1	1

Discussion

The bloodless field is an integral part of limb surgery. However, routine use of pneumatic tourniquets, with commonly accepted pressures and durations, may cause neuromuscular injury which could interfere with post-operative rehabilitation. Few data are available regarding pressure and time thresholds for muscle and nerve injury beneath the tourniquet. This paucity of information relates, in part, to difficulties performing controlled studies of tourniquet compression on conically-shaped animal limbs.

Tourniquet models

Tourniquet compression studies are difficult to perform on the limbs of small animals. Animal limbs are not cylindrical in shape; they are generally conical in the sagittal plane and oblong in the transverse plane. Since standard (ie., rectangular) tourniquets fit poorly on such limbs (Dobner, discussion following Gersoff et al. 1989), previous investigators have applied very high cuff pressures to assure distal ischemia (Lundborg 1970, Enger 1977). A prerequisite for adequate experimental study of neuromuscular injury beneath the pneumatic tourniquet is the development of a well-controlled, clinically relevant, and reasonably convenient animal model.

A curved tourniquet was designed specifically to fit the conically-shaped rabbit hindlimb. A "stellate" pattern, with marked, asymmetrical skin ridging, was observed beneath the inflated tourniquet on cross-sectional images, and a similar pattern was observed in a human thigh compressed by a tourniquet. This pattern may relate to the anatomy of underlying tissues, for example osseofascial compartmental anatomy, or to the materials and designs of these specific tourniquets. Such changes have not been described previously, and previous theoretical models (Griffiths and Heywood 1973, Auerbach 1984, Hodgson 1987, Rydevik et al. 1989) do not predict such asymmetrical tissue deformation beneath the tourniquet. These models should be tested with analyses of in-vivo and in-vitro displacement patterns. The pathophysiologic significance of such deformation, and potential implications regarding tourniquet design, are not known.

The rabbit thigh deformed in the transverse plane from an initially oblong shape to a more circular shape during inflation of a curved tourniquet. Addition of a stiff exterior shell produced a more normal cross-sectional configuration. The shell also minimized distal migration of the tourniquet during inflation, thereby controlling the zone of tissue compression. The curved tourniquet and stiff exterior shell were associated with the lowest AOP compared to the straight cuff and no-shell configurations. This finding may relate either to more uniform and efficient transmission of cuff pressure to the deeper tissues of the thigh, or to an essentially wider tourniquet (ie., larger contact area) with the constrained, curved cuff. Mean AOP for this configuration (67 mmHg) was approximately 10 mmHg lower than the mean systolic blood pressure in these rabbits. Previous human studies demonstrated that sub- systolic inflation pressures occluded distal blood flow when a wide cuff was used (Moore et al. 1987, Crenshaw et al. 1988).

The magnitude and distribution of tissue pressures beneath the curved cuff and stiff exterior shell were similar to those in human cadaver studies of tourniquet compression (Hargens et al. 1987, Crenshaw et al. 1988). Tissue pressures were maximal under the mid-position of the inflated cuff, and were lower in the deep tissues than in the superficial tissues of the thigh. The greatest rate of change of tissue pressure was observed at the proximal and distal edges of the tourniquet, which is consistent with experimental observations of an edge effect at the margins of compression (Bentley and Schlapp 1943, Lundborg 1970, Ochoa et al. 1972, Rydevik and Lundborg 1977). Mathematical models (Griffiths and Heywood 1973, Auerbach 1984, Hodgson 1987, Rydevik et al. 1989) predict that shear stress concentration, largest pressure gradients, and maximal tissue deformation occur beneath the tourniquet edges (Lundborg 1989). Little information is available regarding cellular and sub-cellular pathomechanics during tourniquet compression.

Relatively efficient pressure transmission from the cuff to the underlying tissues was observed, in accordance with previous studies of tourniquet compression in dog and human limbs (Shaw and Murray 1982, McLaren and Rorabeck 1985, Hargens et al. 1987, Crenshaw et al. 1988). However, others

describe inefficient transmission of pressure beneath an inflated tourniquet (Lundborg 1970, Mäkitie and Teräväinen 1977, Nitz et al. 1986). This discrepancy may reflect difficulties positioning a pressure-measuring device in small animal hindlimbs, or may reflect specific pressure catheter techniques. In the present study, mean deep tissue pressures under the center of the cuff were 80 mmHg measured with a Slit catheter and 256 mmHg using a transducer- tipped catheter. This difference was probably due to obliteration of a closed fluid space in the loose areolar connective tissues; such changes would decrease the accuracy of pressure recordings using a non-infusion Slit catheter. Intramuscular pressure measurements correlated closely using Slit and transducer-tipped catheters in a closed compartment of a pig hindlimb compressed by a tourniquet (Pedowitz et al. 1990).

^{99m}Technetium pyrophosphate uptake

Radiolabelled pyrophosphate incorporation is used for clinical and experimental assessment of cardiac and skeletal muscle injury (Siegel et al. 1975, Buja et al. 1977, Simpson 1977, Silberstein and Bove 1979, Brill 1981, Hargens et al. 1981, Matin et al. 1983, Valk 1984, Yip et al. 1988). Sensitivity of the technique peaks approximately two days after tissue injury (Buja et al. 1975, Hargens et al. 1981, Labbe et al. 1988). The degree of Tc-99 uptake correlates with the severity of histologic abnormality (Hargens et al. 1981) and cellular enzymatic dysfunction (Labbe et al. 1988). Tc-99 uptake correlates with increased subcellular calcium stores following tissue injury (Dewanjee and Kahn 1976, Wrogemann and Pena 1976, Buja et al. 1977, Oberc and Engel 1977, Farber et al. 1981). Increased uptake may also reflect altered capillary permeability, hyperemia, or affinity of Tc-99 for soft tissue iron deposits, tissue hormone receptors, immature collagen, or soluble denatured macromolecules (Dewanjee and Kahn 1976, see Brill 1981 for review).

An advantage of the Tc-99 method is that the density of radiopharmaceutical incorporation is determined for a relatively large volume of tissue. Other methods, for example electron microscopy (Patterson and Klenerman 1979) may be particularly influenced by the sampling technique. However, several limitations of the Tc-99 method should be noted. Lack of local perfusion (ie., no-reflow) could result in low Tc-99 uptake in severely injured tissue. In addition, this method is not suitable for assessment of chronic injury patterns. Also, a specific relationship between tissue uptake ratio and functional deficit has not been determined. However, the measured Tc-99 ratios provide a quantitative, relative index of skeletal muscle injury which facilitates comparisons between various experimental protocols.

Increased Tc-99 uptake is reported to be limited to regions of severe tissue injury and necrosis (Buja et al. 1977, Hargens et al. 1981). In the present studies, Tc-99 uptake ratios were increased in all experimental groups, even though histologic evidence of severe tissue injury and necrosis were not observed after the shorter duration and lower pressure protocols. Therefore, the Tc-99 technique may be sensitive to less severe forms of tissue injury than have been recognized previously. In addition, Lieber and co- workers (1990) demonstrated approximately 70 percent reduction of peak isometric force generation in rabbit tibialis-anterior muscle two days after two hours of tourniquet ischemia (mean Tc-99 uptake ratio of 1.70 in the present studies). Electron microscopic studies are needed to determine the anatomic basis for such marked, and clinically relevant, functional abnormalities following two hours of ischemia.

Pressure and time thresholds for muscle injury

Previous studies of muscle injury beneath the tourniquet examined various tourniquet durations with a given cuff pressure (Patterson and Klenerman 1979, Patterson et al. 1981) or various cuff pressures with a given tourniquet duration (Gersoff et al. 1989). The present study examined effects of both variables upon skeletal muscle injury beneath and distal to a tourniquet using controlled and quantitative techniques.

There were no differences in Tc-99 uptake between thigh and leg regions following one hour of tourniquet application. Thigh uptake was greater than leg uptake after two hours of compression at 200 or 350 mmHg and after four hours of tourniquet inflation with all cuff pressures. Regional muscle necrosis was observed in thigh muscles following two hours of 350 mmHg compression, while regional necrosis was not observed after two hours of 125 mmHg tourniquet application. Taken together, these findings suggest that two hours may be a time threshold for skeletal muscle injury induced by tourniquet compression at clinically relevant cuff inflation pressures. It should be noted, however, that muscle necrosis would probably be induced by a very high cuff pressure applied for shorter durations.

Pale tissue was observed within a rim of intense blue staining on gross cross- section of some thigh muscles

subjected to four hours of compression. Such lack of staining probably reflects a region of no-reflow (Strock and Majno 1969) following extended tourniquet compression (see below). Since uptake of Tc-99 is dependent upon tissue perfusion, these areas may have low focal uptake. However, regional uptake was always markedly increased in these animals, since the samples also included regions of blue muscle (ie., perfused and abnormal tissue).

Effects of reperfusion intervals

Methods for safe, extended tourniquet hemostasis are of great importance as the complexity and duration of reconstructive and microsurgical extremity procedures increase. Previous investigators recommend intermittent reperfusion (ie., re-breathing) intervals to permit extended tourniquet application (Bruner 1951, Bunnell 1956, Wilgis 1971, Chiu et al. 1976, Heppenstall et al. 1979, Newman 1984, Sapega et al. 1985). Wilgis (1971) suggested that reperfusion duration should reflect the period of preceding ischemia, for example ten and fifteen minutes following sixty and ninety minutes of tourniquet inflation, respectively. To date, there are no specific studies of effects of reperfusion intervals upon muscle injury beneath the tourniquet.

Chiu et al. (1976) found no increase in systemic creatine phosphokinase (CPK) when three hours of tourniquet inflation was interrupted by hourly, fifteen minute reperfusion intervals. Heppenstall et al. (1979) reported similar findings using hourly, ten minute reperfusion intervals. Sapega et al. (1985) found no difference in CPK elevation or intracellular metabolic recovery between five and fifteen minute reperfusion intervals with a three hour tourniquet time in dogs. However, Newman (1984) observed greater derangement and slower recovery of metabolic parameters with hourly, five minute reperfusion intervals compared to hourly, ten minute reperfusion intervals in rats. Ten minute reperfusion intervals were chosen for the present study based upon these animal studies and previous clinical recommendations (Bruner 1951, Bunnell 1956, Sanders 1973).

In the thigh muscle of the four hour, reperfusion study, Tc-99 uptake was affected by both cuff inflation pressure and reperfusion; the two variables were not independent. This interaction may relate either to lack of effects of reperfusion using 350 mmHg cuff pressure, or to lack of differences in regional uptake between 125 mmHg and 350 mmHg tourniquet groups following four hours of continuous inflation. In other words, a high tourniquet pressure may counteract the beneficial effects of reperfusion intervals, and/or, continuous compression for an extended duration may counteract the beneficial effects of a low cuff pressure.

With 125 mmHg pressure, uptake in the thigh was decreased by ten minutes of reperfusion after either one or two hours. However, with a 350 mmHg tourniquet, reperfusion after two hours tended to increase the uptake, while reperfusion at hourly intervals tended to decrease muscle injury. In addition, marked variation of uptake ratios was observed in the 125 mmHg tourniquet, one reperfusion interval group. Thus, two hours may be a time threshold for beneficial or harmful effects of reperfusion intervals, which is consistent with a two hour threshold for continuous tourniquet compression injury.

Ten minutes of reperfusion after one hour of 350 mmHg tourniquet application showed no difference between thigh and leg regional uptake. It should be noted that uptake in the leg was somewhat higher with reperfusion after one hour compared to two hours of continuous tourniquet application. The pathologic and functional implications of this small difference is unknown.

In the four-hour tourniquet studies, the reperfusion protocol did not have any effect upon uptake in the leg distal to the cuff. However, uptake in the leg was affected by the tourniquet pressure. Effects of cuff pressure upon distal uptake were not observed in studies of continuous compression. Release of metabolic byproducts, activation of polymorphonuclear leukocytes, or partial proximal vascular occlusion could affect distal muscle injury in the setting of reperfusion intervals (see below).

Clinical considerations: The data suggest that if two or more hours of tourniquet hemostasis are needed, compression injury may be decreased by reperfusion at hourly intervals. Hourly reperfusion would also allow for extended hemostasis if it became desirable during the surgical procedure. Reperfusion after a longer initial period of cuff inflation (ie., two hours) could exacerbate skeletal muscle injury with subsequent tourniquet compression.

However, reperfusion intervals could prolong anesthesia time, increase blood loss, or cause hemorrhagic staining and edema (Sapega et al. 1985), which could interfere with microsurgical repair or wound closure. Critical levels of venous pH and pO2 for hypocoagulability (Rutherford et al. 1966) and increased capillary permeability (Hendley and Schiller 1954, Webb 1965), respectively, are reached within 60 to 90 minutes of limb ischemia (Dery et al. 1965, Wilgis 1971, Larsson et al. 1977). Reactive hyperemia of muscle and skin occurs after two hours of tourniquet

ischemia (Kennedy et al. 1981). Thus, hourly reperfusion could actually minimize blood loss and edema formation compared to less frequent reperfusion intervals.

Enzyme- and immuno-histochemical analysis

Inter-individual variability was observed in the severity of histologic abnormalities, and similar variability was noted in some cases using the Tc-99 technique. These findings probably reflect basic pathogenic mechanisms of tourniquet-induced muscle injury (see below). In some specimens, gross fiber necrosis and cellular infiltration were observed in close proximity to relatively normal tissue. Thus, careful sampling criteria should be employed for ultrastructural analyses of tourniquet compression injury.

Regional necrosis was observed in four of ten thigh muscle samples following 350 mmHg compression, and was not seen in any muscle samples after 125 mmHg tourniquet application. A striking topographic pattern was observed in samples with regional fiber necrosis. To our knowledge, similar patterns have not been described previously. Necrosis tended to predominate at the center of fascicles or at the intersection of several fascicles, with relatively normal fibers at the periphery of these areas. Since topographic necrosis was limited to the compressed muscle of the higher tourniquet pressure group, mechanical and physiologic factors probably interact in the production of tourniquet compression injury.

The topographic injury pattern probably relates to the vascular arrangement of skeletal muscle. Myrhage (1977) studied the vascular anatomy of cat skeletal muscle, and described "secondary" arteries which run parallel to the muscles fibers and are approximately one to two mm apart. These arteries represent a basic unit of vascular supply for approximately a one mm2 cross-sectional area of muscle tissue. Roughly similar distances and areas of regional necrosis were observed in the present study. Topographic necrosis could relate either to persistent ischemia due to "no-reflow" (Strock and Majno 1969) in these regions, and/or to reperfusion-mediated damage related to local vascular dysfunction. These mechanisms will be discussed in further detail below.

Alternatively, topographic necrosis could reflect uneven distribution of mechanical forces within the compressed tissue, resulting in local pressure-related fiber damage. Such a pattern could reflect the connective tissue composition and distribution of a particular muscle, with "protective effects" provided in certain fascicles. However, there was no macroscopic or microscopic evidence to support this hypothesis.

Obviously large and rounded fibers were observed in some of the compressed thigh muscles. These fibers were histochemically identified as Type IIB, corresponding to fast glycolytic fibers (FG) in functional classification schemes (Mastaglia and Walton 1982). Selectively damaged FG fibers have been observed after tourniquet ischemia (Caizzo et al. 1990, Jennische 1985) and following eccentric exercise (Lieber and Fridén 1988). Clinical abnormalities of gait and quadriceps muscle function were consistent with selective loss of Type IIB fibers after tourniquet-aided knee arthrotomy (Krebs 1989). The large, Type IIB fibers observed in the present study may reflect differential sensitivity of muscle fiber types to tourniquet compression, or may represent a compounding effect of their selective vulnerability to ischemia. Detailed histomorphometric findings will be presented separately (Fridén et al. 1991).

Implications regarding previous studies

Serum CPK abnormalities have been used to study time limits for distal muscle ischemia, using tourniquet pressures of 300 or 350 mmHg in dogs (Chiu et al. 1976, Heppenstall et al. 1979, Sapega et al. 1985). One hour of tourniquet application did not cause abnormal CPK levels, while two hours did. In the present study, there was no difference in leg regional Tc-99 uptake between one and two hours of continuous ischemia. However, thigh uptake differences were observed between one and two hours of continuous compression with a 350 mmHg tourniquet. In addition, patterns of CPK changes after tourniquet application with reperfusion intervals (Chiu et al. 1976, Heppenstall et al. 1979, Sapega et al. 1985) are similar to patterns of Tc-99 uptake in compressed thigh muscle in the present study. Appropriate consideration should be given to time limits for muscle ischemia which were based upon serum CPK changes after tourniquet compression.

Others have examined systemic effects of hindlimb ischemia induced by tourniquet application (Klausner et al. 1988, Klausner et al. 1989a,b, Paterson et al. 1989). Klausner et al. (1988) noted pulmonary leukosequestration and increased pulmonary vascular permeability following two hours of bilateral, 300 mmHg tourniquet application in sheep. Paterson et al. (1989) demonstrated that four hours of bilateral arterial tourniquet application in rats (tourniquet pressure not specified) was associated with elevated plasma levels

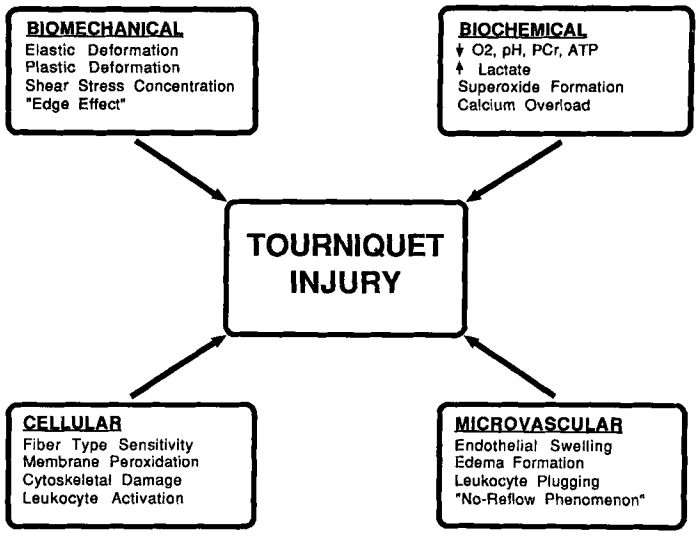


Figure 11. Interactive mechanisms influence tourniquet-induced neuromuscular injury.

of thromboxane B2 and a marked oxidative burst of neutrophils. However, Thörne et al. (1989) observed leukosequestration in the lung and liver following two minutes of mechanically-induced soft-tissue trauma of pig hindlimbs. In view of the findings of the present investigation, studies of systemic effects of limb ischemia should address the potentially confounding effects of injury caused by compression beneath the tourniquet.

Mechanisms of tourniquet-induced muscle injury

Observed patterns of muscle injury induced by tourniquet compression and ischemia probably reflect basic pathogenic mechanisms (Barie and Mullins 1988). Interrelations of pressure and time are reported for injury of peripheral nerves (Lundborg 1970, Rydevik 1979, Rorabeck 1980, Nitz and Dobner 1986, Dahlin et al. 1986, Nitz et al. 1989) and spinal nerve roots (Olmarker et al. 1989, Pedowitz et al. 1992b). Various biochemical, biomechanical, microvascular, and cellular factors probably determine thresholds for tissue compression injury (Figure 11).

The tourniquet causes decreased blood flow in tissues beneath (Dahn 1967) and distal to the cuff (Klenerman and Crawley 1977, Santavirta et al. 1978). A small amount of residual blood flow may be carried by intramedullary vessels (Furlow 1971), however such blood flow is inadequate to maintain aerobic metabolism (Klenerman and Crawley 1977). Reactive hyperemia and arterial vasodilatation follow brief periods of tissue ischemia (Romanus 1977, Larsson and Lewis 1978, Santavirta et al. 1978, Kennedy et al. 1981, Klenerman et al. 1982, Barie and Mullins 1988, Authier 1988). Tissue edema follows reperfusion after thirty minutes to four hours of skeletal muscle ischemia (Paletta et al. 1960, Strock and Majno 1969, Miller et al. 1979, Klenerman et al. 1982, Korthius et al. 1985, Silver et al. 1986, Suval et al. 1987, Dreyfuss and Smith 1988, Gidlöf 1988, Soussi 1989). Edema formation may interfere with postischemic tissue nutrition (Lundborg 1970, Rydevik and Lundborg 1977, Olmarker et al. 1989).

Metabolic changes are well-described for tissues subjected to tourniquet ischemia (Dery et al. 1965, Solonen et al. 1968, Wilgis 1971, Haljamäe and Enger 1975, Enger 1977, Jennische et al. 1978, Larsson and Bergström 1978, Miller et al. 1978, Modig et al. 1978, Santavirta et al. 1978, Heppenstall et al. 1979, Miller et al. 1979, Klenerman et al. 1980, Rorabeck 1980, Jennische et al. 1982, Nakahara 1984, Newman 1984, Cotellessa et al. 1984, Hagberg et al. 1985, Sapega et al. 1985, Heppenstall et al. 1986, Patel et al. 1987, Benzon et al. 1988, Soussi 1989). Levels of oxygen, phosphocreatine, glycogen, and ATP decrease, while the concentrations of lactate, carbon dioxide, and other metabolic by-products increase as metabolism shifts to

predominantly anaerobic pathways. Intracellular pH and tissue pO_2 are decreased within 30 minutes of skeletal muscle ischemia, and critical levels of high energy phosphates (ie., phosphocreatine depletion) are reached after approximately three hours of ischemia (Newman 1984, Sapega et al. 1985). Limb temperatures decrease during tourniquet ischemia (Solonen and Hjelt 1968, Lundborg 1970, Swanson et al. 1991), with associated slowing of cellular metabolic processes (Solonen and Hjelt 1968).

The rate and degree of metabolic recovery after tourniquet release depend upon the duration of preceding ischemia (Wilgis 1971, Heppenstall et al. 1979, Newman 1984, Sapega et al. 1985). Cellular metabolic recovery in skeletal muscle occurs in 5-10 minutes after one hour of ischemia, 5–20 minutes after two hours of ischemia, 10–60 minutes after three hours of ischemia, and may be incomplete following four hours of tourniquet ischemia (Enger 1977, Newman 1984, Sapega et al. 1985, Heppenstall et al. 1986, Soussi 1989). The ischemic sensitivity of skeletal muscle is related to vascular supply (Folkow and Halicka 1967, Gray and Renkin 1978) and fiber type composition. "Fast" (ie., white) muscles may be more sensitive than "red" (ie., slow) muscles (Mortimer et al. 1970, Patterson and Klenerman 1979, Gardner et al. 1984). Fast, glycolytic muscle fibers may be particularly sensitive to temporary ischemia (Jennische 1985, Caizzo et al. 1988, 1990).

Skeletal muscle force production decreases after one to three hours of continuous ischemia with reperfusion (Patterson et al. 1981, Gardner et al. 1984, Chervu et al. 1989). Marked isometric force decrement in rat gastrocnemius muscle (mean 66% of control force) was observed two weeks after a one hour tourniquet (Fish et al. 1989). Larger deficits correlated with increasing ischemic duration up to four hours (Fish et al. 1989).

Various interventions limit the degree of post-ischemic tissue injury. Pre-ischemic extremity cooling decreases tissue edema (Paletta et al. 1960) and histologic abnormalities (Swanson et al. 1991), improves muscle reperfusion (Nakahara 1984), and limits serum myoglobin changes (Ikemoto et al. 1988), perhaps by decreasing tissue metabolic demand (Bruner 1970). Heparin decreased edema (Paletta et al. 1960) and skeletal muscle necrosis (Wright et al. 1988) after ischemia with reperfusion. Regional methylprednisolone administration decreased metabolic abnormalities induced by proximal tourniquet application in dogs (Goto et al. 1988). Other pharmacologic substances, such as allopurinol, catalase, and superoxide dismutase, may limit pathogenic events during tissue reperfusion (Korthius et al. 1985, Lee et al. 1987).

Heppenstall et al. (1986) found that metabolic derangement was more severe in skeletal muscle subjected to compartment syndrome compared to ischemia distal to a tourniquet. Synergistic effects (Heppenstall et al. 1986) of compression and ischemia upon cellular deterioration seem relevant to tourniquet compression injury, although the mechanisms of such synergism have not been clarified. Energy depletion results in inability to break actin-myosin bonds, with tissue rigor, increased stiffness, and perhaps greater sensitivity to mechanical deformation (Lieber and Fridén 1988). Ischemia causes membrane dysfunction, loss of ionic homeostasis, and intracellular calcium overload (Trump et al. 1980, Farber et al. 1981, Lange et al. 1984, Van der Vusse et al. 1989). Increased intracellular calcium activates phospholipases and proteases; these enzymes may cause sarcolemmal and/or cytoskeletal damage with associated loss of structural integrity (Oberc and Engel 1977, Trump et al. 1980, Hattori and Takahashi 1982, Van der Vusse et al. 1989). Ischemia and reperfusion-induced cytoskeletal degradation may contribute to myocardial damage when mechanical deformation (cardiac contraction) occurs after adjacent infarct (Steenbergen et al. 1987, Van der Vusse et al. 1989).

Functional and structural microvascular abnormalities may cause no-reflow or slow-reflow after tourniquet deflation (Harman 1948, Mäkitie 1977, Jennische and Hansson 1986). Reperfusion impairment occurs after thirty minutes to six hours of skeletal muscle ischemia (Strock and Majno 1969, Larsson and Lewis 1978, Kennedy et al. 1981, Korthius et al. 1985, Suval et al. 1987, Blebea et al. 1987). Two hours of tourniquet ischemia induced significantly greater impairment of reperfusion than one hour of ischemia in rat tibialis anterior muscle (Gidlöf 1988). These observations correlate with a two hour threshold effect for tourniquet compression injury. Abnormal microvascular permeability (ie., Evans blue extravasation) has been observed after graded compression and tissue reperfusion in skeletal muscles (Pedowitz et al. 1991a, 1992d), peripheral nerves (Lundborg 1970, Rydevik 1979), and spinal nerve roots (Olmarker et al. 1989). Increased permeability could relate to mechanically-induced endothelial disruption. Endothelial swelling (Gidlöf 1988, Gidlöf et al. 1988), tissue edema (Strock and Majno 1969), and leukocyte alterations (Amundson et al. 1980, Bagge et al. 1980, Braide et al. 1984, Schmid-Schönbein 1987) may precipitate microvascular obstruction.

After extended periods of ischemia, tissue reperfusion and rapid influx of molecular oxygen results in the production of toxic materials, such as superoxide radicals and hydrogen peroxide (Del Maestro et al.

1980, Del Maestro et al. 1981, Korthius et al. 1985, Parks et al. 1983, Parks and Granger 1986, Jarasch et al. 1986, Soussi 1989, Smith et al. 1989). Free radical formation may lead to membrane lipid peroxidation, degradation of glycocalyx, and increased skeletal muscle vascular permeability (Svingen et al. 1979, Del Maestro 1981, Freeman and Crapo 1982, Parks et al. 1983, Korthius et al. 1985, Malis and Boneventre 1986, Lee et al. 1987, Soussi 1989). Superoxides are produced via enzymatic degradation by xanthine oxidase within skeletal muscle vascular endothelium (Korthius et al. 1985). Increased xanthine oxidase activity (Smith et al. 1989) and increased enzyme substrates (Soussi 1989) are noted after two hours of skeletal muscle ischemia.

Neutrophils also contribute to free radical formation (Harlan 1985, Engler 1987, Belkin et al. 1989), and neutrophil activation results in nearly a 100-fold increase in oxygen consumption (Mehta et al. 1988). Increased levels of activated neutrophils are associated with higher mortality rate following rat hemorrhagic shock (Barroso-Aranda and Schmid-Schönbein 1989). Leukocyte activation may be induced by endothelial injury and by mechanical shear (Barroso-Aranda and Schmid-Schönbein 1989). These triggers could be proportional to the degree of mechanical deformation (ie., the tourniquet inflation pressure).

Several findings in the present studies suggest that microvascular phenomena are of particular importance in the pathogenesis of tourniquet-induced muscle injury. Scattered fiber necrosis was observed after one hour of tourniquet compression and after longer periods of distal ischemia; this could be related to flow disturbance at the capillary level. Regional necrosis was noted in muscle compressed for two hours with 350 mmHg cuff pressure. Since cellular infiltration and tissue calcification were present in the center of these necrotic regions just two days after compression, these findings suggest delayed reflow (as opposed no-reflow) and/or reperfusion-mediated injury in regions of tissue supplied by "secondary arteries" (Myrhage 1977). Four hours of tourniquet compression was associated with zones of absent Evans blue extravasation, which probably reflect persistent, regional no-reflow phenomenon. Thus, the extent and distribution of tourniquet-induced muscle fiber necrosis may reflect various degrees of local and regional microvascular abnormality related to the magnitude and duration of tissue compression.

Function and structure of compressed nerves

Previous investigators noted abnormal toe-spread two to four weeks after two hour tourniquet application in rabbits and rats (Lundborg 1970, Nitz et al. 1986). In the present study, abnormal toe-spread was observed in limbs with reduced conduction velocity, but with relatively uniform compound motor action potential propagation. Dahlin et al. (1989) recently observed in rabbits greater conduction abnormalities in peroneal nerve than in tibial nerve compressed with a miniaturized compression device (200 or 400 mmHg). These effects may relate to differences in anatomic location or in the connective tissue composition of the two nerves (Dahlin et al. 1989). Tourniquet application on rabbit thighs may particularly effect the common peroneal nerve, due to the nerve's superficial course in the distal thigh. Alternatively, the discrepancy between toe-spreading and the neurophysiologic findings could reflect injury of the neuromuscular junction, which was not specifically assessed in the present study.

Fowler et al. (1972) noted decreased nerve conduction velocity (NCV) without a fall in muscle action potential two days after tourniquet application in baboons. Decreased NCV may be secondary to changes in myelin impedance or membrane permeability without loss of axonal continuity (Ochoa et al. 1972). Nitz et al. (1989) observed intramyelin cleavage and increased sheath thickness (suggestive of an intra-myelin edema) after tourniquet compression.

Using the present techniques, NCV reflects function of the largest and fastest conducting motor fibers (Fowler et al. 1972). NCV distal to the tourniquet returned to control values one day after 350 mmHg tourniquet application, but was still decreased two days after 1000 mmHg cuff inflation. The etiology of distal fiber disruption in the 1000 mmHg compression group is not clear; perhaps it reflects indirect mechanical deformation, such as stretch, caused by extremely high cuff inflation pressure.

Normalization of CMAP amplitude against distal amplitude facilitates relative functional assessments along a segment of nerve (Gilliatt 1980). The technique does not reveal non-functioning neuromuscular units distal to the region of nerve stimulation, for example deficit at the neuromuscular junction. CMAP amplitudes were normalized against control values for corresponding stimulation positions, since CMAP amplitude decreased with more proximal stimulation in the non-compressed limb (ie., recording dispersion). Direct recording of compound nerve action potentials would allow for specific evaluations of recording dispersion, sensory nerve dysfunction, and differential

fiber sensitivity to compression and ischemia; such techniques were beyond the scope of the present investigation.

Recovery of action potential propagation was noted one day after 350 mmHg tourniquet application (Figure 8), although functional (toe spread) and structural (see below) abnormalities were still detected in these nerves. In contrast, complete conduction block was observed in all limbs two days after 1000 mmHg compression. In these nerves, the greatest deficit was located beneath the distal cuff margin, which is consistent with an edge effect at the cuff border. However, these recordings could also reflect uniform injury beneath the tourniquet, with rapid return of function just distal to the compressed region.

Ochoa et al. (1972), using individual teased fiber preparations, observed paranodal myelin invagination after 1000 mmHg tourniquet compression of the medial popliteal nerve of baboons. These often-cited histologic abnormalities have not been duplicated in tourniquet compression studies (Gilliatt, personal communication, 1988). Gross myelin changes were not observed after 500 mmHg tourniquet application (Ochoa et al. 1972). This is consistent with the findings of the present study, where dramatic myelin damage was observed in nerves compressed by a 1000 mmHg tourniquet, but few myelin abnormalities were observed after 350 mmHg tourniquet application. Thus, myelin invagination is probably not a major mechanism of nerve injury with clinically relevant tourniquet pressures and durations.

After two hours of 350 mmHg tourniquet application, electron microscopy revealed scattered axonal degeneration in fibers beneath and distal to the tourniquet. These observations are consistent with electromyographic evidence of axonal degeneration and regeneration following routine clinical tourniquet application (Saunders et al. 1979, Weingarden et al. 1979, Dobner and Nitz 1982, Nitz and Dobner 1989). Two hours of 300 mmHg and 400 mmHg tourniquet compression in rats produced an average degeneration of 15 percent and 45 percent of the myelinated nerve fibers (Nitz et al. 1989).

Mechanisms of tourniquet-induced nerve injury

Functional and structural changes are well described for peripheral nerves subjected to tourniquet ischemia. Complete conduction block is usually observed within 15-45 minutes of tourniquet inflation (Lundborg 1970, Caruso et al. 1973, Lundborg et al. 1973, Nielsen and Kardel 1974, Yates et al. 1981, Hurst et al. 1981, Rorabeck 1980, Rorabeck and Kennedy 1980, Lundborg 1989, Chabel et al. 1990). Distal nerve conduction recovers to baseline values within 2 to 30 minutes following tourniquet times less than two hours (Lundborg 1970, Nielsen and Kardel 1974, Rorabeck 1980, Hurst et al. 1981). Incomplete functional recovery follows six to eight hours of proximal tourniquet application, while no recovery may be observed after ten hours of continuous nerve ischemia (Lundborg 1970).

A nerve's ischemic sensitivity may be affected by the distribution of its fiber diameters. Smaller and non-myelinated fibers may be more sensitive to ischemia (Lundborg 1970, Dahlin et al. 1989), however some report that ischemic sensitivity is not related to axon diameter (Mäkitie and Teräväinen 1977, Nitz et al. 1989). Differential axonal sensitivity could relate to variations in the diffusion distance, metabolic demand, or in the nutritional supply of the various fiber types (see Lundborg 1970 and 1989 for review). Degenerative changes in peripheral nerves are observed after four to six hours of tourniquet ischemia (Lundborg 1970, Tountas and Bergmann 1977). Mäkitie and Teräväinen (1977) noted abnormalities of motor endplates after two hours of tourniquet application, and suggested that the neuromuscular junction may be particularly sensitive to ischemia.

Grundfest (1936) studied effects of compression upon frog sciatic nerve enclosed in a high pressure container. At extreme pressure conditions (1000 atmospheres) the nerve continued to conduct. However, uniform tissue compression (ie., deep sea diving) is not relevant to the situation of local nerve compression (Gilliatt 1980). Denny-Brown and Brenner (1944) believed that nerve dysfunction was related to the degree of local ischemia induced by compression. Others reported that mechanical deformation, per se, was most important in the causation of conduction block (Eckhoff 1931, Bentley and Schlapp 1943). Rorabeck (1980) noted a three hour time threshold for incomplete early recovery of nerve conduction velocity after 500 mmHg tourniquet compression in dogs. Functional (Nitz and Dobner 1986) and structural (Nitz and Dobner 1989) abnormalities are observed one to five weeks after one to three hours of 200 to 400 mmHg tourniquet application in rats.

Although myelin invagination is probably not a basic mechanism of tourniquet- induced nerve injury with clinically relevant cuff inflation pressures, other processes may cause structural and functional abnormalities. Nitz et al. (1989) suggest that tourniquet compression causes increased microvascular

permeability and intraneural edema formation, with persistent tissue ischemia and subsequent nerve degeneration. This hypothesis is supported by intraneural microvascular abnormalities and edema formation induced by acute, graded compression of peripheral nerves (Rydevik and Lundborg 1977, Rydevik and Nordborg 1980, Rydevik et al. 1981) and of spinal nerve roots (Olmarker et al. 1989). Nerve compression may cause increased endoneurial fluid pressure, with further compromise of local tissue nutrition (Lundborg et al. 1975, Lundborg et al. 1983). Thus, a vicious cycle of compression — edema — nutritional disturbance — increased edema — etc., may be started by the initial nerve insult. Abnormal axoplasmic transport (Rydevik et al. 1980), cellular infiltration (Nitz et al. 1989), and intraneural fibrosis (Lundborg 1970, Rydevik et al. 1976, Rydevik and Lundborg 1977) may affect chronic injury patterns.

The etiology of edema formation in compression neuropathy is incompletely understood. Mechanical deformation may cause direct endothelial disruption with increased protein permeability (Lundborg 1970, Rydevik and Lundborg 1977). Mast cells may contribute to microvascular abnormality, and increased local mast cell concentration has been observed after tourniquet compression (Nitz et al. 1989). However, these cells could aggregate in response to tissue injury without being responsible for the pathologic event. Leukocyte-mediated superoxide radical formation could cause increased permeability after compression and ischemia.

An edge effect of tissue injury may be secondary to maximal pressure gradients or shear stress concentration at the boundaries of applied compression. There was severe myelin deformation beneath the borders of a 1000 mmHg tourniquet in the present and previous studies (Ochoa et al. 1972). Using lower compression pressures (50 to 400 mmHg), greatest abnormalities of microvascular permeability were observed at the margins of compression in peripheral nerves (Rydevik and Lundborg 1977) and spinal nerve roots (Olmarker et al. 1989). In some cases in the present study, localized neurophysiologic deficits occurred near a transversely-oriented neurovascular bundle or fascial structure. These observations could reflect focal deformation of the nerve by a stiffer structure during tourniquet compression. This phenomenon could be important when nerves are compressed by a tourniquet against bone (radial nerve on humerus) or connective tissue (femoral nerve in the adductor canal), or following injury with disturbed local anatomy (Kurihara and Goto 1990).

Tourniquet sensitivity—nerve versus muscle

Some investigators report that skeletal muscle is more sensitive than peripheral nerve (Korthals et al. 1985, Sapega et al. 1985) while others believe that nerve is more affected than muscle (Chervu et al. 1989). Others report that the neuromuscular junction is most sensitive to ischemia (Thomason and Matzke 1975, Mäkitie and Teräväinen 1977). It is inappropriate to compare nerve and muscle directly, since these tissues possess unique functional and structural characteristics. It is of greater relevance to consider patterns of tissue injury as they may affect post-operative rehabilitation. Little information is available regarding the relative sensitivities of muscle and nerve to mechanical deformation.

One hour of tourniquet application was associated with increased Tc-99 uptake in thigh and leg muscles. Previous studies demonstrate reduction of muscle force after one hour of tourniquet compression (Gersoff et al. 1989) or ischemia (Fish et al. 1989). Regional necrosis was observed after two hours of tourniquet compression. The force capacity of these muscles is probably greatly reduced, since Lieber et al. (1990) observed approximately 70 percent reduction of tibialis-anterior force two days after two hours of ischemia distal to a tourniquet. Muscle functional deficits would be particularly important during the early post-operative period when surgeons emphasize range of motion and muscle rehabilitation, for example after arthroscopy or total knee arthroplasty. However, skeletal muscle has a remarkable regenerative capacity (Allbrook et al. 1966, Carlson 1978); nearly complete skeletal muscle regeneration has been noted three weeks after ischemia-induced fiber necrosis (Jennische 1986).

In certain clinical situations, early neurologic dysfunction may be more important than skeletal muscle injury. In the present study, physiologic and morphologic changes of peripheral nerves were present two days after tourniquet application. Morphologic abnormalities were observed six weeks after clinically-relevant tourniquet application (Nitz et al. 1989). Electromyographic (EMG) abnormalities persist up to six months after tourniquet application in humans (Saunders et al. 1979, Weingarden et al. 1979, Dobner and Nitz 1989, Nitz and Dobner 1989).

After an upper extremity reconstructive procedure, nerve compression injury may interfere markedly with rehabilitation, while biceps or triceps muscle damage may not be of great clinical significance. In later periods of recovery, peripheral nerve injury is probably more important than skeletal muscle injury, due to the nerve's limited regenerative capacity (Mäkitie and

Teräväinen 1977, Saunders et al. 1979, Weingarden et al. 1979, Nachemson 1988, Dobner and Nitz 1989, Nitz and Dobner 1989, Lundborg 1989). Fortunately, permanent neurologic injuries are rare with modern tourniquet techniques (Flatt 1972, Middleton and Varian 1974).

Clinical implications

For a given patient, the cuff pressure needed to occlude arterial flow is affected by various factors, including systolic blood pressure (Van Roeckel and Thurston 1985), limb circumference (Van Roeckel and Thurston 1985, Moore et al. 1987, Crenshaw et al. 1988, Pedowitz et al. 1992c), limb shape and local anatomy (Rorabeck and Kennedy 1980), vascular status (Klenerman and Lewis 1976, Jeyaseelan et al. 1981), and the width of the applied tourniquet (Muirhead and Newman 1986, Moore et al. 1987, Crenshaw et al. 1988, Pedowitz et al. 1992c). Cuff design may also influence the tourniquet pressure needed to induce surgical hemostasis (Pedowitz et al. 1992c).

Increased awareness of the deleterious effects of tissue compression has resulted in clinical recommendations for use of lower tourniquet inflation pressures (Sanders 1973, Klenerman and Hulands 1979, Klenerman 1980, Shaw and Murray 1982, Reid et al. 1983, McLaren and Rorabeck 1985, Hargens et al. 1987, Moore et al. 1988, Lundborg 1989, Crenshaw et al. 1989, Gersoff et al. 1989). The lowest possible cuff inflation pressure should be facilitated by direct determination of the arterial occlusion pressure (AOP) for a given patient and a given tourniquet. To be clinically useful, these protocols should be quick, reliable, minimally traumatic, and must produce a surgically acceptable bloodless field. Dopplers, pulse oximeters, and photoplethysmographs are used widely for noninvasive vascular evaluation; these devices differ in simplicity, sensitivity, and expense (Morris et al. 1989). The present study was not intended to define or recommend the optimal equipment for clinical application.

AOP measurements should be similar with inflation of the cuff to the point of no pulsatile flow, or with deflation of the cuff until flow resumes (Dahn et al. 1967). The tourniquet pressure should be changed slowly (for example 1 mmHg per second) in order to minimize errors using either technique. Approximately 15 percent of our cases were rated as having either fair or poor hemostasis, although acceptable hemostasis was achieved quickly by raising the cuff inflation pressure. Most of the failures of hemostasis were caused by either too rapid deflation of the cuff during measurement of AOP (ie., falsely low AOP determination) or by increased systolic blood pressure due to surgical stimulation or a change in level of anesthesia (Kaufman and Walts 1982, Valli and Rosenberg 1985, Chabel et al. 1990). These problems could be minimized by adjusting the cuff pressure according to intraoperative changes in systolic blood pressure (McEwen and McGraw 1982). Alternatively, 75 mmHg could be added to the AOP (instead of 50 mmHg) to provide a larger buffer during surgery (Reid et al. 1983).

Curved tourniquets occluded flow at lower pressures than straight tourniquets of equal width, which was probably due to better cuff fit and more efficient pressure transmission on conically-shaped limbs. Alternatively, curved cuffs may cause an effectively wider region of tissue compression, or the observed differences could relate to variable material composition of these particular straight and curved tourniquets. Wider tourniquets were associated with lower AOP than narrower tourniquets; previous investigators report similar findings (Muirhead and Newman 1986, Moore et al. 1987, Crenshaw et al. 1988).

In the present study, mean cuff pressures of 184 and 208 mmHg were used to induce surgical hemostasis in upper and lower extremity surgeries, respectively. Such tourniquet pressures are probably lower than those in general clinical use. Although tourniquet-induced neuromuscular injury should be decreased by use of the lowest possible cuff inflation pressure, careful prospective studies are needed to examine effects upon post-operative morbidity and functional recovery, and to address the cost-benefit implications of institution of modified clinical protocols.

Summary

A rabbit model was developed which facilitates controlled, experimental studies of tissue injury beneath and distal to a pneumatic tourniquet. Non-uniform tissue deformation was observed beneath inflated tourniquets; such patterns were not predicted by previous mathematical models. Two hours was a time threshold for tourniquet compression injury; depending upon the cuff inflation pressure, greater muscle injury was induced beneath the tourniquet than distal to it. A topographic pattern of necrosis was observed after two hours of tourniquet compression, which may relate to the microvascular anatomy of skeletal muscle and to pathogenic events during tissue reperfusion. With a four hour total tourniquet time, skeletal muscle injury beneath the cuff was significantly decreased by hourly, ten minute reperfusion intervals. A reperfusion interval after two hours of 350 mmHg cuff inflation tended to exacerbate muscle injury.

Physiologic and morphologic nerve abnormalities were induced by a two hour, 350 mmHg tourniquet. Axonal degeneration may correlate with EMG changes after clinical tourniquet application. Paranodal myelin invagination is probably not an important mechanism of injury at clinically relevant tourniquet inflation pressures. Wide cuffs, limb shaped cuffs, and direct determination of the minimal necessary inflation pressure facilitated the use of lower tourniquet pressures in extremity surgery.

In conclusion, tourniquet application, at clinically relevant cuff inflation pressures and durations, induces greater neuromuscular injury beneath the tourniquet than distal to it. Investigators of systemic effects of limb ischemia should be aware of compression injury induced by pneumatic tourniquet models. Surgeons must weigh the advantages of a bloodless field against the disadvantages of tourniquet-induced neuromuscular injury.

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