# Total and Regional Blood Flows in Latissimus Dorsi Muscles Transplanted into the Rectus Femoris Site of Rabbits

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**Abstract**

The objective of this study was to measure total and regional blood flows at rest and during twitch contractions in skeletal muscle transplanted into the site of a structurally and functionally different muscle. The latissimus dorsi (LTD) muscle was transplanted into the site of the rectus femoris (RFM) muscle in rabbits. After 90 days, blood flows were measured at rest and during twitch contractions at stimulation frequencies of 2 and 4 Hz using radioactive microspheres. Total blood flows at rest and at 2 Hz were significantly higher ($p < 0.05$) in LTD grafts (12.1 ± 2 and 27.6 ± 1 ml/100 g/min) compared to control LTD muscles (5.4 ± 2 and 12.5 ± 3 ml/100 g/min), but were not different at 4 Hz (26.5 ± 3 ml/100 g/min in grafts vs 32.0 ± 3 ml/100 g/min in controls). When expressed regionally, blood flows in the grafts were higher than the control muscles in the proximal and belly regions of the muscle at rest and during contractions. Compared to control muscles, graft blood flow in the distal region was higher at rest, not different at a stimulation frequency of 2 Hz and lower at 4 Hz (p 0.05). Blood flows in LTD grafts during twitch contractions were considerably lower than flows previously reported in both control RFM muscles and neurovascular-anastomosed RFM grafts. These findings show that blood flow responses of activated LTD muscle grafts in the RFM site resemble more closely the responses of control LTD muscle rather than the muscle indigenous to that site.

**Key words:** skeletal muscle transplantation, radioactive microspheres, twitch contractions, blood flow, rabbits.

*BAM 2 (2): 127-134, 1992*

Rapid advances in microsurgical techniques have enabled surgeons to correct a variety of skeletal muscle deficiencies through the transplantation of viable skeletal muscle using nerve and blood vessel anastomoses [4, 5, 6, 9, 10, 16, 24]. By limiting the time of muscle ischemia, injury and degeneration of the muscle fibers can be minimized [22]. A particular skeletal muscle is often selected as an acceptable donor because it is easily accessible, has a single neurovascular pedicle which facilitates the reattachment of nerves and blood vessels, and/or because the removal of the muscle does not compromise overall function and/or appearance [5, 9, 10, 17, 20, 24]. Muscles used for transplantation purposes, however, may differ in architectural, biochemical and functional characteristics from the muscle originally in the recipient site [5, 17, 19].

Muscles transplanted with neurovascular anastomoses will change or adapt biochemically to a new site [3, 21] but not necessarily in terms of architecture or fiber length [8]. Consequently, skeletal muscle transplanted into the location of a different muscle may not adapt sufficiently to act as a total functional replacement.

The specific objective of this study was to determine if blood perfusion in a muscle transplanted into the location of a structurally and functionally dissimilar skeletal muscle "adapts" to the requirements of the new site. To pursue this objective, we used tracer labeled microspheres to measure total and regional blood flows in neurovascular-anastomosed latissimus dorsi (LTD) muscle grafts, transplanted into the rectus femoris (RFM) muscle site in rabbits. The LTD muscle is commonly used as a donor muscle to treat

muscle deficiencies in humans because of its accessibility and dominant single vascular pedicle [5, 17, 20].

Based on a previous study that demonstrated no adaptation of fiber architecture or fiber length in LTD muscle transplanted into the RFM site [8], we hypothesized that no differences in blood flows at rest and during twitch contractions would exist between the LTD muscle transplants and control LTD muscles. A comparison of the results of this study is made to previously reported blood flows in control RFM muscles and neurovascular- anastomosed RFM muscle autografts [2].

## Materials and Methods

Eighteen female New Zealand white rabbits, ranging in weight from 3.1 to 3.7 kg, were used in this study. Five rabbits were used for the determination of blood flows in control LTD muscle at rest and in response to twitch contractions at 2 and 4 Hz. Thirteen rabbits had a portion of the left LTD muscle transplanted into the site of the rectus femoris muscle in the left leg. All operations, postoperative care and experimental procedures were conducted with institutional approval and in accordance with the policy statement outlining the care and use of animals published by the American Physiological Society.

### *Grafting Procedure*

The animals were sedated with xylazine (10 mg/kg; i.m.) 10 min prior to being anesthetized with ketamine chloride (50 mg/kg; i.m.). Anesthesia was supplemented with ketamine chloride, as required. All surgical procedures were performed under sterile conditions.

For transplantation of the LTD muscle, the muscle was exposed through a 12-14 cm skin incision beginning in the axilla and extending caudally and dorsally, paralleling the anterior free border of the muscle. The panniculus carnosus muscle was excised with the skin. The origin of the LTD muscle from the thoracodorsal fascia and vertebral spinous process was cut. The muscle's insertion on the humerus was divided, exposing the thoracodorsal neurovascular pedicle. The circumflex scapular artery and vein were identified, ligated and divided, leaving the thoracodorsal vessels attached to their origin from the subscapular artery and vein. The thoracodorsal nerve and subscapular artery and vein were divided at their origins from the axillary vessels. A moist sponge was placed around the muscle and the muscle was removed from the operative field.

The RFM muscle was exposed and removed from the leg [2]. The LTD muscle was brought into the thigh wound and positioned with the muscle's insertion placed proximally to facilitate microsurgical repair. Microvascular anastomoses were performed between the thoracodorsal vessels of the LTD muscle and the vessels previously supplying the RFM muscle. Anastomoses were performed under binocular magnification using 10-0 nylon sutures. Neurorrhaphy between the thoracodorsal nerve and the nerve to the RFM muscle was accomplished in a similar fashion. After completion of the anastomoses, microvascular clamps were released and viability of the muscle was assessed by observation of bleeding from the cut edge of the muscle. The LTD tendon of insertion was sutured to the previously divided origin of the RFM muscle and inguinal ligament, using 4-0 nylon suture. The muscle was placed under tension and trimmed in order to fit in the RFM site. The distal portion of the muscle was sutured to the insertion of the RFM muscle and patellar tendon, using 4-0 nylon suture. The fascia was reapproximated over the transplant and skin incisions on the back and thigh were closed using interrupted sutures of 4- 0 nylon. Each rabbit received a subcutaneous injection of the antibiotics trimethoprim (4.5 mg/kg) and sulfadiazine (22 mg/kg) immediately following the operation and daily for six days. The animals were returned to their cages for 90 days where they were supplied with water ad libitum and 125 g/day of Purina rabbit chow. One rabbit developed an abscess in the region of the transplant at 50 days and was sacrificed.

### *Measurement of Blood Flow*

Blood flows of LTD grafts and control LTD muscles were compared at rest and during twitch contractions at 2 and 4 Hz. We have previously shown that, under these conditions, maximum blood flow is achieved at a stimulation frequency of 4 Hz [2]. Blood flow measurements were made with tracer-labeled microspheres (15 um dia., New England Nuclear, Boston, MA). The procedure for measuring total and regional blood flows in skeletal muscle grafts in the rectus femoris site in rabbits has been described in detail elsewhere [2]. Briefly, animals were anesthetized via the marginal ear vein with a 15% solution of urethane (1.5 g/kg) and tracheotomized. A cannula was advanced through the right carotid artery into the left ventricle. Correct placement of the cannula tip was verified by continuously recording blood pressure and observing the characteristic waveform of the left ventricular pressure. A second cannula was placed in the right femoral artery.

To prepare the animal for measurements of blood flow, the muscle graft was carefully exposed and isolated. The neurovascular pedicle of each graft was examined to ensure that the artery and vein were patent and that the nerve was intact and appeared healthy. The distal tendon of the muscle graft was severed and attached to a force transducer (Grass, model FT-10). A skin pouch was formed around the muscle and was filled with heated (37°C) mineral oil. The left knee and both hips of the rabbit were immobilized with steel pins, anchored to a steel platform. The muscle grafts were stimulated with square wave impulses, 1 msec in duration, through two platinum electrodes, one inserted into either end of the muscle. Stimulus intensity (80- 100 volts) and muscle length were adjusted to provide maximum isometric tension.

To measure blood flow in the control LTD muscles, the rabbit was anesthetized and placed on its right side. The left humerus was abducted to its fullest range of motion and was secured in this position. A small incision was made in the axillary region and the insertion of the muscle

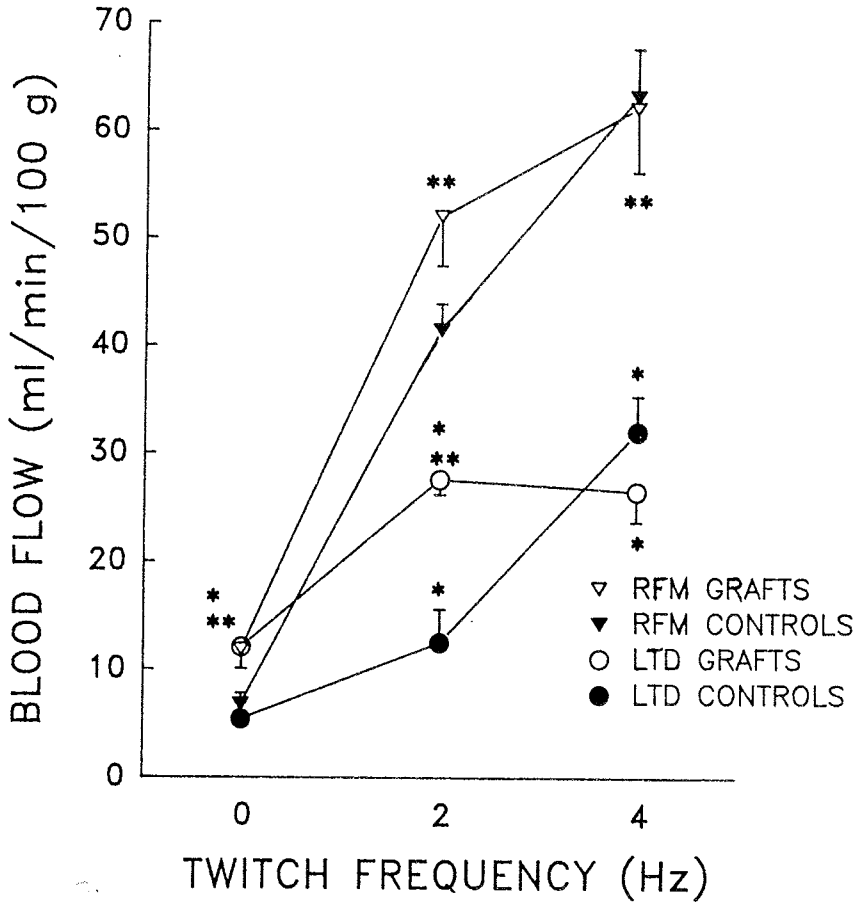


*Figure 1. Total blood flows at rest and during twitch contractions in control latissimus dorsi (LTD) muscles, neurovascular-anastomosed LTD grafts in the RFM site, control rectus femoris (RFM) muscles and neurovascular-anastomosed RFM grafts at rest and during twitch contractions at 2 and 4 Hz. Values given are means ± SE. Sample sizes at rest, 2 and 4 Hz, respectively: LTD control, n = 4, 4, 4; LTD graft, n = 6, 5, 4; RFM control, n = 7, 6, 4; and RFM graft n = 4, 5, 5.*

** Significantly different from RFM control muscles.*

*** Significantly different from control LTD muscles.*

*Note: Data for RFM controls and RFM grafts are reproduced, with permission, from Burton et al., Am. J. Physiol. 255: H1043-H1049, 1988.*

on the humerus was severed. At the same time the motor nerve was isolated, tied with thread and severed proximal to the tie. The proximal tendon of the muscle was attached to a force transducer. The left shoulder and both hips were immobilized with steel pins. The muscle was stimulated through the nerve with supramaximal (4-5 volts) square wave impulses, 0.2 msec in duration.

For each experiment, either two or three of the radioisotopes $^{46}$Scandium, $^{103}$Ruthenium, $^{141}$Cerium, and $^{85}$Strontium, were injected into the left ventricle of the rabbit. Muscle stimulation at 2 or 4 Hz lasted for approximately 3 to 3.5 min. Microspheres were injected over a period of 30 sec between 1.5 and 2 min after the start of muscle stimulation. Reference blood samples were withdrawn from the femoral artery at a rate of 1.5 ml/min, beginning 10 sec prior to the injection of the microspheres and continuing for approximately 100 sec. Blood pressure was recorded from the femoral artery before twitch contractions were started, for one minute during twitch contractions and within ten sec after ending the reference blood sample withdrawal.

The order of muscle stimulation was not randomized. Stimulation at a frequency of 2 Hz always preceded stimulation at 4 Hz. In a previous study [2], it was observed that twitch tension did not return to initial tension values at any time following a stimulation protocol of 4 Hz. In order to maximize the use of each animal and to keep the total number of animals used to a minimum, the decision was made to inject three isotopes per experiment and to follow a standard protocol of rest, 2 Hz and then 4 Hz stimulation. At least 15 min was allowed between stimulation protocols to allow the twitch tension to return to, or near initial tension levels. If this did not occur, the experiment was terminated without proceeding to the 4 Hz protocol. At the

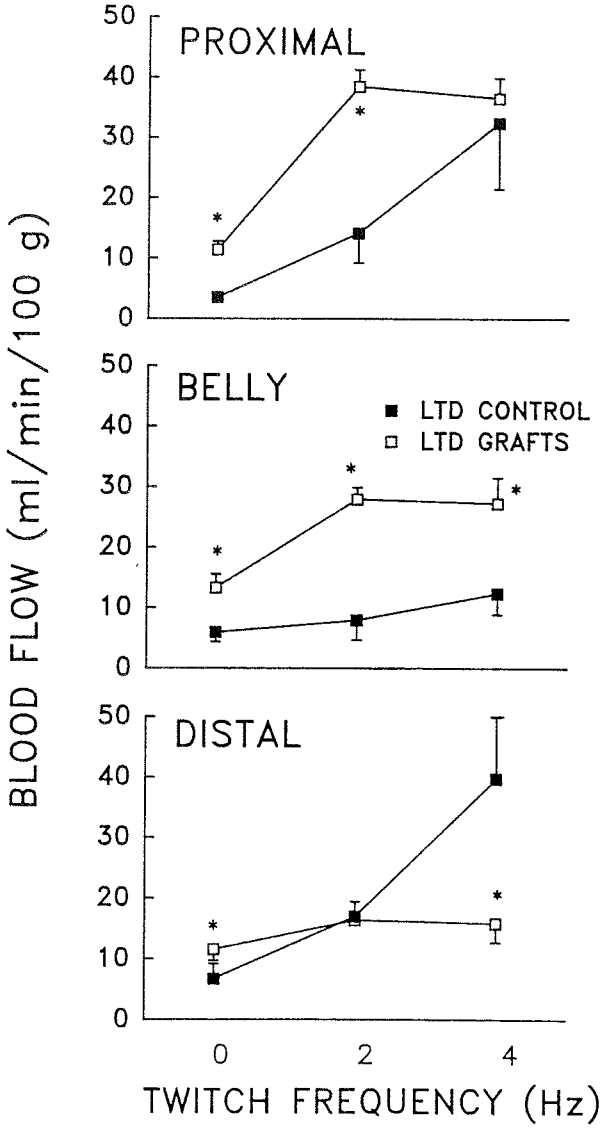


*Figure 2: Regional blood flows at rest and during twitch contractions at frequencies of 2 and 4 Hz in control and grafted LTD muscles. Values given are means ± SE. Sample sizes at rest, 2, and 4 Hz, respectively: LTD control, n = 4, 4, 4; LTD graft, n = 6, 5, 4.*
** Significantly different at p 0.05.*

end of each experiment, the animals were sacrificed with an overdose of urethane. The LTD muscle or graft and both kidneys were removed, weighed and placed in 10% formaldehyde.

To measure the levels of radioactivity, each muscle or graft was divided in half and each of the sections was divided in half and so on until eight sections of approximately equal mass were obtained. Each section was weighed and placed into a scintillation vial. Levels of radioactivity were measured in three samples of tissue from the cortex of each kidney to ensure that the microspheres were adequately mixed within the blood circulation [11]. All tissue samples were assayed in a Tracor (model 1185) gamma scintillation counter. Blood flow was calculated using the equation: $Q^t = (C^t \times Q^r)/C^r$, where $Q^t$ = tissue blood flow in ml/min, $C^t$ = counts/min in the tissue samples, $Q^r$ = the withdrawal rate of the reference arterial sample in ml/min, and $C^r$ = counts/min in the reference arterial sample [11]. Blood flows in each sample were divided by the weight of the sample and expressed as ml/100g/min.

Total blood flows and flows in the proximal, belly and

distal regions of the muscle were compared between LTD grafts and controls. To compare regional blood flows, the first two muscle sections were combined to represent flow in the proximal region of the muscle, the next four sections represented flow in the belly and the last two, flow in the distal region. Deletion of some trials for either non-uniform distribution of the microspheres, failure of twitch tension to return to initial levels, or failure of blood pressure to return to control levels resulted in unequal sample sizes for blood flow values (Fig 1, 2).

For statistical comparisons involving two means, a t-test was used to determine the significance of the differences between the means. When more than two means were compared, differences among groups were determined using a one-way analysis of variance. If a significant F value was obtained, a Scheffe comparison was performed to indicate where the difference occurred. A p value of 0.05 was considered to be significant. All data are presented as means ± SE.

## Results

In four of the twelve experimental rabbits the vascular pedicle was non-existent but the grafts were viable. The grafts were revascularized from the surrounding tissue by means of multiple small blood vessels entering the graft from multifocal origins. The mass of the spontaneously revascularized grafts (2.3 ± 0.7 g) was considerably less than that of the grafts with patent vessels (4.5 ± 0.5 g).

After being trimmed to fit the RFM site, the mass of the transplanted LTD muscles was 6.2 ± 0.4 g compared to the mass of the control muscles of 12.6 ± 0.3 g. Maximum absolute twitch force of the LTD grafts (5.6 ± 0.6 N) was significantly lower than the maximum twitch force of control LTD muscles of 7.3 ± 0.4 N but there was no difference between grafts and control muscles when force was expressed relative to cross-sectional area (8.6 ± 0.6 vs 8.0 ± 0.3 N/cm2, respectively).

The mean arterial blood pressure in resting control animals was 99 ± 2 mmHg, which was not significantly different than the value of 104 ± 2 mmHg in animals with grafts. In both groups, blood pressure decreased significantly during twitch contractions at 2 Hz (88 ± 3 mmHg for controls, 83 ± 2 mmHg for grafted animals) and at 4 Hz (78 ± 2 mmHg for controls and 74 ± 3 mmHg for grafted animals). No significant differences in mean arterial blood pressures were observed between control and experimental animals during contractions at either 2 or 4 Hz. In both groups blood pressure returned to control levels immediately after twitch contractions were terminated.

At rest and during twitch contractions at 2 Hz, blood flows in grafts were significantly higher ($p < 0.05$) than flows in control LTD muscles, whereas no difference in blood flows was observed at 4 Hz (Fig 1). The blood flows in the LTD grafts at both 2 and 4 Hz were considerably lower than flows observed in control RFM muscles and neurovascular-anastomosed RFM autografts in a study conducted previously in this laboratory (Fig 1).

When divided into proximal, belly and distal regions, blood flows in the LTD grafts were significantly higher than flows in control muscles in all regions at rest. During contractions, graft blood flow was higher only in the proximal and belly regions ($p < 0.05$) (Fig 2). At a stimulation frequency of 4 Hz, blood flow in the LTD grafts was not different compared to control muscles in the proximal region, but was higher in the belly and lower in the distal region ($p < 0.05$).

Fig 3 shows the twitch force ($P_t$) at 2 and 4 Hz for control LTD muscles and LTD grafts over 3 min, relative to the maximum force achieved ($P_{tmax}$). At a stimulation frequency of 2 Hz, $P_t/P_{tmax}$ of control muscles had declined to 74 ± 4.5 % by 3 min, whereas graft $P_t/P_{tmax}$ was 90 ± 2.9 %. At 4 Hz $P_t/P_{tmax}$ of controls was 38 ± 7.1 % compared to 53 ± 4.2 % for grafts.

## Discussion

The most significant finding of this study was the observation that during twitch contractions, total blood flows in LTD muscles grafted into the RFM site were not appreciably changed from blood flows in control LTD muscles but were considerably lower than previously reported blood flows in control RFM muscles and in RFM muscle grafts.

The primary objective of skeletal muscle transplantation is to correct a particular muscle deficiency and restore partial or complete functional capabilities [10, 16, 17, 23, 24]. Guelinckx and Faulkner [8] reported that transplantation of the parallel- fibered LTD muscle into the site of the bipennate-fibered RFM muscle resulted in incomplete adaptation of the LTD graft to the new site. Although biochemical changes occurred within the fibers of the transplanted muscle, neither the fiber length nor the angle of pennation of the LTD transplant changed significantly. This resulted in a much lower maximum absolute tetanic force in the LTD grafts. In our study, although blood flow distribution was somewhat altered, there was little change in total blood flow in LTD transplants during twitch contractions compared to the values obtained from LTD controls. Further, despite receiving the neural innervation and vascular supply common to the RFM muscle, blood flow values during twitch contractions remained well below values obtained for either control RFM muscles or RFM grafts [2]. Despite difficulties inherent in making comparisons between muscles of different mass, vascular anatomy, and fiber type distribution, the present study supports the contention of Guelinckx and Faulkner [8] that matching the structural and functional properties of a donor muscle with the properties of the muscle in the recipient site is an important consideration when selecting a muscle for the purpose of transplantation.

The restoration of normal contractile function in muscle transplants, including fatigue characteristics, is dependent largely upon adequate blood perfusion. White et al. [26] reported that maximum blood flow values in extensor digitorum longus muscle autografts in cats (grafted with-

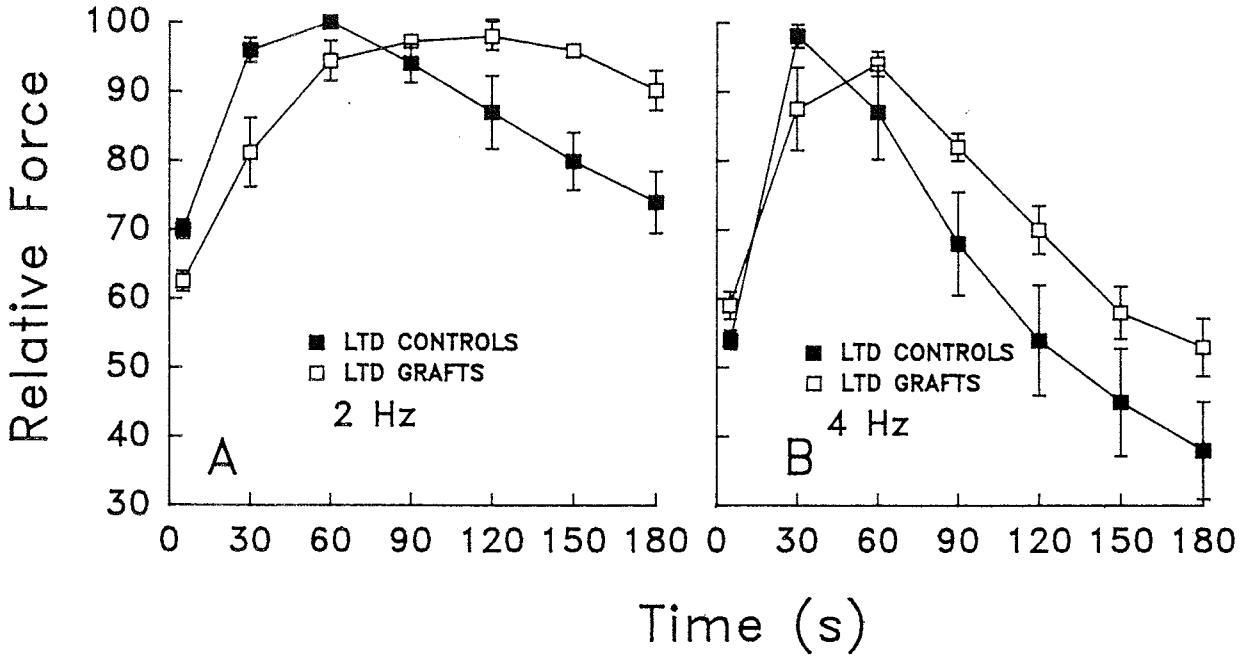


*Figure 3: Decline in force over 3 min for control LTD muscles and grafts during twitch contractions at 2 Hz (A) and at 4 Hz (B). Force is expressed relative to the maximum force achieved. Values given are means ± SE. Sample sizes at rest, 2 and 4 Hz, respectively: LTD control, n = 4, 4, 4; and LTD graft, n = 6, 5, 4.*
*Puencies of 2 and 4 Hz in control and grafted LTD muscles. Values given are means ± SE. Sample sizes at rest, 2, and 4 Hz, respectively: LTD control, n = 4, 4, 4; LTD graft, n = 6, 5, 4.*
** Significantly different at p 0.05.*

out vascular repair) were 30% of the maximum flows obtained in control muscles. The EDL grafts fatigued at twice the rate of control EDL muscles. Although blood flow distribution was not measured by White et al. [26] it is possible that inadequate perfusion of the viable muscle fibers, contributed to the higher fatigue rate. In our study it was evident that LTD grafts were more resistant to fatigue during stimulation at both 2 and 4 Hz. These findings indicate that blood perfusion of the grafts likely was adequate and the oxidative capacity of the muscle fibers was somewhat enhanced. This observation is consistent with the findings of Guelinckx and Faulkner [8] who reported a shift in fiber types from predominately glycolytic Type IIB (67%) in control LTD muscles, to predominately oxidative (12% Type IIB) in 120-day LTD grafts.

Although the fiber type of LTD grafts shifted towards a predominance of oxidative fibers compared to control LTD muscles when transplanted into the RFM site, fiber type distribution of RFM autografts remained essentially unchanged from control RFM muscles (73% Type IIB in RFM controls vs 66% Type IIB in autografts) [8]. Yet, in our study, blood flows in contracting LTD grafts remained well below those observed in RFM grafts [2]. Because blood perfusion of active fibers is proportional to the oxidative capacity of the fibers [13, 15], it was somewhat surprising that flows in the LTD grafts, although higher than LTD controls at rest and during twitch contractions at 2 Hz, were considerably lower than both RFM grafts and control RFM muscles during twitch contractions. Possible explanations for the disparate blood flows between LTD and RFM grafts involves differences in the surgical procedures in grafting the two muscles. Grafting the RFM muscle in its original site includes removal and replacement of the entire muscle. Ninety-five per cent or more of the muscle fibers in these grafts survive the transplantation procedure [7, 23]. Most of the fiber damage is confined to the proximal and distal regions of the muscle [7]. Conversely, the LTD muscle graft is trimmed to approximately half its original mass to fit the RFM site. Damage to a greater number of fibers than in RFM grafts is inevitable. In addition, trimming such a large portion of the muscle obviously disrupts the vascular continuity of the LTD graft. Both of these factors may have hindered or prevented "adaptation" of the vascular bed of the graft.

Generally, blood flows in LTD grafts were higher in the proximal and belly regions, compared to LTD control muscles, but were considerably lower in the distal region at the highest stimulation frequency. A factor that may have contributed to the differences in blood flow distribution between LTD grafts and control LTD muscles is the disruption of secondary feeding vessels during the transplantation procedure. The LTD muscle has one dominant vascular pedicle close to the muscle insertion (proximal end of muscle) and several segmental pedicles close to the origin or distal end [20]. During removal and transplantation of the LTD muscle, only the large dominant pedicle is preserved. The possibility exists that anastomosing only

the dominant vascular pedicle caused a disruption in the vascular bed in the distal portion of the graft. But the overall effect of vascular bed disruption on blood flow characteristics in the grafts remains unclear at this time.

Force development in muscle grafted into the RFM site reach maximum values by 90 days [7] indicating that no further functional adaptation occurs after that time. Our decision to observe blood flow characteristics in 90-day grafts was based on this evidence. However, our observation that an apparent incomplete adaptation in blood flow responses to twitch stimulation existed by 90 days post-transplantation does not preclude the possibility that a longer period may be necessary for blood flow responses to fully adapt to the new environment.

The elevated whole-muscle resting blood flow in the LTD transplants compared to both LTD and RFM control muscles is consistent with the findings of others in cat EDL muscles grafted without neurovascular repair [26] and in neurovascular- anastomosed RFM autografts observed in our laboratory [2]. Higher resting blood flows in neurotomized grafts may be linked to an inadequate return of sympathetic control of vascular tone. In a regenerated vascular system, Lassman [12] has reported that dysfunction of the smooth muscle layer may be associated with inadequate regeneration of both afferent and efferent innervation. The extent of the apparent dysfunction in sensory output from and/or motor input to the vascular bed of transplanted muscles has not been determined.

The observation that 4 of the grafts in which the vascular anastomosis failed, revascularized spontaneously and survived, is contrary to the findings of Watson and Muir [25], and Lavine and Cochran [14], who reported no survival of 6 g muscle transplants in dogs. It is generally accepted that the survival of skeletal muscle grafts larger than 6 g requires a successful vascular anastomosis [1, 2, 14, 18, 25]. In our study, the original mass of the LTD grafts was approximately 6 g. The failed grafts were clearly different than grafts in which the vascular anastomosis was successful, containing more fat and connective tissue and having a mass approximately half the successful grafts. Because the mass and, therefore, potential for force development is much reduced in free grafts of this size, the procedure of choice remains to anastomose blood vessels in the graft to suitable vessels in the recipient site.

In summary, blood flows in neurovascular-anastomosed LTD grafts transplanted into the RFM muscle site in rabbits were slightly higher than control LTD muscles at rest and at a stimulation frequency of 2 Hz, but there was no difference at 4 Hz. When compared to neurovascular-anastomosed RFM grafts and to control RFM muscles, bloods flows in the LTD grafts were considerably lower during twitch contractions. This indicates that, in terms of blood flow responses to twitch stimulation, LTD grafts undergo an incomplete adaptation compared to the patterns observed in either control RFM muscles or RFM grafts.

**Acknowledgements**

The authors thank Jonathan Hartman for his expert technical assistance. This study was supported by research grants NHLBI HL- 34164 and NIDR DE-07687 held by Dr. John A. Faulkner, University of Michigan Medical School.

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