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## The Marc Pro™ Device is a Novel Paradigm Shift in Muscle Conditioning, Recovery and Performance: Induction of Nitric Oxide (NO) Dependent Enhanced Microcirculation Coupled with Angiogenesis Mechanisms

Nicholas DiNubile<sup>1</sup>, Wayne L. Westcott<sup>2</sup>, Gary Reinl<sup>3</sup>, Anish Bajaj<sup>4</sup>, Eric R Braverman<sup>4,5</sup>, Margaret A Madigan<sup>6</sup>, John Giordano<sup>7</sup>, Kenneth Blum<sup>4,6,7,8</sup>

<sup>1</sup>Department of Orthopedic Surgery, Hospital of the University of Pennsylvania Philadelphia, <sup>2</sup>Department of Exercise Science, Quincy College, Quincy, MA <sup>3</sup>Nautilus, Inc. Independence, Virginia, <sup>4</sup>Executive Health, Path Research Foundation NY, <sup>5</sup>Department of Neurosurgery, Weill Cornell College of Medicine, New York, <sup>6</sup>Department of Personalized Medicine, LifeGen, Inc., San Diego, CA, <sup>7</sup>Department of Clinical Pain, G & G Holistic Addiction Treatment, North Miami Beach, FL, and <sup>8</sup>Department of Psychiatry, University of Florida, College of Medicine, Gainesville, FL

**ABSTRACT**

**DiNubile N, Westcott W, Reinl G, Bajaj A, Braverman ER, Madigan MA, Giordano J, Blum K.** The MarcPro™ Device is a Novel Paradigm Shift in Muscle Conditioning, Recovery and Performance: Induction of Nitric Oxide (NO) Dependent Enhanced Microcirculation Coupled with Angiogenesis Mechanisms. **JEPonline** 2011;14(5):10-19. This is a follow-up commentary to a recent paper in this journal on Marc Pro™ electrical device stimulation (MPDS) showing Delayed Onset of Muscle Soreness (DOMS) recovery. We hypothesized that MPDS increases arteriolar diameters, a mechanism involved in the recovery process, and that repeated MPDS would elicit angiogenesis, a mechanism involved in conditioning and improved performance. First, arteriolar diameters were measured in the cremaster muscle of 57 male, anesthetized rats using intravital microscopy before and after MPDS or sham stimulation (SS) at 1 or 2 Hz for periods of 30-60 min. In a separate cohort, the role of nitric oxide (NO) in the response to MPDS was assessed by blocking NO synthase

using topical L-nitro-arginine-methyl ester (L-NAME) at 10<sup>-5</sup> M (Molar). Maximal arteriolar responses to stimulation were compared to pre-stimulation diameters. MPDS both at 1 and 2 Hz resulted in significant arteriolar vasodilation (P<0.05). The arterioles in SS animals demonstrated no changes in diameter. Similarly, microvascular diameters did not change with MPDS following blockade of NO production. Secondly, the effects of repeated MPDS on blood flow and angiogenesis in the rat hind limb were studied. Animals were MPDS-conditioned ("Conditioned") or sham-stimulated ("Sham") (n = 5/group) daily for 3 wk. The contralateral limb in both groups served as the control. Each animal was injected with bromodeoxyuridine (BrDU). After 3 wk, rats were anesthetized and iliac artery blood flow was measured bilaterally before, during, and after acute MPDS. Conditioned limbs elicited a 247% increase in limb blood flow above resting conditions compared to a 200% increase in the control legs receiving only a single application. Sham animals did not demonstrate between-leg differences in flow. Hind limb musculature staining for BrDU revealed angiogenesis in Conditioned vs. Sham groups. Flow changes accompanying MPDS corroborated earlier microvascular findings demonstrating a significant striated muscle arteriolar dilation with MPDS. We are confident that these properties of MPD variant technology derived from animal studies showing NO-dependent enhanced microcirculation, muscle loading and angiogenesis, improved muscle performance, and recovery from concentric and eccentric exercise induced muscle fatigue will be confirmed in larger studies.

**Key Words:** Microvascular Diameters, Muscle Performance, Delayed Onset of Muscle Soreness

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**INTRODUCTION**

The use of electro-muscle stimulators to enhance muscle conditioning, performance, and recovery is well-known (5,8,9,11). The Marc Pro™ electrical device stimulation (MPDS) has been shown to expedite muscle recovery from strenuous exercise (16). The MPDS has received FDA clearance for its ability to improve muscle conditioning and performance and received a U.S. patent (Notice of Allowance). This paper is a follow-up commentary to the most recent paper on MPDS in this journal showing Delayed Onset of Muscle Soreness (DOMS) recovery (16). Specifically, we reported that MPDS significantly improved muscle recovery and muscle endurance from combined concentric and eccentric exercise in healthy recreational exercisers. Fourteen subjects (no prior soreness upon study

entry) performed strength training activity (leg extension exercise with eccentric emphasis) to produce DOMS in the quadriceps muscles. All participants received 1 hr of MPDS on the right leg only following the exercise session. One day later, assessment of muscle soreness revealed significantly less discomfort in the right leg (MPDS) than in the left leg (no MPDS) in all subjects and in responders, respectively ( $P < 0.008$ ;  $P < 0.002$ ). The number of strength repetitions completed with the right leg (MPDS) was significantly greater than the number of repetitions completed with the left leg (no MPDS) in all subjects and in responders, respectively ( $P < 0.03$ ;  $P < 0.008$ ). In the second experiment, 13 subjects (no prior soreness upon study entry) utilized a modestly challenging uphill/downhill hike to produce DOMS in the quadriceps muscles. Following the hike, the subjects' right leg received MPDS for 60 min while the left leg received no MPDS application. Reported soreness was significantly less in the right leg (MPDS) than in the left leg (no MPD) in all participants and in responders, respectively ( $P < 0.0008$ ;  $P < 0.0002$ ). Therefore we are compelled to present specific information that will help explain the known and proposed mechanism of action (MOA) of MPDS.

## OVERCOMING DOMS

The process of overcoming DOMS involves many factors, including but not limited to: (a) loading of bone, fibrous tissue and muscle; (b) nitric-oxide (NO) dependent increase in blood flow; (c) increased formation of new blood vessels or angiogenesis; (d) increase in protein clearance at fatigued loci; (e) increase absorption of cellular lactate; and (f) increase mitochondrial biogenesis [see Figure 1].

In a detailed review (17), a number of mechanisms involved in the endurance and muscle recovery process have been suggested. Interestingly, in the study by Hellsten et al. (3) passive movement enhanced ( $P < 0.05$ ) the eNOS mRNA level fourfold above resting levels. Moreover, their results show that a session of passive leg movement, elevating blood flow and causing passive stretch, augments the interstitial concentrations of vascular endothelial growth factor (VEGF), the proliferative effect of interstitial fluid, and eNOS mRNA content in muscle tissue. The authors proposed that enhanced blood flow and passive stretch are positive physiological stimulators of factors associated with capillary growth in human muscle.

In this commentary, we are also proposing that MPDS overcomes DOMS due to its unique innate properties associated with enhanced microcirculation, dependent on NO and angiogenesis. It is noteworthy that a study by Tedeger et al. (15) on DOMS showed that concentrations of NO were lower in the DOMS leg than control leg, particularly during the first 4 hr of microdialysis. Chronic increases of skeletal muscle contractile activity, such as endurance exercise, lead to physiological and biochemical adaptations in skeletal muscle, including mitochondrial biogenesis, angiogenesis, and fiber type transformation (4,7). These adaptive changes are the basis for the improvement of physical performance.

In addition, endurance exercise stimulates peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha) expression in skeletal muscle, and forced expression of PGC-1alpha changes muscle metabolism and exercise capacity in mice (1). PGC-1alpha plays a functional role in endurance exercise-induced mitochondrial biogenesis and angiogenesis, but not IIb-to-IIa fiber-type transformation in mouse skeletal muscle, and the improvement of mitochondrial morphology and antioxidant defense in response to endurance exercise may occur independently of PGC-1alpha function (11).

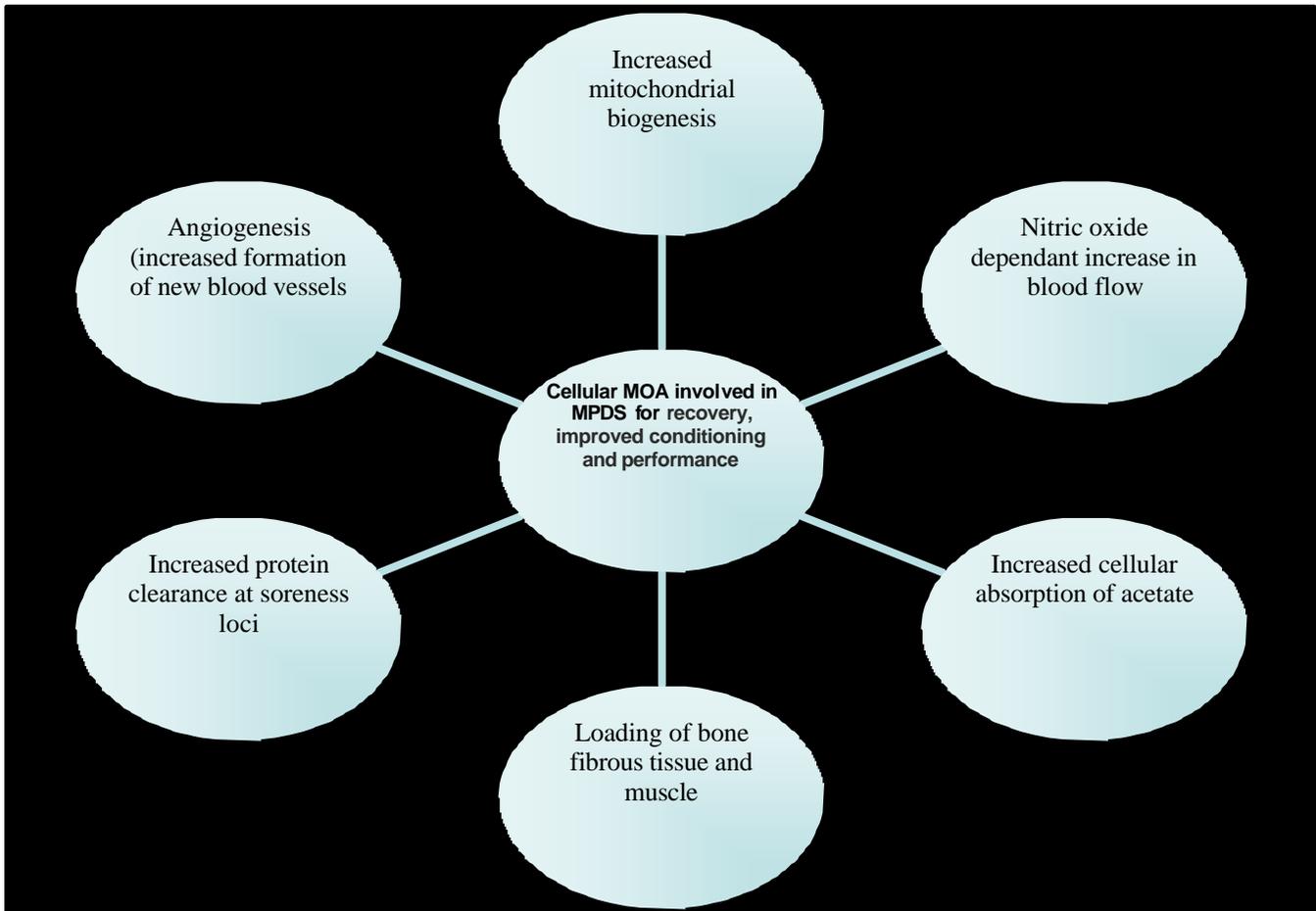


Figure 1. Factors involved in the process of overcoming DOMS.

The physiological mechanisms of the effect from MPDS recently has been systematically investigated in animal studies (12,14). It has been suggested that the physiological effects of MPDS are due to a number of specific properties of MPDS variant resulting in improvements in tissue circulation. These novel properties of the MPDS following stimulation resulted in an acute and long-term increase of microcirculation of rat striated muscle, which is NO-dependent (12). Moreover, muscle recovery activity following long-term repeated MPDS to the hind limb of rats is evident due to the significant systemic induction of new blood vessel formation or angiogenesis (14).

The MPDS is an electrical stimulation modality demonstrated to reduce DOMS following strenuous exercise. The MOA of this modality may be related to improved perfusion to the muscle, with the potential for reducing the extravasations of fluid and minimizing congestion (10). Results suggest that increased extracellular fluid can account in part for the increase in muscle T2 observed during exercise.

### **MICROVASCULAR AND HEMODYNAMIC MECHANISMS OF MPD MUSCLE STIMULATION IS DEPENDENT ON NITRIC OXIDE**

The aim of a number of animal studies conducted at Wake Forest University School of Medicine in the Departments of Orthopedic Research and Physiology and Pharmacology was to directly assess

striated muscle microvascular responses to MPDS. In addition, the effect of repeated stimulation over a 3-wk period on hindlimb blood flow was also assessed. Our laboratory hypothesized that acute electrical stimulation of striated muscle would result in arteriolar vasodilation and that repeated electrical stimulation would result in an increased perfusion capacity in the treated muscle.

To address these questions Smith and colleagues (12-14) performed studies on rats, whereby the microvasculature of 57 male rats was studied using intravital microscopy and electrical stimulation. The rats were anesthetized and the cremaster muscle was prepared for video-microscopy. Platinum electrodes were used for electrical stimulation of the tissue. One arteriole per rat was measured before, during, and after electrical stimulation of the cremaster muscle for up to 2.5 hrs. The cremaster muscle was stimulated at either 1 Hz or 2 Hz for periods of 30 to 60 min. Control rats (n = 15) were not exposed to electrical stimulation during the intravital microscopic evaluations [see Figure 2]. MPDS both at 1 and 2 Hz resulted in significant arteriolar vasodilation (Figure 2). The arterioles in control animals not exposed to electrical stimulation demonstrated no changes in diameter.

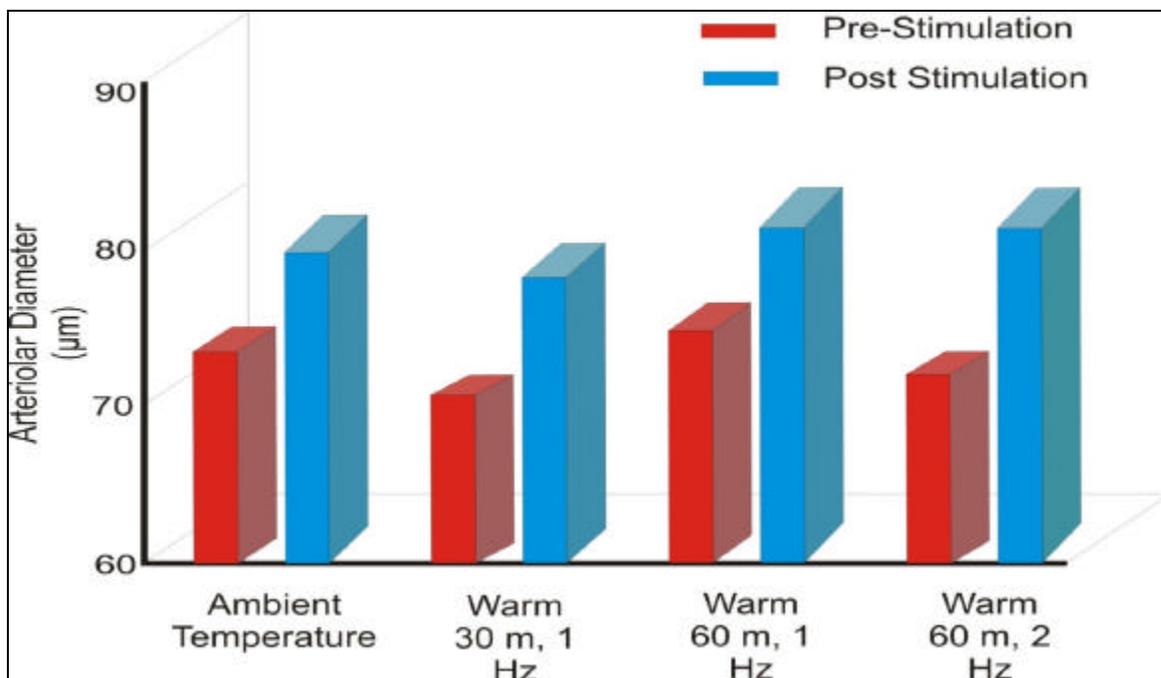


Figure 2. Microvascular diameters before and after electrical stimulation for 30 or 60 min at 1 or 2 Hz stimulation  $P < 0.05$  (pre vs. post) (12,13).

The role of NO in the microvascular response to MPDS was assessed by blocking the NO pathway using L-nitro-arginine-methyl ester (L-NAME), topically applied at  $10^{-5}$  Molar to the cremaster prior to electrical stimulation at 2 Hz (n = 10 rats). Maximal arteriolar responses to MPDS were measured and compared to pre-stimulation diameters. Microvascular diameters did not change with variant MPDS following blockade of NO ( $84.1 \pm 4.5$  µm pre- vs.  $84.7 \pm 4.1$  µm post-stimulation), demonstrating that the possible MOA is related to NO activation within the blood vessels [see Figure 3].

Significant arteriolar dilation induced by MPDS suggests that this treatment modality is associated with significant increases in striated muscle perfusion. Because of Poiseuille's law, the observed increases in arteriolar diameter translate into increases in blood flow through single vessels of 26% to 62%. Therefore, MPDS of striated muscle using clinical stimulation parameters results in a

physiologic response characterized by microvascular arteriolar dilation. In addition, arteriolar dilation is not observed following variant MPDS in the presence of L-NAME blockade of NO production, suggesting that the microvascular response to MPDS is mediated, at least in part, by NO.

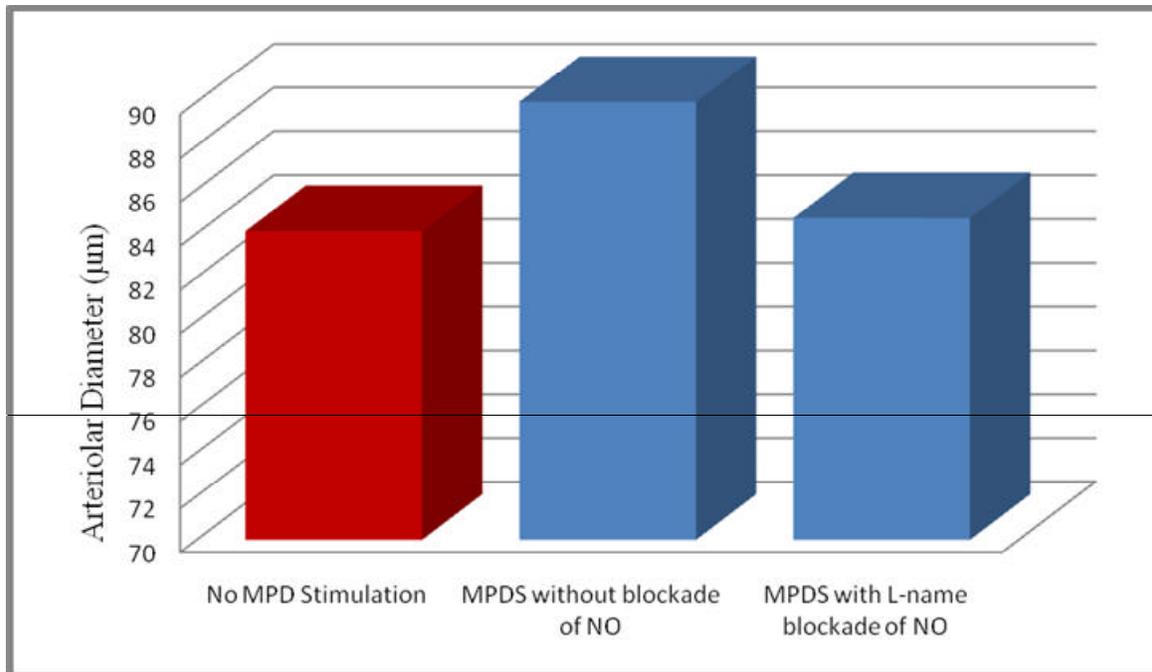


Figure 3. Effect of L-NAME on arteriolar dilation induced by stimulation with Marc Pro™ Device showing blockade of the MPDS induced effect whereby there was no increase of arteriolar diameter above baseline (12,13).

## BLOOD FLOW

In addition [Figure 4], limb blood flow was studied in 10 male rats. Animals were assigned to a stimulation-conditioned group ("Conditioned") or a sham stimulation group ("Sham") (n = 5/group). The Conditioned group was treated with 1 hr of 2 Hz electrical stimulation to the left leg daily (Monday–Friday) for 3 wk. The Sham group was treated with a sham MPD device for an equivalent period. The contralateral limb in both groups served as the control limb. After 3 wk, rats were anesthetized with isoflurane, and iliac artery blood flow was measured bilaterally using ultrasound transit-time flowmetry before, during, and after acute HWDS of each hindlimb for 5 min. Differences between Conditioned or Sham hindlimb blood flows, compared with the control side, were analyzed. MPDS of the Conditioned hindlimb elicited an average 247% increase in limb blood flow above resting conditions from 5 min of stimulation [Figure 4]. The control leg increased blood flow by 200% from 5 min of stimulation. Sham animals did not demonstrate a between-leg difference (Sham leg vs. control leg) (13,14).

In the blood flow image, the straight line indicates the resting blood flow. As soon as stimulation occurs on the conditioned leg, there is an immediate noticeable rise in blood flow in the conditioned hind limb, reaching a peak of 247% above resting state. After MPDS there was an asymptotic fall and return to baseline resting level. When the contralateral non-conditioned leg was stimulated with MPDS a similar but less robust blood flow pattern was obtained reaching a peak level of 200% above the resting blood flow level in the hind limb. This difference of 25% was significant (see Figure 5). It is

conjectured that the difference between the control and conditioned hind limb is due to a vascular reserve or angiogenesis. This rapid rise in blood flow suggests that choosing MPDS as a frontline modality should result in an immediate augmentation of the initiation of the recovery process. It is suggested that this observed rapid rise of blood flow is extremely important in terms of impacting rapid return to the playing field.

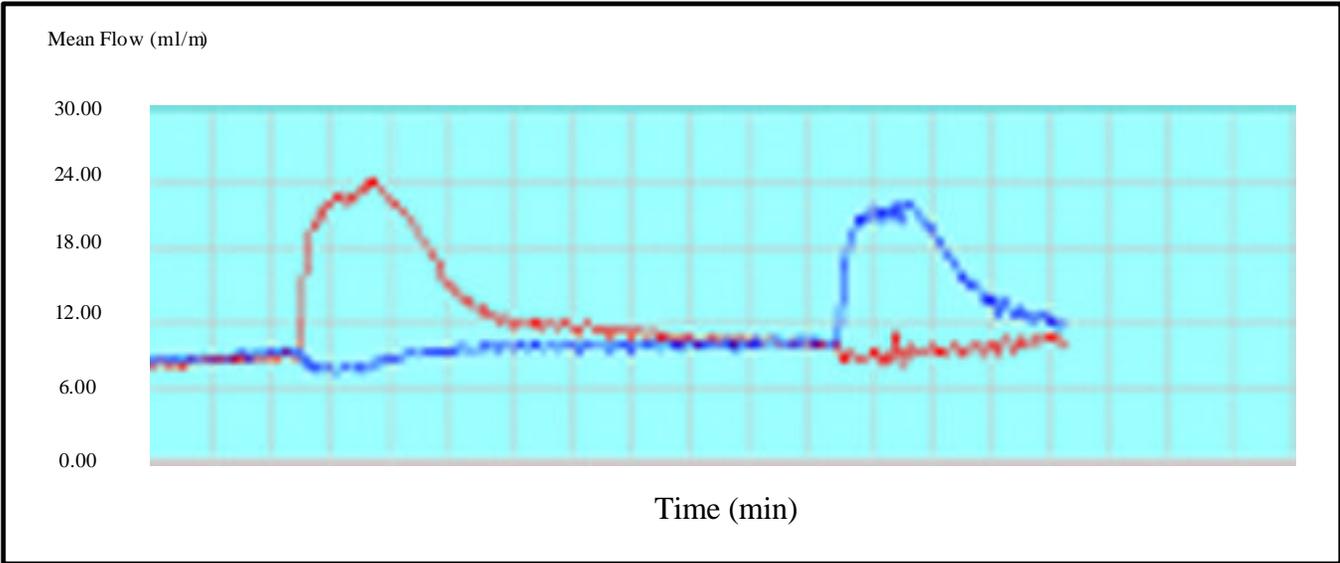


Figure 4. Blood flow image following long-term MPDS using transit-time flowmetry before, during, and after acute MPDS of each hind limb for 5 min (12,13).

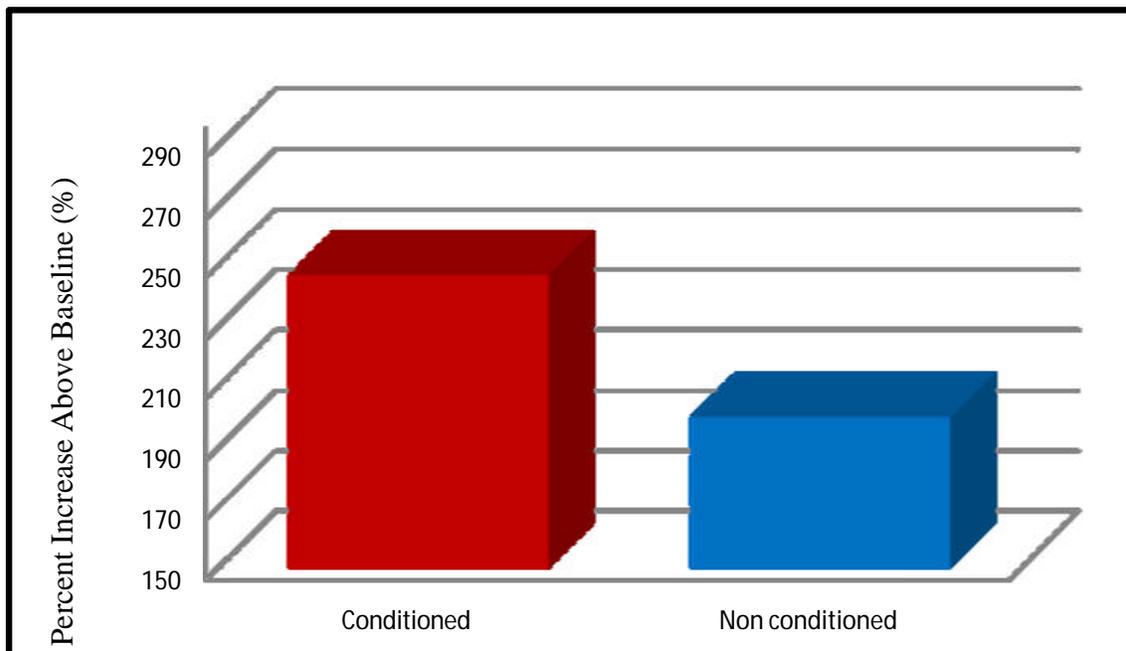


Figure 5. Limb blood flow in MPDS conditioned versus control limbs during electrical stimulation, compared to pre-stimulation baseline values ( $P < 0.05$ );  $P < 0.05$  compared with baseline.  $N = 5$  rats (13,14).

## INDUCTION OF ANGIOGENESIS BY MPDS

Subsequent analysis (12) of the conditioned hind limb for the production of new vessels resulted in a demonstrable increase in new blood vessels proving angiogenesis using bromouridine staining [see Figure 6]. Thus, the difference between the conditioned and non-conditioned hind limb response to blood flow was due in part to MPDS induced angiogenesis. Limb blood flow changes accompanying MPDS corroborated the microvascular findings, demonstrating a significant increase in limb blood flow accompanying MPDS. In addition, repetitive daily exposure to MPDS for 3 wk elicited a 25% greater increase in blood flow in the MPDS conditioned limb compared with the contralateral non-conditioned control limb. This increase in blood flow in the conditioned limb suggests an increased vascular reserve available for augmenting perfusion in the limbs exposed to repetitive MPDS. In fact, the reserve augmentation is due in part to angiogenesis produced by chronic MPDS important for both muscle recovery and enhanced performance.

Recent work supports the relationship of NO production and or function and VEGF. As stated earlier, results by Hellsten et al. (3) show that a session of passive leg movement, elevating blood flow and causing passive stretch, augments the interstitial concentrations of VEGF, the proliferative effect of interstitial fluid, and eNOS mRNA content in muscle tissue. They propose that enhanced blood flow and passive stretch are positive physiological stimulators of factors associated with capillary growth in human muscle.

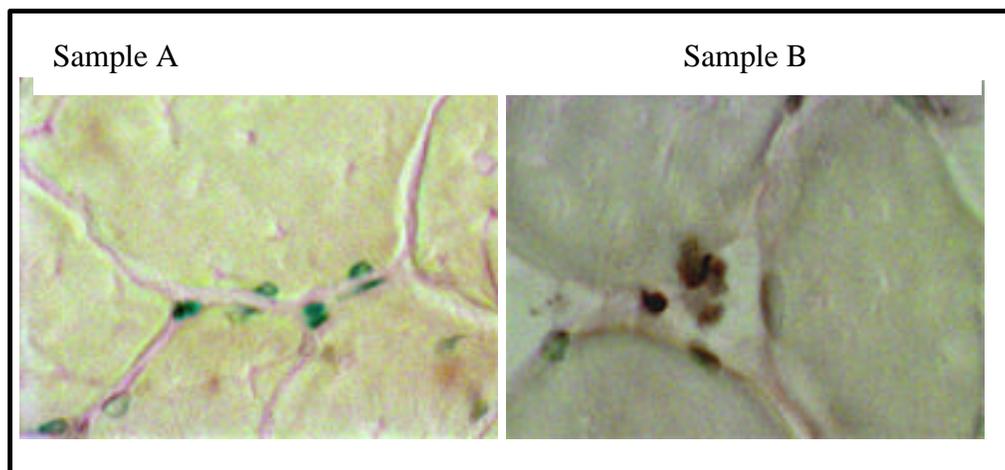


Figure 6. Sample A shows a sham-stimulated limb. Pre-existing vessels stain green. New vessels stain brown. Sample B shows an MPDS limb. New vessels stain brown. Also see pre-existing vessels (green). Images provided by the Department of Orthopedic Research and Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina (12,13).

## SUMMARY AND FUTURE PERSPECTIVE

Understanding the important innate properties of MPDS provides the impetus to utilize MPDS to enhance exercise performance. This commentary was developed to provide those interested in the potential of this novel device to become acquainted with the potential benefits of MPDS.

Skeletal muscle exhibits superb plasticity in response to changes in functional demands. Chronic increases of skeletal muscle contractile activity, such as endurance exercise, lead to a variety of physiological and biochemical adaptations in skeletal muscle, including mitochondrial biogenesis,

angiogenesis, and fiber type transformation. These adaptive changes are the basis for the improvement of physical performance. We propose that the innate properties of variant MPDS may enhance muscle performance and recovery potentially due to NO production, mitochondrial biogenesis, angiogenesis, and fiber type transformation. Moreover, PGC-1alpha plays a functional role in endurance exercise-induced mitochondrial biogenesis and angiogenesis, but not fiber-type transformation in mouse skeletal muscle. Since PGC-1alpha is required for complete skeletal muscle adaptations induced by endurance exercise, we are continuing our research by evaluating the possible production of PGC-1alpha following MPDS (9).

MPD is not designed as a healing device, but rather as a modality to condition healthy muscles, reduce DOMS increase recovery, muscle strength and performance after strenuous exercise. Further investigation is warranted to confirm this novel MOA of MPDS.

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**Address for correspondence:** Kenneth Blum, PhD, Department of Psychiatry, University of Florida, College of Medicine and Mcknight Brain Institute, Gainesville, Florida, USA Email:drd2gene@aol.com

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