Effect of Motor Neuromuscular Electrical Stimulation on Microvascular Perfusion of Stimulated Rat Skeletal Muscle

The purpose of this study was to determine the effect of neuromuscular electrical stimulation (NMES) (2,500-pps sine wave interrupted at 50 bps) on the degree of microvascular perfusion in stimulated skeletal muscle. The tibialis anterior (TA) and extensor digitorum longus (EDL) muscles of 36 male rats were treated with NMES for 30 minutes at current amplitudes sufficient to produce a sustained muscle contraction (motor NMES). Muscle tissue was removed at 0, 5, 10, 15, and 30 minutes after NMES. The perfused vessel/muscle fiber ratio (PV/F) of the stimulated animals at time 0 minutes was greater than that of the unstimulated control animals. A gradual decrease in the magnitude of the PV/F increase was noted over time. Depending on the muscle's fiber-type composition, the PV/F values returned to control levels by 10 to 30 minutes after motor NMES. The results indicate (1) that motor NMES significantly increases the degree of microvascular perfusion in stimulated rat skeletal muscle and (2) that the increased degree of perfusion persists for various lengths of time, depending on the fiber-type composition of the muscle. Thus, if responses in an animal model can be used as indicators of similar human responses, then the results of this study suggest that NMES can be used to increase the degree of microvascular perfusion in human skeletal muscle. [Clemente FR, Matulionis DH, Barron KW, Currier DP. Effect of motor neuromuscular electrical stimulation on microvascular perfusion of stimulated rat skeletal muscle. Phys Ther. 1991;71:397-406]

Key Words: Electrotherapy, electrical stimulation; Hemodynamics; Musculoskeletal system; Perfusion; Rats.

The application of electric current to human tissues to alleviate or improve various maladies dates back to 400 BC. Since that time, electrotherapy has experienced a fluctuating popularity as a treatment agent. In the early 1980s, there was a resurgence of interest in electrotherapy or neuromuscular electrical stimulation (NMES) as a therapeutic modality. This resurgence has stimulated interest in research of the efficacy of NMES. Some reports in the literature indicate that the application of NMES will alter peripheral hemodynamics. Investigators have shown an increased blood flow...
velocity in the arteries that supply the stimulated muscles and a decreased blood flow in the arteries of nonstimulated extremities. Even though blood flow appears to be increased in the vessels supplying the stimulated muscle, nothing is known regarding the specific response of the microvascular bed in these muscles.

The volume of blood that flows past a given point in a vascular bed in a given period of time \( (Q) \) is related to blood flow velocity \( (V) \) and perfused vascular cross-sectional area \( (A) \), according to the equation:

\[
V = \frac{Q}{A}
\]

This equation can be rewritten as

\[
VA = Q
\]

Based on this relationship, blood flow can be increased by increasing either the blood flow velocity or the perfused vascular cross-sectional area. The blood flow velocity is indicative of how fast blood is moving through the blood vessels, but it does not necessarily reflect the spatial relationship between the blood and the parenchymal tissue. The perfused vascular cross-sectional area or the degree of microvascular perfusion is an indicator of the diffusion distance, the spatial relationship between the blood and the parenchymal tissue.

The degree of muscle microvascular perfusion is an indicator of the diffusion distance for oxygen, nutrients, and metabolites to and from the parenchymal tissue. An increased degree of microvascular perfusion reduces the diffusion distance, which improves the availability of oxygen and nutrients to and enhances the removal of metabolites from the parenchymal muscle tissue. The diffusion distance between the blood supply and the muscle tissue markedly influences the function of the muscle. If treatment with NMES does increase the degree of microvascular perfusion, then NMES should decrease the diffusion distance. This decrease in exchange distance could enhance the efficiency of muscle contraction, promote healing of damaged muscles, and improve metabolic exchange in areas of impaired circulation.

Currently, no definitive studies address the microvascular response in skeletal muscle subsequent to NMES. An understanding of microvascular response to NMES is important because tissue function is dependent on an accessible blood supply in the microvascular bed and NMES is used clinically without a clear understanding of its possible effects on the tissue microvascular bed. Thus, the purpose of this investigation was to test the hypothesis that NMES of 2,500 pps frequency modulated to 50 bursts per second \((\text{bps})\) increases the degree of perfusion in the microvascular bed of stimulated skeletal muscle.

**Method and Materials**

**Sample**

Thirty-six male, 12- to 16-week-old Sprague-Dawley rats weighing 300 to 450 g were used in this study. All animals were housed in quarters at the University of Kentucky Tobacco and Health Research Institute. The ambient environment was maintained at 22°C and 48% relative humidity with a 12-hour light/dark cycle. Food and water were provided ad libitum. In order to ensure proper health, all animals were quarantined for 10 days prior to their use in the study.

**Procedure**

All animals were weighed and subsequently anesthetized by intraperitoneal injection \((65\,\text{mg/kg})\) of sodium pentobarbital. Sodium pentobarbital was selected because it has been shown to have little or no effect on the vascular resistance in skeletal muscle. Appropriate anesthesia was maintained for 30 minutes or for the duration of the experimental period for all animals, including the controls. The temperature of each animal was monitored by a rectal probe and maintained at 37°C by radiant heat. After each animal was anesthetized, cannulas were inserted into the right jugular vein, the trachea, and the left common carotid artery. The jugular cannula was used for administration of the vascular label, and the endotracheal tube was inserted to maintain a patent airway. The mean arterial blood pressure and heart rate were measured via the carotid artery cannula and recorded on a strip-chart recorder. These cardiovascular variables were used to monitor the status of the peripheral circulatory system of each animal under resting, nonstimulated conditions and during experimental manipulations.

Each of the 36 animals was randomly assigned to one of six groups. Group 1 consisted of animals that were untreated (absolute controls). Because of their accessibility and distinct muscle fiber-type distributions, the right tibialis anterior \((\text{TA})\) and extensor digitorum longus \((\text{EDL})\) muscles were chosen for stimulation. These muscles of the group 2, 3, 4, 5, and 6 animals were electrically stimulated to evoke a sustained tetanic contraction.

Groups 2, 3, 4, 5, and 6 were defined based on the tissue sampling times of 0, 5, 10, 15, and 30 minutes after NMES, respectively.

**Electrical Stimulation**

All animals except the absolute controls received NMES transcutaneously. The method of stimulation was designed to alter peripheral blood circulation based on protocols used clinically and during experimentation on animals. The animals were positioned supine on a surgery board and secured in place. Carbon silicone electrodes, \(1.0 \times 1.5\,\text{cm}\), were used to adapt the electrical stimulator to the hind limb of the rats. After shaving the right leg, one electrode was positioned over the lateral aspect of the right knee and another was placed anteriorly, just proximal to the right ankle. These electrodes were held in place by rubber strips, which were glued to the skin. The electrical current was produced with an
Electrostim® 180-21 stimulator.* The characteristics of the Electrostim®
180-21 stimulator's current have been described and illustrated pre-
viously. The stimulator emits a continuous sine-wave output with
a carrier frequency of 2,500 pps. The carrier frequency was inter-
rupted at 50 bps. The stimulator delivered 12-second bursts of stim-
uli that were finely ramped so that the current gradually increased over
a 5-second period but had an abrupt ramp decline. Each 12-
second burst was followed by a 10-
second rest interval, producing a
12/-10-second “on/off” ratio.

The NMES was applied at three times
the amplitude needed to produce a
minimal, visible contraction of the TA
muscle (motor NMES). The current
amplitudes were monitored with a
multimeter. In all cases, motor NMES
was applied for 30 minutes.

Muscle Preparation and
Data Collection

At various times after completion of
the NMES (ie, 0, 5, 10, 15, and 30
minutes), the TA and EDL muscles
were removed quickly by sharp dis-
section. These muscles were then
dipped in talcum powder, covered
with OCT (ornithine carbamoyltrans-
ferase) compound, pinned to a piece
of cork, and frozen in isopentane
cooled over liquid nitrogen. The
tissue was transversely sectioned (10
μm) at the midpoint of the muscle
belly.

Muscle fiber types and perfused microvessels were identified on serial
sections. Identification of muscle fiber
types was achieved by staining the
muscle sections for myosin ATPase
(preincubation pH = 4.4). For each
TA muscle, 72 nonoverlapping fields
(0.057 mm²/field) were sampled in
each section. These sampled sections
included 36 fields in the area in which
muscle fiber types were most hetero-
geneous and 36 in the area in which
muscle fiber types were most homoge-
neous. Twenty-seven nonoverlapping
fields were sampled in each EDL mus-
cle section. Fibers that partially pro-
tuded from the reference area (0.057
mm²/field) were counted as one-half
fibers. The proportion of each fiber
type was calculated as a percentage
of the total number of muscle fibers
counted per reference area. Percent-
ages were calculated for the entire
EDL and TA muscle sections and for
the two different fiber-type regions of
the TA muscle sections.

Fluorescein isothiocyanate conjugated
to bovine serum albumin (FITC-BSA)
was used according to the methodol-
dy of McDonagh and Williams to
label the perfused microvessels. The
FITC-BSA solution was continuously
infused over a 1-minute period
through the jugular vein cannula. The
infusion was started 2.5 minutes prior
to collection of the muscle tissue, al-
lowing the FITC-BSA to circulate for
1.5 minutes. To visualize the perfused
microvasculature, the tissue, which
had been labeled with FITC-BSA, was
processed according to a previously
described method. The degree of
microvascular perfusion was evaluated
via fluorescent microscopy using a
photomicroscope with xenon epi-
ilumination and a 490-nm barrier
filter. The same sampling procedure
was used for this assessment as de-
scribed previously for the assessment
of fiber-type composition.

Perfused microvessels were defined
as microvessels (ie, terminal arteri-
ocles, capillaries, and postcapillary
vessules), 5 to 20 μm in diameter, which
contained the fluorescent label. The
perfused microvessels and muscle
fibers present in each field were
counted at a magnification of ×400. A
value of one-half was given to any
muscle fiber or microvessel located
on the field perimeter line. The per-
fused microvessel/muscle fiber ratio
(PV/F ratio) was calculated as the total
number of perfused microvessels in
an area divided by the total number
of muscle fibers in the same area.
This ratio was used as an indicator of
the density or degree of microvascular
perfusion in the skeletal muscle. The
PV/F ratio was calculated for the
whole TA muscle section, for its het-
erogeneous and homogeneous muscle
fiber-type regions, and for the whole
EDL muscle section.

Data Analysis

The mean values of the PV/F ratio
were determined for the TA and EDL
muscles for all animal groups. These
ratios were statistically assessed using
the Fisher's Protected LSD Test to
make all possible pair-wise compar-
sions. Significance was set at the alpha
level of .05, and all data are reported
as means ± 1 standard error of the
mean.

Results

Cardiovascular monitoring indicated
that mean arterial blood pressure
and heart rate were consistent with
physiologic normative values for the
animal model throughout the experi-
mental recording period. These
cardiovascular variables remained
consistent with physiologic norma-
tive values during all experimental
procedures.

Myosin ATPase staining of the TA
muscle sections revealed two distinct
regions of different fiber-type com-
position. These results are shown in
Table 1. The nearly homogeneous
superficial region, up to approxi-
ately 0.81 mm from the surface, was
composed of 1.1% ± 0.4% type I (oxi-
dative) and 98.9% ± 0.4% type II (gly-
colytic) fibers. The deeper, more het-
erogeneous region of the TA muscle,
greater than 0.81 mm from the sur-
face, contained approximately
8.3% ± 0.6% type I and 91.7% ± 0.6%
type II fibers. The EDL muscle was
intermediate between these two re-
ions of the TA muscle, being com-
posed of 96.4% ± 0.4% type II fibers
with a uniform distribution of
3.7% ± 0.4% type I muscle fibers.

*Electrostim® USA Ltd, 1851 Black Rd, Joliet, IL 60435.
1Miles Inc, Diagnostics Div, 1025 Michigan St, Elkhart, IN 46515.
**Table 1.** Fiber-Type Composition of Tibialis Anterior (TA) and Extensor Digitorum Longus (EDL) Muscles (Mean±SEM)

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Type I (%)</th>
<th>Type II (%)</th>
</tr>
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<tbody>
<tr>
<td>Whole TA</td>
<td>5.2±0.4</td>
<td>94.2±0.4</td>
</tr>
<tr>
<td>Superficial TA</td>
<td>1.1±0.4</td>
<td>98.9±0.4</td>
</tr>
<tr>
<td>Deep TA</td>
<td>8.3±0.6</td>
<td>91.7±0.6</td>
</tr>
<tr>
<td>Whole EDL</td>
<td>3.7±0.4</td>
<td>96.4±0.4</td>
</tr>
</tbody>
</table>

The PV/F ratio for the whole TA muscle of the control animals was 0.954±0.036. The nearly homogeneous type II superficial TA muscle region had a PV/F ratio of 0.904±0.023, and the deeper, more heterogeneous TA muscle region had a ratio of 1.010±0.050. In the EDL muscle of the control animals, a PV/F ratio of 0.970±0.024 was calculated. These data are shown in Table 2 and in Figures 1 through 4.

In the motor NMES-time 0 minutes specimens, PV/F ratios were 1.271±0.019 for the whole TA muscle, 1.132±0.049 for the superficial TA muscle region, and 1.366±0.027 for the deep TA muscle region (Tab. 2, Figs. 1–3). Statistical analysis indicated that motor NMES increased the PV/F ratios of the whole TA muscle and of both the superficial and the deep TA muscle regions at time 0 minutes (Figs. 1–3). In the EDL muscle at time 0 minutes after motor NMES, the PV/F ratio was 1.229±0.038 (Tab. 2). This PV/F ratio represents a statistically significant increase when compared with control levels (Fig. 4).

The degree of perfusion (PV/F ratio) was also determined at time intervals of 5, 10, 15, and 30 minutes after termination of the motor NMES of the TA and EDL muscles (Tab. 2, Figs. 1–4). Over time, a gradual return to control values was observed. The PV/F ratio for the whole TA muscle returned to values similar to control levels by 15 minutes post-motor NMES (1.049±0.020) (Fig. 1). In the superficial TA muscle region, and PV/F ratio returned to control levels by 10 minutes after motor NMES (0.930±0.037) (Fig. 2). The PV/F ratios in the deep TA muscle region decreased to a value equivalent to control levels by 30 minutes after motor NMES (1.002±0.009) (Fig. 3). Degree of perfusion in the EDL muscle followed a similar pattern to that of the deep TA muscle region, with the PV/F ratio returning to control values by 30 minutes post-motor NMES (0.901±0.020) (Fig. 4).

**Table 2.** Mean (±SEM) of Perfused Vessel/Fiber Ratio for Tibialis Anterior (TA) and Extensor Digitorum Longus (EDL) Muscles at Various Times (in Minutes) After Motor Neuromuscular Electrical Stimulation (Motor NMES)

<table>
<thead>
<tr>
<th>Source</th>
<th>Perfused Vessel/Fiber Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole TA</td>
</tr>
<tr>
<td>Control</td>
<td>0.954±0.036</td>
</tr>
<tr>
<td>Motor NMES-0</td>
<td>1.271±0.019</td>
</tr>
<tr>
<td>Motor NMES-5</td>
<td>1.176±0.049</td>
</tr>
<tr>
<td>Motor NMES-10</td>
<td>1.139±0.043</td>
</tr>
<tr>
<td>Motor NMES-15</td>
<td>1.049±0.020</td>
</tr>
<tr>
<td>Motor NMES-30</td>
<td>0.948±0.009</td>
</tr>
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</table>
The primary goal of this study was to test the hypothesis that transcutaneous NMES, applied at a frequency of 2,500 pps and modulated at 50 bps, increases the microvascular perfusion of stimulated skeletal muscle. At time 0 minutes, motor NMES produced a significant (P ≤ 0.05) increase in the degree of microvascular perfusion in all muscles analyzed. Although no previous reports of the effects of transcutaneous NMES on microvascular perfusion of skeletal muscle were found, the results of this study are in agreement with the increased degree of perfusion described by other investigators, who used direct muscle stimulation with indwelling electrodes. The increase in degree of microvascular perfusion described in this study supports the view that the blood supply increases during high metabolic demand, such as during muscle contraction.

Muscle contraction has also been noted to cause acute alterations of blood flow. Reports in the literature suggest that these changes might be mediated via a reflex arc. The afferent limb of the suggested arc consists of group III and group IV somatic fibers, which innervate mammalian skeletal muscle. The efferent component of the proposed reflex arc is the sympathetic outflow to the vasculature of the contracting muscle.

Investigators have demonstrated that the afferent limb of this suggested reflex arc can be activated by muscle contraction and that the activation of the group III and IV fibers can produce a pressor response or a depressor response and an increase in blood flow.

Clement and Shepherd reported that muscle contraction can markedly attenuate the vasoconstrictor effects of efferent sympathetic outflow to the active muscle. They suggested that the interaction between the influences of muscle contraction and sympathetic outflow acts to maintain the most efficient and effective ratio of blood flow to oxygen consumption.

Other investigators have proposed additional mechanisms for increasing the degree of microvascular perfusion such as a myogenic reflex, low oxygen tension in the parenchymal tissue, unspecified metabolites of muscle contraction, increased concentration of adenosine, and the release of a neuromodulator or neuropeptide. Many of these mechanisms have been studied in some detail; however, the actual role that each plays in the response of the microvasculature to muscle contraction is still not certain.
After determining that the motor NMES used in this study causes recruitment of microvessels, the effects of motor NMES over time were assessed. The degree of perfusion in the muscles analyzed returned to control levels after varying periods of time following NMES. No reports were found in the literature that describe persistent increases of microvascular recruitment in skeletal muscle following electrical stimulation. Honig and Frierson, however, demonstrated a dilation of arterioles in the gracilis muscle of the dog, which lasted for 12 to 40 minutes after electrical stimulation. Other investigators have shown a direct relationship between arteriolar dilation and increased microvascular perfusion. Based on this relationship, the results of our study are in agreement with previous observations and indicate that transcutaneously applied motor NMES has a varying effect over time on the degree of microvascular perfusion in rat skeletal muscle.

A relationship between the hyperemic response discussed previously and muscle-fiber composition is suggested by the results of this study. These results demonstrate that the time needed for the degree of microvascular perfusion to return to control levels following motor NMES was longer for muscles with a higher percentage of type I muscle fibers (deep TA and EDL muscles) and shorter for those with a lower percentage of type I muscle fibers (superficial TA muscles). According to Berne and Levy, oxidative (type I) muscle fibers continue to require delivery of increased levels of oxygen after exercise in order to reestablish the resting steady-state relationship between oxygen levels and cellular metabolism. This relationship of hyperemic responses suggests that muscle fibers with greater oxidative capacity might require an extended period of increased microvascular perfusion to provide access to the oxygen needed to return to the resting conditions. Other reports indicate that glycolytic (type II) muscle fibers depend on postexercise blood supply primarily for the removal of metabolites. Accumulation of metabolites has an adverse effect on the performance of the muscle, indicating that the rapid removal of these substances would be advantageous to the muscle. After these metabolites are removed, an extended period of increased microvascular perfusion would not be necessary.

The possible correlation between muscle fiber-type composition and the hyperemic response suggests that the PV/F ratios of the deep TA and EDL muscles will return to control levels at different rates because of these muscles' different fiber-type compositions. The design of this study places the return of the PV/F ratios of both of these muscles in the 15- to 30-minute interval. Additional research is needed to determine whether the degree of microvascular perfusion of the deep TA and the EDL muscles returns to control levels during different time periods within the 15- to 30-minute interval.

Another explanation for the findings of this study might be the variations in blood flow between distinct muscle fiber-type regions before, during, and after exercise that are attributed to the metabolic differences between the fiber types. A contributing factor to the differential blood flows could be a difference in the sensitivity of the microvasculature associated with the specific fiber types to oxygen or metabolite concentrations. No experimental evidence exists to support this hypothesis; however, such a difference in sensitivity of the microvasculature would explain the differential blood flow as well as the different recovery pattern observed in this study.

The results of this study indicate that motor NMES does increase the degree of microvascular perfusion in the stimulated skeletal muscle. This recruitment of microvessels will decrease the diffusion distance in the stimulated muscle tissue and enhance the exchange of nutrients and metabolites between the blood and functional tissue. Such a change in the diffusion distance will improve the function of the stimulated muscle.

The increase in the degree of microvascular perfusion observed in this study, together with support from previous investigations, suggests that the noted vascular response was activated by the physical muscle contraction secondary to transcutaneous NMES. The possible involvement of
sensory elements activated by NMES, however, has not been ruled out. Because sensory NMES is used clinically to alter hemodynamics without evidence to its effect, the role of sensory NMES in vascular perfusion needs to be critically evaluated.

Conclusions

The results of this study confirm the proposed hypothesis that NMES (2,500-pps frequency interrupted at 50 bps), applied for 30 minutes at amplitudes that produce sustained tetanic muscle contraction, causes an increase in the degree of microvascular perfusion in the stimulated skeletal muscle. The observed increase persisted for 10 to 30 minutes after the termination of the NMES, depending on the fiber-type composition of the stimulated muscle or muscle region. Muscle contraction induced by the NMES appears to elicit the increase in the degree of microvascular perfusion. If experimentally evoked responses observed in animals can be used as indicators of similar responses in humans, the results of this study suggest that 2,500-pps NMES interrupted at 50 bps can be used clinically to increase the degree of perfusion in the microvascular bed of stimulated human skeletal muscle.

Acknowledgment

We thank Linda Zimmermann for her valuable technical assistance.

References

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**Commentary**

The research article by Clemente et al details an interesting and well-designed study. The investigators used sound, established microcirculatory techniques to study microvessel perfusion during neuromuscular electrical stimulation (NMES). The animal model used was an appropriate one, and the data were analyzed in an appropriate manner.

The investigators demonstrated that the number of perfused microvessels (capillaries and postcapillary venules) was increased following intermittent NMES with "Russian" current (2,500 Hz AC, modulated at 50 bps). The increased perfusion was sustained for varying periods of time in the muscles sampled, depending on relative muscle fiber composition. The assumption that the increased microvascular perfusion enhanced exchange of oxygen and nutrients between the microvessels and the muscle fibers is reasonable.

Although a statistically significant increase in the perfused vessel/muscle fiber (PV/F) ratio was demonstrated, a question that must be asked is: What was the "perfusion reserve"? That is, what was the maximal potential increase in the PV/F ratio? Although there is controversy about the proportion of capillaries that are perfused at rest in skeletal muscle, estimates of one third to one half have been made.1 The maximal increase in PV/F ratio in the present study was about 36%, observed immediately after NMES (time 0 minutes) in the deep tibialis anterior muscle. Assuming that 50% of the available capillaries were perfused at rest, 86% would have been perfused immediately following NMES in this muscle. Thus, it would seem that the increase in PV/F ratio was sizeable, but not maximal.

It is important to distinguish increased microvascular perfusion from increased regional blood flow. The two are often related, but not always. For example, tissues have some ability to maintain (auto-regulate) blood flow, even if perfusion pressure is decreased. This ability is the result of arterioles and capillaries opening up (because of their myogenic properties), thereby decreasing resistance to flow. Thus, in this study, it is conceivable that the number of capillaries perfused (PV/F ratio) could have increased without increased regional blood flow. It would be useful to know the relationship between regional blood flow and microvascular perfusion during NMES. This relationship should be clarified by experiments using techniques such as the isotope-labeled microsphere technique,2 which can provide quantitative measurement of regional blood flow.

The discussion on the relationship between fiber type and the duration of enhanced perfusion is potentially misleading. The authors found that enhanced perfusion was sustained longer in muscles with a high percentage of type I fibers than in muscles with a lower percentage of type I fibers. The investigators correctly suggest that this finding may be due to the fact that type I muscle fibers, which function primarily by aerobic metabolism, need time and enhanced perfusion in order to reestablish a resting steady state. The reader should not conclude, however, that perfusion of type I muscle fibers is always enhanced with NMES, because a muscle with predominantly type I fibers was not tested. The muscles sampled were of mixed composition, but all of them were predominantly made up of type II fibers (91.7%–98.9%). It would be helpful to know how muscles with a high percentage of type I fibers would compare with the muscles tested in this study. The soleus muscle of the rat, for example, has approximately 87% type I fibers3 and could be stimulated transcutaneously.

To address the question of what caused the increased perfusion, consider the physiologic controllers of the microcirculation a little more globally than the investigators have done. Control of the microcirculation can be thought of in terms of intrinsic and extrinsic controllers. The intrinsic controllers are local factors such as carbon dioxide, adenosine, and lactate.

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