FATIGUE-INDEPENDENT ALTERATIONS IN MUSCLE ACTIVATION
AND EFFORT PERCEPTION DURING FOREARM EXERCISE: THE
ROLE OF LOCAL OXYGEN DELIVERY

by

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Abstract

In exercise, the sensations of effort to make the muscles do exercise can contribute to an individual’s decision to stop exercise or decrease exercise intensity. Skeletal muscle fatigue is one mechanism by which the effort required to continue exercise can be increased. In order to continue exercise with fatigued skeletal muscle we must increase the amount of electrical signals sent to the muscle. This increase in electrical signals sent to the muscle is perceived by the exercising individual and is interpreted as increased perception of effort to maintain the same exercise intensity. Another mechanism which may also require increased electrical activity sent to the muscle and therefore may increase perception of effort for the same exercise intensity is exercise where oxygen delivery is suboptimal, resulting in reduced oxygen levels (oxygenation) in the muscle. When human and animal muscle is electrically stimulated, force produced for a given electrical stimulation decreases when muscle oxygenation is reduced. When muscle oxygenation is restored force produced is also rapidly restored for the same electrical stimulation. This rapid adjustment of force for the same electrical stimulation with changes in muscle oxygenation is referred to as the oxygen conforming response (OCR). However, the existence of an OCR in voluntary human exercise has not been established and it remains to be determined if our perceptions of effort will follow changes in electrical signal sent to the muscle as a result of the OCR. We tested these unanswered questions by intermittently reducing forearm blood flow (FBF) during human (n = 16) handgrip exercise. When FBF was reduced, electrical activity and perception of effort increased. Interestingly, upon the first restoration of FBF neither electrical activity nor perception of effort were restored. We interpreted these findings to be consistent with skeletal muscle fatigue development during the first compromise to muscle oxygenation. Upon the second restoration of FBF both electrical activity and perception of effort were restored, which is
consistent with an OCR and its ability to rapidly modify perception of effort in voluntary human exercise. Through its effects on effort perception, the OCR may therefore have important implications for exercise behaviour.
Co-Authorship

This thesis presents the work of Patrick Jonathan Drouin in collaboration with his supervisor, Dr. Michael E. Tschakovsky

Manuscript: Fatigue-Independent Alterations in Muscle Activation and Effort Perception During Forearm Exercise: The Role of Local Oxygen Delivery

Patrick Jonathan Drouin’s contributions were as follows:

- Collaborated with Dr. Tschakovsky on conception and design of the study protocol, as Dr. Tschakovsky was the principal investigator on the grant that funded this research.
- Independently conducted literature review for rationale and contextualization of findings with the relevant literature.
- Responsible for all data acquisition, data analysis and statistical analysis.
- Data interpretation in collaboration with Dr. Tschakovsky.
- Writing of first draft of manuscript, and revision of manuscript based on feedback by Dr. Tschakovsky.
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<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Actin</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>APD</td>
<td>Avalanche Photodiode</td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>C</td>
<td>Compression</td>
</tr>
<tr>
<td>CaO2</td>
<td>Arterial Oxygen Content</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
</tr>
<tr>
<td>CMD</td>
<td>Central Motor Drive</td>
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<tr>
<td>CO</td>
<td>Cardiac Output</td>
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<tr>
<td>CSEP</td>
<td>Canadian Society of Exercise Physiology</td>
</tr>
<tr>
<td>DHPR</td>
<td>Dihydropyridine receptor</td>
</tr>
<tr>
<td>DPF</td>
<td>Differential path-length factor</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>FBF</td>
<td>Forearm Blood Flow</td>
</tr>
<tr>
<td>FDS</td>
<td>Flexor Digitorum Superficialis</td>
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<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
</tr>
<tr>
<td>H⁺</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>HHb</td>
<td>Deoxyhemoglobin</td>
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<tr>
<td>BAC</td>
<td>Brachial Artery Compression</td>
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iEMG – Integrated Electromyography
K⁺ – Potassium
LL – Landmarking Line
M – Myosin
MAP – Mean Arterial Pressure
MBV – Mean Blood Velocity
MRCP – Movement-Related Cortical Potential
MVC – Maximal Voluntary Contraction
NC – No Compression
NH₃ – Ammonia
NIRS – Near Infrared Spectroscopy
O₂D – Oxygen Delivery
O₂Hb – Oxyhemoglobin
OCR – Oxygen Conforming Response
PCr – Phosphocreatine
P – Inorganic Phosphate
P-RPE – Physical Rating of Perceived Exertion
PLFD – Prolonged Low Frequency Force Depression
Q_muscle – Muscle Blood Flow
RMS – Root Mean Square
RPE – Rating of Perceived Exertion
RPM – Revolutions Per Minute
ROS – Reactive Oxygen Species
RyR1 – Ryanodine Receptor

$S_aO_2$ – Arterial Oxygen Saturation

SR – Sarcoplasmic Reticulum

SS – Steady State

SV – Stroke Volume

TEA – Task Effort and Awareness

Tm – Tropomyosin

TnC – Troponin
Perception of effort refers to the subjective awareness of effort expended during a physical task (20, 52, 61, 71). Perception of effort is thought to originate from efferent neural activity (corollary discharge) arising parallel and in proportion to the central motor drive (CMD) determining motor neuron recruitment (20, 28, 44, 52, 54, 59, 64). This corollary discharge terminates in sensory areas within the anterior cingulate and insular cortex of the brain (79–81) where it is perceived as effort. Perception of effort is therefore the conscious awareness of CMD activating motor neurons, where if CMD increases so does perception of effort.

CMD may increase during exercise for several reasons. First, CMD may increase as a result of skeletal muscle fatigue, which is a reversible reduction in skeletal muscle maximal voluntary force output (12) as well as a reduction in force produced for a given motor neuron activation (indirect measure of CMD measured by electromyography; EMG) (59). Because of the latter, in order to maintain a given submaximal force output under fatiguing exercise conditions a greater EMG is required such that perception of effort is increased (59). Thus, skeletal muscle fatigue makes the same exercise be perceived as requiring more effort (feels harder to do) and plays an important role in an individual’s exercise tolerance. Of particular importance is that the development of the fatigue induced compromise to force production occurs in stable oxygen delivery conditions and remains evident for some time after exercise has ended (27, 82).

In contrast, if during constant skeletal muscle motor neuron recruitment (electrically stimulated muscle contractions) muscle oxygenation (the amount of oxygen in the skeletal muscle cell (myocyte), expressed as a partial pressure of a gas) is increased or decreased the force of contraction is also increased or decreased, respectively (32, 42, 83). This phenomenon is known...
as the oxygen conforming response (OCR) (72), and it refers to the rapid adjustment of muscle force production (adenosine triphosphate (ATP) demand) for the same CMD in response to muscle oxygenation-dependent changes in aerobic ATP supply. As opposed to the sustained elevation in EMG/Force characteristic of skeletal muscle fatigue, the OCR is characterized by a rapid restoration of the EMG/Force relationship upon restoration of muscle oxygenation. Given the nature of the OCR, as evident from electrically stimulated human (32, 51) and animal (38) exercise, it is predicted that in order to maintain a constant voluntary contraction force, EMG and therefore perception of effort will rapidly follow changes in muscle oxygenation independent of fatigue. However, this remains unknown.

While a single study has tested the hypothesis that a change in exercising limb perfusion pressure would necessitate an increase in EMG for the same voluntary force output, this single study (38) had several limitations: 1) As opposed to typical rhythmic exercise the authors used prolonged isometric contractions with long intermittent rest. 2) The authors did not measure muscle blood flow nor oxygenation 3). The authors did not report when their measure of EMG was taken following restoration of perfusion pressure which prevents assessment of whether restoration of EMG/Force was rapid enough to be consistent with an OCR. Considering these limitations, the OCR remains to be tested during rhythmic exercise while simultaneously quantifying changes in muscle oxygenation. In addition, the impact of the OCR on perception of effort has not been tested.

Therefore, the purpose of my Master’s thesis was to, for the first time, quantify the response of EMG amplitude to measured changes in muscle oxygenation during voluntary exercise (forearm contractions) in humans, and determine whether perception of effort changes with EMG. We hypothesized that for the same voluntary force output 1) Muscle activation would increase with
decreased muscle oxygenation, 2) Muscle activation would be rapidly restored upon muscle reoxygenation, 3) Perception of effort would follow changes in muscle activation. These hypotheses were tested by manipulating forearm blood flow during forearm exercise via brachial artery compression. Measures of forearm muscle oxygenation (near infrared spectroscopy; NIRS) and EMG activity during these manipulations would allow the necessary simultaneous tracking of muscle oxygenation status and motor drive, respectively. Studying the effect of changes in local oxygen delivery on sensations involved in exercise tolerance separate from fatigue is important for improving our understanding of how local oxygen delivery might contribute to modifying exercise behaviour by its effects on exercise tolerance and performance.
Chapter 2 - Literature Review

This review will provide an overview of mechanisms involved in fatigue progression, the nature of and evidence for the oxygen conforming response, and identify the key differences between the two. The recently proposed concept of a sensory tolerance limit will be introduced and discussed as a key determinant of exercise tolerance. The contribution of perceived effort vs. exertion will be proposed as a model that contributes to this point of exercise termination. Finally, I will propose a new hypothesis, linking together the possible effect of muscle oxygenation on perception of effort during exercise.

2.1 Skeletal Muscle Fatigue

Skeletal muscle fatigue is a reversible reduction in skeletal muscle maximal voluntary force (12) as well as force produced for a given motor neuron activation (33, 59). The force decrement associated with skeletal muscle fatigue can be of two distinct but interacting origins; central and peripheral fatigue. The progression of general fatigue in a given exercise task reflects the combined effect of central and peripheral fatigue and can be assessed by measuring 1. The progressive decline in MVC’s performed at intervals during the exercise task or 2. By measuring the time to exhaustion in a given exercise task, which would reflect the point where the muscle force production capacity has declined to that required for the exercise task. A key limitation of both fatigue assessment tests is that they can be greatly influenced by a participants’ motivation (70).

2.1.1 Overview of excitation contraction coupling

Prior to looking at mechanisms of fatigue, it is first important to understand the proper function of the excitation contraction coupling process. Excitation contraction coupling in skeletal
muscle (see Fig. 2-1) first begins with an action potential from the brain conducted via the spinal cord and motor nerve, leading to the neuromuscular junction where the motor neuron release of acetylcholine stimulates muscarinic receptors on the motor end plate, initiating a myocyte membrane action potential which travels along the surface membrane of the myocyte (25). In many places, the membrane forms a tubule (transverse, or t-tubule) that penetrates deep into the myocyte. The myocyte action potential travels down the t-tubule membrane and causes a charge movement in the dihydropyridine receptor (DHPR) and ryanodine receptor (RyR1) which combine to form the Ca$^{2+}$ release unit (25). This charge movement causes a release of Ca$^{2+}$ from the sarcoplasmic reticulum through a ~12nm gap between the terminal cisternae of the sarcoplasmic reticulum and the T-tubule (22). The released Ca$^{2+}$ flows through electron dense junctional feet formed by four identical subunits which spread across the gap (22). This release of Ca$^{2+}$ increases cytosolic [Ca$^{2+}$] from 100nM prior to action potential to 5-10 μM post action potential (22). The released Ca$^{2+}$ then proceeds to bind to troponin (TnC) on tropomyosin (Tm) filaments that were blocking actin strong binding sites. Once Ca$^{2+}$ is bound to TnC, Tm moves out of the way, permitting strong-binding between actin and myosin (22) (see Fig. 2-1).
Figure 2-1. Excitation contraction coupling process, adapted from Davis et al. (2004). A, Actin; ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; M, Myosin; P<sub>i</sub>, inorganic phosphate.
2.1.2 - Low frequency fatigue

Skeletal muscle fatigue determined by steps in excitation contraction coupling (peripheral fatigue) can be differentiated into low and high frequency fatigue. Low frequency fatigue is a long lasting reduction in skeletal muscle contraction force which is apparent when skeletal muscle is stimulated at low frequencies (47, 58). Many forms of activity can cause low frequency fatigue, so “low frequency” is referring not to the stimulation causing fatigue, but to the stimulation frequencies at which force is compromised. Recently, the term low frequency fatigue has therefore been renamed “prolonged low frequency force depression” (PLFD) (3). Repeated, intense use of muscles leads to a decline in performance known as muscle fatigue. Many muscle properties change during fatigue including the action potential, extracellular and intracellular ions, and many intracellular metabolites. A range of mechanisms have been identified that contribute to the decline of performance. The traditional explanation, accumulation of intracellular lactate and hydrogen ions causing impaired function of the contractile proteins, is probably of limited importance in mammals (3). Alternative explanations that will be considered are the effects of ionic changes on the action potential, failure of SR Ca\(^{2+}\) release by various mechanisms, and the effects of reactive oxygen species. Many different activities lead to fatigue, and an important challenge is to identify the various mechanisms that contribute under different circumstances. Most of the mechanistic studies of fatigue are on isolated animal tissues, and another major challenge is to use the knowledge generated in these studies to identify the mechanisms of fatigue in intact animals and particularly in human diseases.

Jones (47) described PLFD to have three main features. First, muscle force is most greatly affected by low frequency stimulation. Second, full recovery can take a long time, extending from hours to even days in severe cases. Third, PLFD can be apparent despite the absence of metabolic
or electrical disturbance in the muscle. The exact mechanism of PLFD is unknown, however, it is believed that the reason for this prolonged low frequency force depression is muscle damage, and therefore time, in the form of protein turnover, is required to repair the muscle in order to permit proper force production (47). Additionally, Jones et al. (46) demonstrated that PLFD could also be the result of a decrease in [Ca\(^{2+}\)] release for a given action potential. This hypothesis was later confirmed by Westerblad, Duty and Allen (77) who demonstrated a decrease in [Ca\(^{2+}\)] release for a given action potential in isolated mouse skeletal muscle. This reduction in [Ca\(^{2+}\)] per action potential leads to a decrease in affinity of troponin for Ca\(^{2+}\) thereby impairing the strong binding state between actin and myosin (see Fig. 2-1).

2.1.3 - High frequency fatigue

As opposed to low frequency fatigue (prolonged low frequency force depression), high frequency fatigue is fatigue occurring in response to a high frequency of stimulation. Jones (47) summarized the findings by Bigland-Ritchie, Jones and Woods (13), Jones, Bigland-Ritchie and Edwards, (48) and Jones and Bigland-Ritchie, Dawson, Johansson and Lippold (14) which identified three important defining features of high frequency fatigue. First, loss of force caused by high frequency stimulation can be quickly reversed by reducing the frequency of stimulation. Second, force loss will be accompanied by a decrease in amplitude and speed of waveform muscle action potentials. Third, force loss is worsened if extracellular [Na\(^{+}\)] is decreased or [K\(^{+}\)] increased (3, 13, 48). Therefore, Jones (47) suggests that high frequency fatigue is caused primarily by an extracellular increase in [K\(^{+}\)] leading to a prevented action potential propagation along the myocyte surface membrane, thereby inhibiting action potential transmission along the t-tubular system. The inhibition of action potential transmission inhibits Ca\(^{2+}\) release, thereby reducing Ca\(^{2+}\) binding to troponin and in turn reducing the available strong-binding sites contributing to a reduced force of
contraction (31, 47, 78) (Fig. 2-1). As opposed to low frequency fatigue, high frequency fatigue has a quick recovery time, with a half time of 5-10 min and full recovery time of 30 min (3).

2.1.4 - Peripheral fatigue

More generally, fatigue can be grouped into central and peripheral fatigue. Peripheral fatigue is fatigue which occurs specifically at or distal to the neuromuscular junction (36) and was described above as low and high frequency fatigue. During exercise several metabolites (ATP, adenosine diphosphate (ADP), phosphocreatine (PCr), reactive oxygen species (ROS) and inorganic phosphate (P_i)) have been proposed to play a role in muscular fatigue onset. Allen et al. (3) provide a good overview of these fatigue inducing metabolites. Specifically, during exercise an increase in [P_i] has been proposed to have a few inhibitory effects on excitation contraction coupling processes. First, increased [P_i] has been proposed to interfere with cross bridge cycling (4). Debold, Romatowski and Fitts (23) demonstrated that as muscle [P_i] increased, so did the [Ca^{2+}] required to achieve a muscle contraction. This effect was dependent on muscle temperature and muscle fiber type, where specifically in fast fibers, increased [P_i] was associated with reduced muscle force production at 30°C and not 15°C. This study completed by Debold et al. (23) provides support for a synergistic effect between reduced release of Ca^{2+} and increased [P_i] on impaired muscle force production. Specifically, increased [P_i] is proposed to cause a reduction in force per cross bridge (23). Second, increased [P_i] has been proposed to reduce the free Ca^{2+} available in the sarcoplasmic reticulum (SR) (76). Specifically, the increased [P_i] has been shown to enter the SR where when [P_i] reaches sufficiently high levels, P_i and Ca^{2+} would precipitate in the SR and thereby reduce the available free Ca^{2+} for release from the SR per action potential (26, 50, 76). Support for P_i and Ca^{2+} precipitating came from findings identifying a phosphate permeable
channel in the sarcoplasmic reticulum (50) as well as no change in total SR \([\text{Ca}^{2+}]\), suggesting \(\text{Pi}\) and \(\text{Ca}^{2+}\) precipitated (26).

Importantly, a study completed by Vestergaard-Poulsen et al. (74) combined \(^{31}\text{P}\) nuclear magnetic resonance spectroscopy and electromyography during fatiguing human calf ergometer exercise. At the onset of exercise, there was a rapid increase in \(\text{Pi}\) and a rapid decrease in \(\text{PCr}\) and \(\text{pH}\), where EMG amplitude increased only once muscle \(\text{pH}\) declined below 6.75-6.85. Additionally, median EMG frequency declined linearly during exercise and was strongly correlated with \(\text{pH}\) (\(r = 0.82\)). The decreased EMG frequency and increased EMG amplitude, respectively implies that muscle conduction velocity has reduced (62, 69) and larger motor units or the same motor units are being recruited more frequently to maintain the current exercise intensity (30, 37, 62). Therefore, during fatiguing exercise, motor unit recruitment must increase to maintain force output, however, with this increase in motor unit recruitment there is increased build-up of fatigue related metabolites.

Recovery from exercise induced skeletal muscle fatigue is exercise task dependent. Carroll, Taylor and Gandevia (18) provide a good overview of recovery from fatigue. In general, their literature review demonstrates a rapid but incomplete recovery of maximal voluntary force after exercise is terminated. This recovery occurs within 15-30 sec and has been suggested to be as a result of re-perfusion of the muscle, where recovery is inhibited if the muscle is kept ischemic (18). Specifically, during sustained MVC exercise, maximal voluntary force declines rapidly, where force falls to below 50% of initial levels within 1-2 min. Full recovery from a sustained MVC is very slow where only 80% MVC may be recovered by 4-5 min (35) and may only fully recover by 30 min after exercise termination (18). Importantly, a slow recovery can be seen in sustained submaximal contractions, where an increase in EMG amplitude is required to increase
motor unit recruitment during a fatiguing exercise challenge (2). After completing such exercise, maximal voluntary force is not yet fully recovered by 20-30 min after exercise cessation (18). Importantly, peripheral fatigue and the slow recovery thereof occurs under normal muscle oxygenation conditions (82). However, in order to see recovery, exercise intensity must be decreased.

In summary, skeletal muscle fatigue is the result of a build-up of metabolites, which in high enough concentrations can result in impaired excitation contraction coupling processes, leading to a reduction in force for a given CMD. Recovery from skeletal muscle fatigue is slow, where depending on the task, full recovery can extend from minutes to hours or even days (18). Importantly, recovery from skeletal muscle fatigue necessitates a reduction in exercise intensity.

2.2 - Skeletal Muscle Oxygen Conforming Response

As opposed to skeletal muscle fatigue, oxygen conforming refers to the rapid adjustment of muscle force production (ATP demand) for the same motor neuron activation, in response to changes in muscle oxygenation (aerobic ATP supply) so that the cell environment remains stable. In other words, the skeletal muscle’s inherent response to changes in oxygenation is to adjust ATP demand accordingly. Importantly, the oxygen conforming response occurs in both directions: downregulation of force with decreased muscle oxygenation and upregulation of force when muscle oxygenation is increased.

The first evidence of an oxygen conforming response was demonstrated by Hobbs et al. (38) in 1987. Within an in situ cat design, Hobbs and McCloskey (38) tested the hypothesis that type I and IIa fibers would be highly sensitive to changes in perfusion pressure. Hobbs and McCloskey (38) therefore controlled blood flow and measured force production during stimulated exercise. Hobbs and McCloskey (38) demonstrated that, within a muscle composed of 100% type
I fibers (cat soleus), force production is highly sensitive to changes in blood flow. Of particular importance is that this oxygen conforming response is rapidly reversible. Hobbs and McCloskey (38) clearly demonstrated the reversible nature of the oxygen conforming response by decreasing blood flow and then returning blood flow to previous levels. In the highly non-fatigable cat soleus muscle, force production always returned to the same level when blood flow was restored (38).

Within the same study, Hobbs and McCloskey (38) tested the hypothesis that a fall in local blood pressure would necessitate an increase in muscle activation to maintain the same force in an in vivo human design. Hobbs and McCloskey (38) tested this hypothesis by having participants complete constant (~20% MVC) plantar flexor exercise in three different positions; heart level, 30° and 60° above heart level. Hobbs and McCloskey (38) concluded that integrated electromyography (iEMG) increased as muscle perfusion pressure decreased. Importantly, iEMG was not different from starting position when the leg was returned to heart level. To further confirm that this effect was not caused by mechanical changes associated with leg tilting, the authors used leg cuff occlusion during the transition from above heart to heart level. It was found that iEMG did not recover until leg cuff occlusion was removed, and perfusion pressure restored. Therefore, Hobbs and McCloskey (38) concluded that the increases in iEMG when the leg was tilted above heart level was as a result of changes in muscle blood flow and perfusion pressure. Hobbs and McCloskey (38), however, did not measure muscle blood flow or muscle oxygenation.

A decade later, in 1996, Fitzpatrick, Taylor and McCloskey (32) looked at the effect of consistent electrical stimulation on muscle force production in humans. These investigators used bipolar stimulation of the ulnar nerve to cause adductor pollicis contraction (result is adduction of the thumb) under three different conditions; heart level, below heart level and above heart level. When the arm was raised above the heart (i.e. low perfusion pressure), force for thumb adduction
decreased by 22% while when the arm was moved below the heart (i.e. high perfusion pressure), force of thumb adduction increased by 8% compared to heart level. These data show the direct effect of changes in perfusion pressure on ATP supply and demand during exercise, demonstrating increased force under supposedly increased oxygenation and decreased force under supposedly decreased oxygenation. Importantly, when the forearm was returned to heart level, force always and rapidly (within ~20 sec) returned to that normally observed at heart level, indicating that no fatigue had occurred. In other words, the muscle’s inherent response to changes in oxygenation is to adjust ATP demand by modifying force of contraction, for a given CMD. Notably, in order to see force return to that normally seen at heart level, exercise termination was not required.

More invasive animal in situ experiments have demonstrated that reductions in force at a given motor drive prevent changes in the myocyte cytosolic environment. Hogan, Kurdak and Arthur (42) demonstrated the tight relationship between developed force (ATP utilization) and oxygen delivery (ATP supply). Hogan et al. (42) measured the force of the gastrocnemius in mongrel dogs through stimulation of the sciatic nerve under a control condition, hypoxemic condition and ischemic condition, with normal blood flow and PaO₂ for the first three minutes preceding each experimental procedure. Under ischemia and hypoxemia, oxygen delivery (O₂D) was reduced equally and significantly from the control condition. Force of contraction was also reduced equally and significantly from control. However, there was no change in the metabolic disturbance of the muscle as verified by measures of ATP, ADP, PCr, Pᵢ, NH₃, redox ratios and lactate. Hogan et al. (42) demonstrated that force generation (ATP demand) responds to altered O₂D (aerobic ATP supply) to ensure a stable myocyte cytosolic environment.
In summary, the oxygen conforming response (OCR) refers to the rapid adjustment of muscle force production (ATP demand) for the same motor neuron activation, in response to changes in O$_2$D (aerobic ATP supply) so that the cell environment remains stable.

2.3 - Muscle fatigue vs. Oxygen Conforming

In the previous two sections, we described both skeletal muscle fatigue and the OCR and it is now evident that they could both be seen as a compromise to force for the same motor drive. However, skeletal muscle fatigue (or peripheral fatigue) is a compromise in force as a result of fatigue-related metabolites (such as P$_i$) for the same CMD. Importantly, the compromise in force associated with peripheral skeletal muscle fatigue remains evident for some time following exercise cessation. Alternatively, the OCR is a bi-directional alteration in force production for a given motor drive. Compared to the compromise in muscle fatigue, the OCR demonstrates a compromise in muscle force production for the same CMD in a reduced O$_2$D condition, despite no change in metabolic disturbance of myocyte cytosolic environment (i.e. no change in ATP, ADP, PCr, P$_i$, ammonia (NH$_3$), redox ratios and lactate). The OCR is bi-directional, where for a given motor drive, muscle force production is reduced in a reduced O$_2$D condition, or muscle force is increased in an increased O$_2$D condition. Importantly, the OCR change in force production is rapidly (within ~20 sec) reversed when muscle oxygenation is reversed to its original level of muscle oxygenation. Furthermore, in order to see force return to that normally seen at heart level, exercise cessation is not required. Therefore, the OCR is a distinct mechanism from exercise induced skeletal muscle fatigue.
2.4 - (Voluntary) Exhaustion

Now that it is clear that fatigue and the OCR are distinct phenomena, how might they be important for exercise tolerance? Exhaustion is the voluntary termination of exercise, occurring at a sensory tolerance limit which occurs prior to a peripheral critical threshold (1, 43, 53, 54). The sensory tolerance limit theory was proposed by Gandevia (33), and suggests that as exercise continues, neural inhibitory feedback from working muscles, respiratory muscles, frank pain in either exercising or non-exercising muscles as well as corollary discharge associated with motor drive, will contribute to a sensory tolerance limit (44). At this sensory tolerance limit the individual will no longer be willing to continue exercising, leading to voluntary exhaustion (44).

The peripheral critical threshold hypothesis states that exercise will not continue beyond a specific and individual level of peripheral fatigue (6, 33, 43). More specifically, the peripheral critical threshold suggests that the build-up of fatigue related metabolites during exercise, such as H⁺, Pᵢ and ADP will eventually reach a level where negative sensory feedback will contribute to the voluntary termination of exercise. Voluntary termination occurs at this specific individual level of metabolite build-up and peripheral fatigue (8). Eventually, the build-up of fatigue related metabolites will contribute to an inability to contract the skeletal muscle, by inhibiting the excitation-contraction coupling process or by providing increasingly severe negative sensory feedback, via group III/IV afferents (7, 44, 53, 60). Therefore, rather than continuing exercise to the point where excitation-contraction coupling processes are “catastrophically” inhibited, strong negative sensory feedback is sent to and sensed within the brain to promote the discontinuation of exercise prior to causing bodily harm (1). Consequently, exercise is terminated prior to a critical peripheral threshold, at a sensory tolerance limit (8, 44). Therefore, sensations during exercise are important determinants of exercise continuation or termination, and therefore exercise tolerance.
The concept of a sensory tolerance limit has been eloquently demonstrated by Amann et al. (8) and may provide support for the concept of the oxygen conforming response acting to limit exercise tolerance. Specifically, the response of the quadriceps muscle group to sustained exercise at 85% of work rate peak at 60 revolutions per minute (RPM) until task failure (cadence below 50 RPM for ≥ 10 s) (termed Leg2 condition) was compared with this same muscle group response to the same exercise, but where it was preceded by the contralateral leg performing this exercise to task failure (termed Leg2-post). This modality was used to increase total afferent feedback in Leg2-post compared to Leg2. Results from the Amann et al. (8) study demonstrated reduced time to exhaustion in Leg2-post, despite reduced peripheral fatigue in Leg2-post at exhaustion. These authors concluded that this was evidence of a sensory tolerance limit, where the negative afferent feedback from the exhausted leg1 contributed to limiting exercise performance in Leg2-post. However, the authors do not consider the impact of the significantly lower blood flow (~1L/min difference) in Leg2-post following the exhausting bout in leg1 during the first minute of exercise in contributing to this sensory tolerance limit. Specifically, if blood flow to the second leg is reduced in Leg2-post compared to the leg2, then the oxygen conforming response would predict that increased EMG activity would be required to maintain the same work-rate. Specifically, during this first minute, increased CMD would be required to continue to maintain the current exercise intensity. This increased CMD would be expected to increase corollary discharge and contribute to this sensory tolerance limit as demonstrated by the main effect of condition on rating of perceived exertion (RPE). Thus, while feedback from the exhausted leg1 may contribute to negative sensory feedback from group III/IV afferents, the oxygen conforming response occurring in Leg2-post would predict an increase in corollary discharge further contributing to this sensory tolerance limit.
Importantly, the reduced % increase in iEMG reported in Leg2-post may be caused by an invalid data comparison. Specifically, iEMG is being reported as a percentage increase from the 1\textsuperscript{st} minute of exercise. Due to the reduced blood flow in Leg2-post and based off the OCR we would predict an increase in CMD to maintain the current exercise intensity in Leg2-post following the exhausting bout in leg\textsubscript{1} compared to Leg\textsubscript{2}. Therefore, if iEMG in Leg2-post is 2 mV (arbitrary value) during the first minute and Leg\textsubscript{2} is 1.6 mV and in both there is an absolute max iEMG of 4 mV, %iEMG in Leg2-post would be 200% whereas Leg\textsubscript{2} would be 250%. Consequently, reporting iEMG as a percentage increase from the 1\textsuperscript{st} minute of exercise would be underestimating the maximal value attained in Leg2-post. iEMG in leg2 may therefore not have been reduced compared to control.

Findings from Amann et al. (8) indicate a potential gap in the literature, where the oxygen conforming response may be implicated in exercise tolerance separate from fatigue, though this remains unknown. If, in the study by Amann et al. (8), EMG amplitude is in fact higher in the second leg compared to control, we would predict increased CMD required to continue exercising. Increased CMD would contribute to negative sensations making exercise feel more difficult for a given workload, thereby leading to a sensory tolerance limit and voluntary exhaustion earlier within the same exercise.

2.5 - Effort vs. Exertion and How to Measure Them

If sensations of exercise play a key role in exercise tolerance, what specifically are these sensations? RPE, as created by Borg, is a psychophysiological measure which requires both physical and psychological sensations to provide a rating of subjective perception of exercise intensity (15). Importantly, when Borg first developed the RPE scale he used the words effort and exertion interchangeably (15, 16). Furthermore, the words effort and exertion are used
interchangeably in the literature, where either no definition has been provided or inconsistent
definitions have been used (52). Though effort and exertion are synonyms of each other, the
present section focuses on the unique physiological differences between effort and exertion,
helping to understand how changes in muscle oxygenation may contribute to sensation of effort
during exercise.

Borg’s rating of perceived exertion is a tool used to measure overall sensation of discomfort
or fatigue during exercise (15, 16), therefore, it may not be suitable to measure specific sensations
during exercise, without first modifying the verbal anchors and or the descriptors related to the
scale being used. It would seem important to modify scale descriptions to ensure participants can
discriminate between the perception of different sensations experienced during exercise. In this
context, Swart et al. (71) modified Borg’s 15-point RPE scale to measure only physical sensations
during exercise, excluding any psychological/psychic contribution to perception of effort, and
termed this the physical-rating of perceived exertion (P-RPE). Furthermore, Swart et al. (71)
developed a novel scale designed to measure the magnitude of psychological/psychic sensations
of effort and the extent to which the subject was consciously aware of these sensations; termed the
task effort and awareness (TEA).

To ensure that participants could correctly distinguish between what the P-RPE measured
vs. what the TEA measured, Swart et al. (71) created a questionnaire to assist in understanding the
difference between the perception of effort and exertion. Accurate response to this questionnaire
(9 of 10 questions correct) was required before engaging in the exercise protocol. Swart et al. (71)
tested the hypothesis that effort and exertion could be distinguished during exercise using the P-
RPE and TEA scales, and that they could be disassociated from each other. Swart et al. (71) had
seven trained male cyclists complete a 100 km maximal time trial with interspersed 1km maximal
sprints. TEA and P-RPE were measured every 5 km throughout the time trial, and at the end of every sprint. During this maximal 100 km time trial, P-RPE and TEA increased linearly over the duration of the trial ($r = 0.93$) and were strongly correlated ($r = 0.80$). Importantly, during maximal 1 km sprints, TEA achieved maximal levels in all sprints, whereas P-RPE only achieved maximal levels when approaching the second to last sprint. This distinction between P-RPE and TEA during a maximal effort sprint, suggests that perception of effort and exertion are indeed distinct sensations, and should be investigated as different sensations during exercise.

### 2.6 - Perception of Effort

If perception of effort then is a distinct sensation during exercise, what is known about it? Perception of effort has been defined as the amount of mental or psychic energy being given to a task (71). Based off the sensation of innervation theory, it is proposed that the sense of effort is the conscious awareness of motor drive or motor command sent to the active muscle (49, 79). This CMD is believed to be directed to both the muscle and sensory areas within the brain via a copy of central motor command that is not directly involved in the current motor task; known as corollary discharge (63, 64, 80). de Morree, Klein and Marcora (59) proceeded to clarify that perception of effort is correlated with motor drive. Using electroencephalography (EEG), with electrodes attached to various sites of the scalp known to pick up signals of brain structures involved in motor control de Morree et al. (59) measured movement-related cortical potentials (MRCP) during forearm exercise at two different intensities (25% or 35% of MVC) under two different conditions (fatigued or non-fatigued). The amplitude associated with these MRCP’s were considered a direct neurophysiological measure of CMD. However, to confirm that perception of effort was a neural correlate of CMD, the amplitude of the MRCPs needed to increase with both force of contraction and muscle fatigue, as well as be significantly correlated with RPE under both
fatigued and non-fatigued conditions. de Morree et al. (59) concluded that MRCP amplitude correlates with perception of effort and that perception of effort is the conscious awareness of central motor command (59).

The findings of de Morree et al. (59) clarify the involvement of corollary discharge in the perception of effort, however, they do not provide direct insight into the supraspinal sites involved in this perception. It has been suggested that corollary discharge comes directly from the primary motor cortex (19, 68), however, it has also been proposed that these discharges may originate from sites upstream of the primary motor cortex, though only indirect evidence of this has been provided (19). Because of the difficulties involved in investigating brain function during exercise, it is difficult to identify the specific sites involved in the perception of effort. Therefore, this area will require investigation in the future.

It is however believed that the sensation of effort is interpreted in the anterior cingulate cortex, thalamus and insular cortex (79–81). Specifically, the anterior cingulate cortex and the insular cortex were identified as sites of effort sensation because they were activated during static handgrip exercise independent of afferent feedback from group III and IV afferents or feedback from changes in blood pressure sensed via baroreceptors (52, 79). These findings are interpreted to suggest that central motor command activated these sensory areas independent of afferent feedback and therefore effort sensation is independent of afferent feedback. Yet, a review by Gandevia (34) explained that small diameter muscle afferents can reduce central motor command, and that this was because of group III/IV afferents targeting areas upstream from the motor cortex. Therefore, while afferent feedback is not directly involved in perception of effort, afferent feedback may modify CMD and in turn affect perception of effort during fatiguing exercise.
In summary then, perception of effort increases with an increase in CMD, therefore, whether CMD increases as a result of an increase in fatigue-related metabolites compromising skeletal muscle force production or via a reduction in skeletal muscle oxygenation, perception of effort should be increased. This increased perception of effort for a given workload would bring the individual closer to the sensory tolerance limit and in this way could contribute to the voluntary termination of exercise.

2.7 - Impact of Perfusion Pressure on Exercising Muscle Blood Flow

We turn our attention now to exercising muscle blood flow, a key determinant of muscle oxygenation, and how it is affected by alterations in local exercising muscle perfusion pressure. During submaximal exercise, muscle blood flow increases in proportion to metabolic demand \((73)\), reaching a steady state within 1.5-2 min \((66)\). Convective O\(_2\)D is equal to muscle blood flow \((\dot{Q}_{\text{muscle}})\) multiplied by arterial O\(_2\) content \((C_aO_2)\). The magnitude of O\(_2\)D relative to metabolic demand for oxygen determines the partial pressure of oxygen in the capillaries and therefore in the muscle fibre. Therefore, muscle oxygenation can be increased or decreased during exercise at a fixed metabolic demand by increasing or decreasing muscle blood flow \((32, 82, 83)\).

Exercising skeletal muscle blood flow is a function of the local arterial – venous perfusion pressure gradient \(\times\) vascular conductance. The local arterial pressure is a function of the systemic arterial pressure generated by the balance of cardiac output and peripheral blood flow, and the local hydrostatic pressure due to gravity. The latter decreases with increasing distance above heart level, and increases with increasing distance below heart level. Thus for the same amount of skeletal muscle vasodilation during exercise, blood flow will be greater if the exercising muscle is below vs. above heart level. This perfusion pressure challenge has been repeatedly demonstrated
in a number of studies that manipulated exercising forearm position between above and below heart level during forearm exercise (9, 10, 32, 75, 82, 83).

Given that oxygen delivery/demand matching is regulated, it might be expected that at a given exercising muscle metabolic demand, compensatory changes in vascular tone would occur to offset the effects of altered perfusion pressure and restore the original blood flow. However, it appears that such a response may depend on the individual (10). Specifically, Bentley et al. (9) identified a vasodilator phenotype, where certain participants responded to this same perfusion pressure challenge by vasodilating and defending forearm blood flow and therefore exercise capacity. Bentley et al. (10) then proceeded to follow this study up with a study to further explore the concept of a vasodilator phenotype or inter-individual differences in the mechanisms governing muscle oxygen delivery. This study confirmed a dichotomous vasodilatory response, where 13 individuals responded to the perfusion pressure challenge with compensatory vasodilation and restoration of blood flow, whereas 8 individuals did not. When individuals do not compensate in the face of altered perfusion pressure, sustained changes in the oxygen delivery vs. metabolic demand are incurred, as evidenced by the findings of Walker et al. (75). A key finding of Walker et al. (75) was that these changes are reproducible with repeated changes in perfusion pressure. These results demonstrate the dependence of the exercising limb blood flow on perfusion pressure.

Another methodological approach which may be successful in reducing forearm blood flow and which may be able to overcome any issues associated with the vasodilator phenotype is brachial artery compression (65). Pyke et al. (65) clearly demonstrated that by pneumatically compressing the brachial artery proximal to the antecubital crease, forearm blood flow could be controlled during a flow-mediated dilation test. Therefore, brachial artery compression should be considered an appropriate method of controlling forearm blood flow.
In summary, it is clear that skeletal muscle fatigue and the oxygen conforming response are fundamentally distinct phenomena. Skeletal muscle fatigue is characterized by a sustained elevation in the amount of muscle activation required to maintain a given force output. Importantly the recovery from skeletal muscle fatigue can take hours to days and requires exercise intensity to be reduced. Alternatively, the oxygen conforming response is characterized by a rapid restoration (within 20 s) of the EMG/Force relationship in response to changes in muscle oxygenation, and it does not require reduced exercise intensity. Therefore, to distinguish skeletal muscle fatigue from the oxygen conforming response, it is necessary to demonstrate the rapid restoration of the EMG/Force relationship upon muscle re-oxygenation. To date, the few studies to have investigated the oxygen conforming response have been completed in electrically stimulated muscle exercise models. Stimulated models are fundamentally different from voluntary exercise, therefore, it remains to be determined if the oxygen conforming response exists in voluntary human exercise.

Additionally, it is clear that sensations during exercise can play an important role in an individual’s exercise tolerance and performance. Specifically, perception of effort, which is thought to be the subjective awareness of CMD activating motor neurons, can be modified by skeletal muscle fatigue and make the exercise feel harder to do. Whether perception of effort can be altered by an oxygen conforming response has not been investigated. Therefore, the proceeding sections will act to test the hypothesis that the oxygen conforming response exists and is able to modify perception of effort in voluntary human exercise.
2.8 – Summary (Problem Statement)

Principle Proposition:

1. As opposed to the sustained compromise in EMG/Force characteristic of skeletal muscle fatigue, the OCR is characterized by a rapid restoration of the EMG/Force relationship upon restoration of muscle oxygenation.

2. Perception of effort is the conscious awareness of CMD activating motor neurons, where if CMD (measured with EMG) increases so does perception of effort.

Interacting Proposition: Considering the limitations of the study designed by Hobbs and McCloskey (38), the OCR remains to be tested during rhythmic exercise while simultaneously quantifying changes in muscle oxygenation. In addition, the impact of the OCR on perception of effort has not been tested.

Speculative Proposition: Given the nature of the OCR, as evident from electrically stimulated human (32, 51) and animal (38) exercise, it is predicted that in order to maintain a constant voluntary contraction force, EMG and therefore perception of effort will rapidly follow changes in muscle oxygenation independent of fatigue.

Purpose: The purpose of my Master’s thesis was to, for the first time, quantify the response of EMG amplitude to measured changes in muscle oxygenation during voluntary exercise (forearm contractions) in humans, and determine whether perception of effort changes with EMG.

Hypothesis: We hypothesized that for the same voluntary force output 1) Muscle activation would increase with decreased muscle oxygenation, 2) Muscle activation would be rapidly
restored upon muscle re-oxygenation, and 3) Perception of effort would follow changes in muscle activation.

**Significance:** Studying the effect of changes in local oxygen delivery on sensations involved in exercise tolerance separate from fatigue is important for improving our understanding of how local oxygen delivery might contribute to modifying exercise behaviour by its effects on exercise tolerance and performance.
Chapter 3 - Manuscript

Fatigue-Independent Alterations in Muscle Activation and Effort Perception During Forearm Exercise: The Role of Local Oxygen Delivery


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ABSTRACT

The oxygen conforming response (OCR) of skeletal muscle refers to a down regulation of muscle force for a given muscle activation when oxygen delivery is reduced, which is rapidly reversed when oxygen delivery is restored. We tested the hypothesis that the OCR exists in voluntary human exercise and results in compensatory changes in muscle activation to maintain force production, thereby altering perception of effort. In 16 humans, electromyography (EMG), oxyhemoglobin (O$_2$Hb) and deoxyhemoglobin (HHb; near infrared spectroscopy), forearm blood flow (FBF) and task effort awareness (TEA) were measured during steady state rhythmic handgrip exercise with alternating 2-min periods of free flow and brachial artery compression (BAC) used to reduce FBF by ~50% of steady state (SS). BAC successfully reduced FBF (C1, -55% (percent reduction), P<0.001; C2, -58%, P<0.001) and O$_2$Hb (C1, -40%, P<0.001; C2, -39%, P<0.001) during both compression periods compared to steady state free flow (FBF = 226±144; O$_2$Hb =57±17%). EMG during C1 (1.58±0.39, P<0.001) increased compared to SS (1.31±0.33) though it was not restored during the subsequent no compression (NC1, 1.48±0.38, P=0.477). EMG increased during C2 (1.73±0.50) compared to NC1 (1.48±0.38, P=0.013) and then returned to NC1 levels during NC2 (1.50±0.39, P=0.016). TEA (SS, 2.2±2.3; C1, 3.9±2.5; NC1, 3.4±2.7; C2, 4.6±2.7; NC2, 3.9±2.8) followed changes in EMG. Noteworthy, during the second compromise and then restoration of muscle oxygenation, EMG and TEA were rapidly restored to pre-compromise levels. We interpreted these findings to support the existence of an OCR and its ability to rapidly modify perception of effort.

KEYWORDS: Oxygen conforming response, local oxygen delivery, muscle activation, perception of effort, voluntary exercise
INTRODUCTION

Skeletal muscle fatigue, resulting from the build-up of fatigue related metabolites, can result in impaired excitation contraction coupling processes, resulting in reduced force per muscle activation (Force/EMG (electromyography)) (3, 4, 23). This necessitates increased motor neuron activation to maintain force output when skeletal muscle is fatigued. Importantly, perception of effort is thought to originate from efferent neural activity arising parallel and in proportion to central motor drive (CMD) determining motor neuron recruitment (20, 28, 44, 52, 54, 59, 64). Therefore, the increase in CMD required to maintain force output when the skeletal muscle is fatigued is sensed and perceived as an increase in the subjective awareness of effort (6, 8, 20, 57, 59). Thus, by increasing the perceived effort during exercise, skeletal muscle fatigue can play an important role in an individual’s exercise tolerance (33).

Another mechanism that could increase CMD at a given skeletal muscle force output is the down-regulation of Force/EMG that can accompany reduced muscle $O_2$D (32, 38, 42, 51). This phenomenon is known as the oxygen conforming response (OCR) (39, 40, 72). As opposed to the sustained compromise in the Force/EMG relationship characteristic of skeletal muscle fatigue (59, 82), the OCR is characterized by a rapid (within ~20 sec) restoration of the Force/EMG relationship with restoration of muscle oxygenation (32, 38, 51). This OCR phenomenon has been observed in electrically stimulated skeletal muscle contraction models in humans (32, 51) and animals (38). An important limitation of the electrical stimulation models cited (32, 51) is that supramaximal stimulation is being used, therefore, as opposed to typical exercise which follows the size principal of motor recruitment, all motor units are recruited for every contraction in their stimulated exercise designs (24, 37). Considering this limitation supramaximal stimulation models do not replicate voluntary exercise, where a subpopulation of motor units are recruited to produce
the required force in submaximal exercise. Because of this, a plausible response to reduced oxygenation in voluntary exercise could be to rotate activated motor units, thereby reducing the O$_2$ demand of a given motor unit. This would not necessarily result in increased muscle activation, and therefore not increase perception of effort. Considering these limitations, the existence of an OCR in voluntary human exercise and its role in modifying perception of effort remains unknown.

Therefore the objectives of this study were to determine 1) whether an OCR effect can occur in voluntary human exercise and 2) whether perception of effort follows OCR-mediated alterations in EMG. In order to complete these objectives, muscle activation, muscle blood flow, muscle oxygenation, and perception of effort were measured during rhythmic non-fatiguing voluntary handgrip exercise with intermittent compromise to O$_2$D. We hypothesized that for the same voluntary force output 1) muscle activation would increase with decreased muscle oxygenation, 2) muscle activation would be rapidly restored upon muscle re-oxygenation, and 3) perception of effort would follow changes in muscle activation.

METHODS

Participants

Sixteen healthy participants (8 men, 8 women, see Table 1 for anthropometric data) were recruited for this study. Participants were instructed to adhere to the following prior to testing: to forego strength training exercise for 24 hours before, abstain from alcohol, smoking and caffeine consumption for 12 hours before, and for eating 4 hours before testing. All experimental procedures were approved by the Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board, and conformed to the standards set by the Declaration of Helsinki. On the first of two visits, participants provided verbal and written consent as well as
completed the CSEP Get Active Questionnaire form (see Appendix A) prior to voluntary participation in this study.

**Instrumentation**

**Surface Electromyography.** Surface EMG was measured via four mini wireless bipolar electrodes (25 mm x 12 mm x 7 mm) with a 10 mm inter-electrode distance (Delsys Trigno Wireless EMG system, Natick, MA, USA). Prior to sensor placement the approximate location of EMG sensor placement was cleaned of any hair and thoroughly scrubbed with alcohol wipes. The electrodes were then placed over the 4 muscle bellies of the right flexor digitorum superficialis (FDS). The 4 muscle belly sites were identified by following the guide designed by Bickerton et al. (11) for injection of botulin toxin into the 4 muscle bellies of the FDS. Briefly, a landmarking line (LL) was drawn between the right medial epicondyle and the pisiform. The length of the LL was recorded and the approximate distance from the medial epicondyle to the 4 muscle bellies was marked (FDS4; 49%, FDS3; 54%, FDS2; 72%, FDS5; 76%) (11). Now, from the marks made along the LL, measurements were made laterally to the exact location of each muscle belly and were then marked (FDS4; 0.7cm, FDS3; 1.7cm, FDS2; 1.4cm, FDS5; 0.6cm) (11). EMG electrical activity was measured in Volts (V) and up-sampled to an even 2000 Hz/channel.

**Force.** Force of handgrip contraction was recorded via a handgrip force transducer connected to PowerLab /8SP (ADInstruments, Colorado Springs, USA), collected at a frequency of 200 Hz/channel.

**Forearm Blood Flow.** During exercise, brachial artery blood velocity and diameter were measured continuously. Brachial artery blood velocity was measured with a 4-MHz pulsed flat Doppler probe (Model 500V 131 Transcranial Doppler, Multigon Industries, Mt. Vernon, NY)
attached to the skin over the brachial artery proximal to the ante-cubital fossa of the exercising arm. Brachial artery diameter was measured using a linear echo ultrasound probe, positioned over the brachial artery ~5cm proximal to the Doppler probe, operating at 13-MHz in 2D mode (Vivid-I GE Medical Systems, London Ontario, Canada).

**Muscle Oxygenation.** Muscle oxygenation was measured using a commercially available near infrared spectroscopy (NIRS) system (Oxymon, Artinis Medical Systems, Einsteinweg, Netherlands). The NIRS device provided measurements of change in oxyhemoglobin ($O_2$Hb) and deoxyhemoglobin (HHb). This study used a single channel consisting of one optical fiber operating at three wavelengths (874, 761 and 803 nm) where the light was transmitted into the muscle and received by an avalanche photodiode (APD). NIRS data was collected at a sample rate of 10 Hz with an inter-optode distance of 25 mm, differential path-length factor (DPF) of 3.59, with participant specific power and gain settings, ensuring optimal signal performance. The NIRS probes were placed longitudinally between the two distal and two proximal mini-EMG sensors, where the sites were shaved and cleaned prior to securing the probe in place.

**Perception of Effort.** The task effort awareness (TEA) scale, a 15-point scale developed by Swart et al. (71), was used to measure perception of effort; the amount of attention, mental effort and difficulty experienced while maintaining the prescribed physical task (see Appendix A). Participants were able to see the scale continuously throughout exercise, and were asked to report their TEA 45 and 90 seconds into each segment starting at steady state. Participants were asked to speak the number that represented their current perception of effort.

In order to ensure proper reporting of perception of effort in isolation from peripheral afferent feedback associated with discomfort (5) all participants were required to complete a 10-point questionnaire (see Appendix A) used to establish difference between sense of effort and
physical sensations. This questionnaire was completed on “Day 1” during screening and then repeated on “Day 2” prior to beginning exercise. Clarification of the answers were provided when needed. As per Christian et al. (20) the example that “a brief maximal effort requires a maximal conscious effort despite only inducing a small amount of peripheral discomfort” was explained to every participant (61).

Central Hemodynamic Measures. Heart rate was monitored with a three-lead electrocardiogram (ECG) with electrodes attached to the skin in standard CS₅ placement (21). Arterial oxygen saturation (Saₐ₀₂) was monitored with a pulse oximeter (Nellcor N-395; Covidien-Nellcor, Boulder, CO) placed over the index finger of the participants non-exercising hand. A finger photoplethysmograph (Finometer MIDI, Finapres Medical System, The Netherlands) was used to measure heart rate (HR), mean arterial blood pressure (MAP), and to provide estimates of stroke volume (SV), cardiac output (CO) and total peripheral resistance via ModelFlow™ (Finapres Medical Systems, The Netherlands).

Experimental Design

This was a within-participant repeated measure design in which all participants completed a minimum of six exercise tests during a two day collection period. Screening and fatigue threshold identification occurred on one day, while the control and brachial artery compression (BAC) protocol occurred on another day. All data collections were completed a minimum of 24 h apart. Each participant completed both data collection sessions at the same time of day, though time of day differed between participants. All data collections were completed with the participant laying supine on a table with their arms resting on tables at heart level, abducted approximately 70° to their respective sides. All testing sessions were completed in a temperature-controlled room (19-
21°C) and isometric handgrip exercise was completed following a contraction/relaxation duty cycle of 2 s/2 s as guided by visual force output and metronome cues.

**Reducing Muscle Oxygenation.** To reduce muscle oxygenation, local forearm blood flow was reduced via manual brachial artery compression proximal to the antecubital fossa. A single researcher was tasked with reducing MBV by ~50% of the steady state MBV measured during a control trial completed on day 2. The researcher completing brachial artery compression used live feedback from the MBV measured via 4-MHz pulsed flat Doppler probe and displayed on a monitor. The researcher was provided with a visual target between 40-60% of steady state MBV on LabChart 7 (ADInstruments Inc., Colorado Springs, CO, USA) for simplified MBV tracking. Brachial artery compression was completed by a single researcher throughout the length of the study.

**TEA Familiarization.** Prior to completing exercise on data collection sessions one or two, participants were asked to read a description of both the physical-rating of perceived exertion (P-RPE) and TEA scale. Following completion of the readings, participants were asked to complete a 10-point True/False questionnaire, testing their understanding of the TEA scale. Any incorrect answers were clarified prior to continuing to exercise testing. Additionally, participants were asked their TEA during all exercise sessions on day 1 in order to orient them to the concept prior to the control and BAC protocol completed on day 2.

**Experimental Protocol and Measurement Details**

**Identification of non-fatiguing exercise work rate.** Participants were asked to complete three 3 s MVC’s, each separated by 1 min. EMG and force output was recorded during MVC’s to provide an estimate of maximal EMG activity and force output. Subsequently, forearm fatigue
threshold identification was accomplished using a non-continuous incremental ramp protocol. All participants were asked to remain still for 1 min while silent EMG values were taken then they were asked to complete 13 min of rhythmic forearm handgrip exercise targeting 10% of their highest MVC. Throughout this protocol EMG data was recorded continuously on the exercising arm from the 4 muscle bellies of the FDS. Participants were given a 5 min break where a regression analysis of EMG amplitude was completed for each muscle. The regression analysis was used to identify fatigue onset, where fatigue was evident if the EMG amplitude regression slope was significant positive. This fatigue onset or fatigue threshold has been suggested to represent the aerobic-anaerobic transition point, indicating the point where ATP demand exceeds the rate of supply from primarily aerobic sources (29). See Figure 3-1 for a full description of this protocol.

![Figure 3-1](image)

**Figure 3-1.** Fatigue threshold identification on Day 1. Participants began forearm handgrip exercise at 10% of their maximal voluntary contraction (MVC). Following 13 min at this intensity, participants were asked to relax for 5 min while EMG data was analyzed to check the EMG amplitude regression slope for this %MVC. If the EMG amplitude slope was significant (YES), participants decreased %MVC by 5% and the 13 min test was repeated. If the EMG amplitude slope was not significant (NO), participants increased %MVC by 10% and the 13 min test was repeated. The protocol was terminated once a YES-NO had been identified (in that order).
Confirming the highest level %MVC sustainable for 13 min; the length of the experimental protocol on day 2. If a NO-YES-YES occurs, %MVC that is sustainable will be assumed to be the first NO. Fatigue threshold = YES-NO or NO-YES-YES. This %MVC was confirmed on day 2.

**Control.** On day 2, participants completed three 3 s MVC’s, each separated by 1 min. Participants then completed a control exercise trial. This control was used to confirm the %MVC identified on day 1 does not cause fatigue over the duration of the 13 min test. In order to ensure no fatigue was present, the same fatigue identification analysis as used in the identification of non-fatiguing exercise work rate was used. See Figure 3-2 for a summary of this protocol.

![Figure 3-2](image)

**Reduced muscle oxygenation protocol.** Once confirmation of the fatigue threshold was completed and successfully passed, FBF was manipulated during the 13 min rhythmic forearm handgrip exercise at the confirmed non-fatiguing exercise work rate (see Fig. 3-3). During this exercise, participants had their right arm resting at heart level on a cushioned table. Prior to beginning the experimental manipulation, a single researcher designated to complete BAC, located the brachial artery proximal to the antecubital fossa.

As Figure 3-3 depicts, participants were asked to reach steady state exercise. Steady state was achieved by completing 5 min of rhythmic handgrip exercise at the confirmed non-fatiguing exercise work rate. At the beginning of the 5th and 9th minute of exercise brachial artery compression was completed for 2 min periods. Participants were asked to maintain force of
contraction throughout where the only change during exercise was BAC. Following completion of the first bout of the BAC protocol, a 10 min break was provided, then the BAC protocol was repeated. See Figure 3-3 for summary.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>NC</th>
<th>NC-SS</th>
<th>C</th>
<th>NC</th>
<th>C</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

**Figure 3-3.** BAC protocol. Steady state forearm handgrip exercise was completed at each participant’s individually determined %MVC work rate. Exercise began with 5 minutes of no compression (NC) handgrip exercise following a contraction:relaxation cycle of 2s:2s. Starting at minute 5 and 9, brachial artery compression (C) was maintained for 2 minutes. This protocol was completed twice for all participants. SS, Steady state; T, Task effort awareness measurement.

**Data Acquisition**

**Identification of non-fatiguing exercise work rate.** EMG data was recorded continuously and each muscle contraction was used to complete the regression analysis required for identification of an individual’s maximal non-fatiguing exercise work rate.

**Control.** Confirmation was completed using the same method as that described during the identification of non-fatiguing exercise work rate protocol. Additionally, all recorded data was averaged into 1 min 30 s time bins. Specifically, data was averaged going from 15 s to 1 min 45 s into a new segment. Two measures of TEA (45 s apart) were averaged to provide one value for each segment.

**Reduced muscle oxygenation protocol.** All recorded data was averaged into 1 min 30 s time bins. Specifically, data was averaged starting from 15 s to 1 min 45 s into a new segment. Two measures of TEA (45 s apart) were averaged to provide one value for each segment.
Data Analysis

Identification of non-fatiguing exercise work rate. Using the EMG data collected through the incremental exercise test, a linear regression of the EMG root mean square (RMS) was created for each exercise intensity. Similar to Camic et al. (17) the fatigue threshold was identified by finding the lowest work-rate to have a significant positive EMG RMS slope coefficient, and then reducing work rate by 5% to identify the highest work rate that does not have a significant positive EMG RMS slope. This work rate was used for all tests completed on day 2.

EMG analysis was completed using MATLAB 2017a (Mathworks, Natick, Massachusetts, USA). Raw EMG data was processed by de-trending the raw EMG data, converting it into absolute values and then subtracting the baseline noise from the signal. Once cleaned the signal was filtered using a butterworth filter (2 Hz cut off). The EMG activity from the MVC was then processed to identify the peak EMG activity. Subsequently the filtered EMG exercise data was processed to identify the on/off cycles for every muscle contraction. On/off cycles were identified using an on/off EMG threshold equal to 5% of the peak EMG amplitude seen during the participants maximal MVC. Once the on/off cycles were identified and grouped into vectors, these vectors were used to quantify the amplitude and mF of each on/off cycle. Amplitude was quantified by measuring the RMS of each on/off cycle from the raw EMG data. Alternatively, mF was measured by completing a fast Fourier transformation (FFT) taking raw EMG signal from the time domain into the frequency domain, and then calculating the median frequency. Any bad contractions (i.e. time of contraction being less than 1.5 seconds or greater than 2.5 seconds) were removed. Using function “regstats” a linear regression of amplitude was tested to be different from 0, further visual analysis was used to confirm fatigue onset.
**Control and BAC protocols.** During the control and BAC tests, EMG, NIRS, Echo and Doppler ultrasound were measured continuously. The control analysis was the same for EMG RMS as that completed during the identification of non-fatiguing work rate protocol. Data from both trials of the BAC protocol were averaged together to provide one representative data set for the BAC protocol.

**Forearm blood flow.** Brachial artery diameters were quantified using *Measurements from Arterial Ultrasound Imaging* (MAUI, Hedgehog Medical). FBF was calculated as MBV (cm/s) · 60 s/min·$\pi$ [brachial artery diameter (cm)/2]$^2$.

**Muscle Activation.** EMG was quantified by completing the same analysis for EMG completed during the identification of non-fatiguing exercise work rate described above. After completion of the steps mentioned above, the EMG RMS of each muscle belly was averaged together to get one value for each BAC protocol. This EMG RMS data was then divided by the average force output over the same period. This was done in order to account for any involuntary changes in force output between perfusion pressure conditions.

**Force output.** Force output data was analyzed to confirm contractions were consistently completed throughout FBF conditions. Specifically, code was written to first clean the force data by zeroing the baseline voltage. Next, the on/off cycles for every contraction were identified by setting a threshold of 1 kg. With on/off vectors identified, the average kg output was calculated over each cycle. Time of each contraction was calculated and any contraction less than 1.5 s or greater than 2.5 s was removed from the analysis, as per EMG analysis above. Force was measured in kg where it was then converted to Newtons and then presented as a percentage of the individuals MVC.
Muscle Oxygenation. NIRS data was measured in arbitrary units. In order to provide a meaningful relative comparison, all arbitrary units were compared to an estimated maximal and minimal level of both $O_2$Hb and HHb. In order to obtain estimates of maximal and minimal $O_2$Hb and HHb, arm cuff occlusion was completed for 5 min followed by 2 min of rest. Measures of maximal HHb and minimal $O_2$Hb were calculated as an average of the highest and lowest values measured (respectively) prior to cuff release. Measures of minimal HHb and maximal $O_2$Hb were calculated as an average of the lowest and highest values measured (respectively) during the 2 min period following cuff release. When negative values were present, the lowest measured value was added to all measured values. Addition of the lowest value to all values provided a measure of 0-100% HHb and $O_2$Hb. All values were then calculated as a percentage of the maximal measured value, providing estimates of change in muscle HHb and $O_2$Hb.

Perception of effort. TEA was recorded 45 and 90 seconds into each segment starting at steady state (see figure 3-3). These two values were averaged together to provide a single value for TEA in each segment.

Statistical Analysis

Identification of non-fatiguing work rate. A linear regression of EMG RMS was completed for all exercise intensities completed during the maximal non-fatiguing exercise work rate identification. A $P < 0.01$ was required to indicate a significant slope.

Control and BAC protocols. To test the effects of condition (control and BAC) and time on EMG RMS/Force, TEA, FBF, $O_2$Hb, HHb and mF, two way repeated measures ANOVA’s with sex as a between subject factor were completed. When assumptions of sphericity were violated, a Greenhouse-Geisser correction was used. When assumptions of normality were not
met, a Friedman’s nonparametric test was run to identify main effects. In the case of a significant F-statistic, pairwise comparisons were assessed with Bonferroni correction. A $P < 0.05$, $P < 0.01$ and $P < 0.005$ was required for parametric tests, pairwise comparisons between conditions and non-parametric post-hoc tests respectively. Statistical analyses were performed using SPSS version 24.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD.

RESULTS

Participants

21 participants began this study, two were excluded due to inadequate EMG signal, one was excluded due to bifurcation of the brachial artery proximal to the site of compression, thereby making complete brachial artery compression and forearm blood flow measurements impossible, one dropped out after Day 1 and finally one was excluded from analysis due to the manipulation not being successful in reducing muscle oxygenation. Therefore, 16 (8 women, 8 men) participants completed this study (see Table 1 for anthropometric data) (see Appendix B for individual response data).

<table>
<thead>
<tr>
<th>Table 1. Age, anthropometric measures and non-fatiguing work-rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Height, cm</td>
</tr>
<tr>
<td>Weight, kg</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>%MVC</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI, Body Mass Index; MVC, Maximal Voluntary Contraction.
Identification of non-fatiguing work rate

Completion of the incremental exercise test to identify a participant’s specific maximal non-fatiguing exercise work rate, identified an average non-fatiguing work rate of 26 ± 6% MVC.

Was force output consistent throughout BAC exercise?

Force output was not effectively maintained throughout the length of the test, $X^2 (4) = 16.400, P = 0.003$. Post-hoc tests determined that force output was significantly higher in steady state (SS, 21.24 ± 4.6%) compared to C2 (20.56 ± 4.3%, $P = 0.001$) and NC2 (20.87 ± 4.5%, $P = 0.002$) (see Table 2). See figure 3-6 for a representation of raw hand grip data during BAC.

Table 2. Quantification of muscle Activation and force output during BAC protocol

<table>
<thead>
<tr>
<th>Variable</th>
<th>SS</th>
<th>C1</th>
<th>NC1</th>
<th>C2</th>
<th>NC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMG RMS, %MVC</td>
<td>28.3 ± 11</td>
<td>33.8 ± 12*</td>
<td>31.9 ± 12*</td>
<td>36.5 ± 15*†</td>
<td>32.1 ± 12*</td>
</tr>
<tr>
<td>Force, %MVC</td>
<td>21.2 ± 4.6</td>
<td>20.9 ± 4.5</td>
<td>21.0 ± 4.7</td>
<td>20.6 ± 4.3*</td>
<td>20.9 ± 4.5*</td>
</tr>
<tr>
<td>mF, Hz</td>
<td>157 ± 18</td>
<td>156 ± 21</td>
<td>154 ± 19</td>
<td>157 ± 21</td>
<td>154 ± 18</td>
</tr>
</tbody>
</table>

Values are means ± SD. C, compression; EMG, electromyography; mF, median frequency; MVC, maximal voluntary contraction; NC, no-compression; RMS, root mean squared; SS, steady state. *Significantly different from SS. †Significantly different from NC1. All $P < 0.05$.

Did FBF change over time during the control trial?

Control FBF was significantly different over time ($F(2.040, 26.523) = 7.556, P = 0.002$) (see Table 3, Figure 3-4 for pairwise comparisons).

Did HHb change over time during the control trial?

Control HHb was not significantly different between time points ($F(1.977, 27.672) = 1.348, P = 0.276$).
Did $O_2$Hb change over time during the control trial?

Control HHb was not significantly different between time points ($F(1.938, 27.130) = 2.323$, $P = 0.119$).

Did EMG/Force remain constant throughout the control trial?

Control EMG/Force was not significantly different between time points ($F(4, 56) = 0.817$, $P = 0.519$). There was no time*sex interaction ($F(4, 56) = 1.294$, $P = 0.283$).

Did TEA remain constant throughout the control trial?

There was a significant ($F(1.273, 17.816) = 13.825$, $P = 0.001$) increase in TEA over time during the control protocol (see Table 3, Figure 3-5 for pairwise comparisons). There was no time*sex interaction ($F(1.273, 17.816) = 0.396$, $P = 0.587$).

Was FBF reduced during brachial artery compression?

There was a significant effect of time on FBF during the BAC protocol ($X^2 (4) = 57.05$, $P < 0.001$). Within BAC FBF was successfully reduced during C1 and C2. Additionally, blood flow was restored following cessation of brachial artery compression at NC1 and NC2 (see Table 3 and Figure 3-4, panel C for within condition pairwise comparisons) (see Table 4 for between condition within time pairwise comparisons). See figure 3-6 for a representation of raw FBF data during BAC.

Was muscle oxygenation decreased during brachial artery compression?

$O_2$Hb. There was a significant effect of time on $O_2$Hb during the BAC protocol ($F(1.278, 19.168) = 42.2$, $P < 0.001$). Within BAC $O_2$Hb was successfully reduced during C1 and C2. Following cessation of brachial artery compression, $O_2$Hb was restored at NC1 and NC2 (see Table
HHb. There was a significant effect of time on HHb during the BAC protocol ($X^2 (4) = 44.5, P < 0.001$) Within BAC HHb was successfully increased during C1 and C2. Following cessation of brachial artery compression, HHb was restored at NC1 and NC2 (see Table 3 and Figure 3-4, panel B for within condition pairwise comparisons) (see Table 4 for between condition within time pairwise comparisons).

**Did EMG RMS/Force increase with brachial artery compression?**

There was a significant effect of time on EMG RMS/Force during the BAC protocol ($F(2.442, 34.194) = 17.135, P < 0.001$). Within BAC EMG RMS/Force was increased at C1 and C2, however, EMG RMS/Force was not restored following C1 at NC1, though EMG RMS/Force was restored following C2 at NC2 (see Table 3 and Figure 3-5, panel B). There was no main effect of sex ($F(1,14) = 0.868, P = 0.367$) or time*sex interaction ($F(2.442, 34.194) = 0.426, P = 0.696$) on EMG RMS/Force (see Table 4 for between condition within time pairwise comparisons). See figure 3-6 for a representation of raw EMG data during BAC.

**Did TEA follow changes in EMG/Force?**

There was a significant effect of time on TEA during the BAC protocol ($F(1.620, 22.684) = 15.299, P < 0.001$). Brachial artery compression led to an increase in TEA at C1 and C2, however, TEA was not restored following C1 at NC1, though TEA was restored following C2 at NC2 (see table 3 and figure 3-5, panel A). There was no main effect of sex ($F(1,14) = 0.009, P = 0.927$) or a time*sex interaction ($F(1.620, 45.637) = 1.178, P = 0.316$) on TEA.
Table 3. Measures of hemoglobin, blood flow, muscle activation and effort sensation over time within BAC and control protocols

<table>
<thead>
<tr>
<th>BAC Protocol</th>
<th>FBF (ml/min)</th>
<th>SS (226 ± 144)</th>
<th>C1 (102 ± 62)</th>
<th>NC1 (277 ± 172)</th>
<th>C2 (114 ± 68)</th>
<th>NC2 (294 ± 189)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.001 †</td>
<td>P = 0.001 †</td>
<td>P &lt; 0.001 †</td>
<td>P = 0.001 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1 (102 ± 62)</td>
<td>P &lt; 0.001 †</td>
<td>P = 0.005</td>
<td>P &lt; 0.001 †</td>
<td>P = 0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NC1 (277 ± 172)</td>
<td>P &lt; 0.001 †</td>
<td>P &lt; 0.001 †</td>
<td>P &lt; 0.001 †</td>
<td>P = 0.001 †</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C2 (114 ± 68)</td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.001 †</td>
<td></td>
</tr>
<tr>
<td>O₂Hb (%)</td>
<td>C1 (34 ± 15)</td>
<td>SS (57 ± 17)</td>
<td>P &lt; 0.001 *</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1 (34 ± 15)</td>
<td>P = 0.061</td>
<td>P &lt; 0.001 *</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
<td></td>
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<tr>
<td></td>
<td>C1 (61 ± 16)</td>
<td>P = 0.336</td>
<td>P &lt; 0.001 *</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
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<tr>
<td></td>
<td>C2 (37 ± 14)</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.890</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
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<tr>
<td>HHb (%)</td>
<td>C1 (34 ± 15)</td>
<td>SS (68 ± 17)</td>
<td>P &lt; 0.001 *</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1 (34 ± 15)</td>
<td>P = 0.124</td>
<td>P &lt; 0.001 *</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1 (61 ± 16)</td>
<td>P = 0.017 *</td>
<td>P = 1.000</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C2 (37 ± 14)</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.379</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.029 *</td>
<td></td>
</tr>
<tr>
<td>EMG RMS/Force</td>
<td>C1 (1.58 ± 0.39)</td>
<td>SS (68 ± 17)</td>
<td>P = 0.017 *</td>
<td>P = 1.000</td>
<td>P &lt; 0.001 *</td>
<td>P = 1.000</td>
</tr>
<tr>
<td></td>
<td>C1 (1.58 ± 0.39)</td>
<td>P = 0.479</td>
<td>P &lt; 0.001 *</td>
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</tr>
<tr>
<td></td>
<td>C1 (1.48 ± 0.38)</td>
<td>P = 1.000</td>
<td>P &lt; 0.001 *</td>
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<td>P &lt; 0.001 *</td>
<td>P = 1.000</td>
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<tr>
<td></td>
<td>C2 (1.73 ± 0.50)</td>
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<td>P = 0.379</td>
<td>P &lt; 0.001 *</td>
<td>P = 1.000</td>
<td>P &lt; 0.001 *</td>
</tr>
<tr>
<td>TEA</td>
<td>C1 (3.9 ± 2.5)</td>
<td>SS (68 ± 17)</td>
<td>P &lt; 0.001 *</td>
<td>P = 1.000</td>
<td>P &lt; 0.001 *</td>
<td>P = 1.000</td>
</tr>
<tr>
<td></td>
<td>C1 (3.9 ± 2.5)</td>
<td>P = 0.017 *</td>
<td>P = 0.379</td>
<td>P &lt; 0.001 *</td>
<td>P = 1.000</td>
<td>P &lt; 0.001 *</td>
</tr>
<tr>
<td></td>
<td>C1 (3.4 ± 2.7)</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.022 *</td>
<td>P = 0.007 *</td>
<td>P = 0.022 *</td>
<td>P = 0.007 *</td>
</tr>
<tr>
<td></td>
<td>C2 (4.6 ± 2.7)</td>
<td>P &lt; 0.001 *</td>
<td>P = 0.022 *</td>
<td>P = 0.007 *</td>
<td>P = 0.022 *</td>
<td>P = 0.007 *</td>
</tr>
</tbody>
</table>

Control Protocol FBF (ml/min) C1 (187 ± 107) | C2 (201 ± 114) | NC2 (201 ± 125) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS (178 ± 104)</td>
<td>P = 0.237</td>
<td>P = 0.021 *</td>
</tr>
<tr>
<td></td>
<td>C1 (187 ± 107)</td>
<td>P = 1.000</td>
<td>P = 0.240</td>
</tr>
<tr>
<td></td>
<td>C1 (196 ± 116)</td>
<td>P = 0.300</td>
<td>P = 1.000</td>
</tr>
<tr>
<td></td>
<td>C2 (201 ± 114)</td>
<td>P = 1.000</td>
<td>P = 1.000</td>
</tr>
<tr>
<td>TEA</td>
<td>C1 (2.3 ± 2.4)</td>
<td>SS (1.8 ± 2.3)</td>
<td>P &lt; 0.005 *</td>
</tr>
<tr>
<td></td>
<td>C1 (2.3 ± 2.4)</td>
<td>P &lt; 0.048 *</td>
<td>P &lt; 0.005 *</td>
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<tr>
<td></td>
<td>C1 (2.7 ± 2.7)</td>
<td>P = 0.063</td>
<td>P = 0.016 *</td>
</tr>
<tr>
<td></td>
<td>C2 (3.2 ± 2.8)</td>
<td>P = 0.011 *</td>
<td>P = 0.005 *</td>
</tr>
</tbody>
</table>

Values are means ± SD. BAC, brachial artery compression; C, compression; EMG, electromyography; FBF, forearm blood flow; HHb, deoxyhemoglobin; NC, no-compression; O₂Hb, oxyhemoglobin; RMS, root mean squared; SS, steady state; TEA, task effort and awareness. *Denotes significant difference for parametric test. †Denotes significant difference for non-parametric tests.
**Figure 3-4.** Percent oxyhemoglobin (%O$_2$Hb; panel A) and percent deoxyhemoglobin (%HHb; panel B) in response to no manipulation (control) and to brachial artery compression (BAC) targeting a 50% reduction in forearm blood flow (FBF; panel C) for control and brachial artery compression (BAC) conditions during non-fatiguing handgrip exercise. SS, steady state; compression, C; no compression, NC. for control and brachial artery compression (BAC) conditions during non-fatiguing handgrip exercise. SS, steady state; C, compression; NC, no compression. *Significantly different from control at a given measurement time. #Significantly different from SS within a condition. †Significantly different from C1 within a condition. ‡Significantly different from NC1 within a condition. &Significantly different from C2 within a condition. Significance P < 0.05 for all comparisons except BAC FBF where P < 0.005.
Figure 3-5. Task effort awareness (TEA) and electromyography root mean square (EMG RMS) per unit of force for control and brachial artery compression (BAC) conditions during non-fatiguing handgrip exercise. SS, steady state; C, compression; NC, no compression. *Significantly different from control at a given measurement time, P<0.01. †Significantly different from SS within a condition, P<0.05. ‡Significantly different from C1 within a condition, P<0.05. ‡‡Significantly different from NC1 within a condition, P<0.05. &Significantly different from C2 within a condition, P<0.05.
Did EMG median Frequency change with brachial artery compression?

There was not a statistically significant effect of time on mF (F(4, 60) = 1.394, P = 0.247). See Table 2.

Was EMG RMS/Force different between Control and BAC?

Compared to control brachial artery compression led to an increase in EMG RMS/Force during C1 and C2. Compared to control EMG RMS/Force was not different during NC1 and NC2 in the BAC protocol (see table 4 and figure 3.4, panel B).

Was TEA different between Control and BAC?

Brachial artery compression led to an increase in TEA during C1 and approached significance in C2. TEA was not different during NC1 and NC2 during BAC compared to control (see table 4 and figure 3.4, panel A).
Figure 3-6. Individual response demonstrating the increase in EMG upon brachial artery compression-evoked compromise to forearm blood flow (represented by brachial artery blood flow velocity) and the rapid reduction of EMG when oxygen delivery was restored. Top panel: electromyography (EMG); Middle panel: brachial artery blood velocity; Bottom panel: hand grip force output.
Table 4. Measures of hemoglobin, blood flow, muscle activation and effort sensation compared between BAC and control within time

<table>
<thead>
<tr>
<th></th>
<th>BAC</th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td>SS</td>
<td>C1</td>
</tr>
<tr>
<td><strong>FBF (ml/min)</strong></td>
<td>(210 ± 133)</td>
<td>(94 ± 56)</td>
</tr>
<tr>
<td>SS (178 ± 104)</td>
<td>P = 0.003*</td>
<td></td>
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<tr>
<td>C1 (187 ± 107)</td>
<td></td>
<td></td>
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<tr>
<td>NC1 (196 ± 117)</td>
<td>P &lt; 0.001*</td>
<td></td>
</tr>
<tr>
<td>C2 (202 ± 125)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC2 (201 ± 126)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>O₂Hb (%)</strong></td>
<td>(57 ± 17)</td>
<td>(34 ± 15)</td>
</tr>
<tr>
<td>SS (44 ± 20)</td>
<td>P = 0.019</td>
<td></td>
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<tr>
<td>C1 (45 ± 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC1 (46 ± 21)</td>
<td>P = 0.008*</td>
<td></td>
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<tr>
<td>C2 (46 ± 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC2 (47 ± 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HHb (%)</strong></td>
<td>(46 ± 11)</td>
<td>(66 ± 13)</td>
</tr>
<tr>
<td>SS (51 ± 13)</td>
<td>P = 0.035</td>
<td></td>
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<tr>
<td>C1 (52 ± 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC1 (52 ± 14)</td>
<td>P &lt; 0.001*</td>
<td></td>
</tr>
<tr>
<td>C2 (53 ± 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC2 (53 ± 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EMG RMS/Force</strong></td>
<td>(1.3 ± 0.33)</td>
<td>(1.58 ± 0.39)</td>
</tr>
<tr>
<td>SS (1.3 ± 0.34)</td>
<td>P = 0.681</td>
<td></td>
</tr>
<tr>
<td>C1 (1.35 ± 0.36)</td>
<td></td>
<td></td>
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<tr>
<td>NC1 (1.36 ± 0.35)</td>
<td>P = 0.005*</td>
<td></td>
</tr>
<tr>
<td>C2 (1.41 ± 0.41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC2 (1.40 ± 0.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TEA</strong></td>
<td>(2.2 ± 2.3)</td>
<td>(3.9 ± 2.5)</td>
</tr>
<tr>
<td>SS (1.8 ± 2.3)</td>
<td>P = 0.428</td>
<td></td>
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<tr>
<td>C1 (2.3 ± 2.4)</td>
<td></td>
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<tr>
<td>NC1 (2.7 ± 2.7)</td>
<td>P = 0.385</td>
<td></td>
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<tr>
<td>C2 (3.2 ± 2.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC2 (3.6 ± 2.8)</td>
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Values are means ± SD. BAC, brachial artery compression; C, compression; EMG, electromyography; FBF, forearm blood flow; HHb, deoxyhemoglobin; NC, no-compression; O₂Hb, oxyhemoglobin; RMS, root mean squared; SS, steady state; TEA, task effort and awareness. *Denotes significant difference.
DISCUSSION

The purpose of this study was to test the existence of an OCR in voluntary human exercise and to interrogate whether the OCR plays a role in modifying perception of effort. The key findings from the current study are 1) EMG/Force increased during both bouts of compromised forearm muscle O$_2$D. 2) EMG/Force rapidly returned to pre-compromise values following the second period of compromised forearm muscle O$_2$D (i.e. measurement NC2 following C2), though this was not the case following the initial compromise to forearm muscle O$_2$D (i.e. measurement NC1 following C1). 3) Perception of effort reflected changes in EMG/Force, such that TEA increased and decreased with EMG/Force. 4) Perception of effort increased over time during a control trial without an increase in EMG/Force.

We interpret the above findings to support the existence of an OCR in voluntary human exercise and its ability to rapidly modify perception of effort. The increased EMG/Force required to maintain voluntary force of contraction under conditions of reduced muscle oxygenation and the rapid restoration of EMG/Force upon muscle reoxygenation (measurement NC2 following C2) is consistent with the OCR seen in stimulated human (32, 51) and animal exercise (38–41). In contrast, the lack of restoration in EMG/Force with muscle oxygenation restoration following the first compromise to muscle O$_2$D (measurement NC1 following C1) is not consistent with an OCR. Rather, this response is consistent with mechanisms of skeletal muscle fatigue (45, 82). Importantly, perception of effort followed changes in EMG/Force whether EMG/Force increased due to OCR or hypothesized skeletal muscle fatigue mechanisms. Therefore, similar to skeletal muscle fatigue, we interpret these findings to support the potential for an OCR to impact exercise tolerance. Finally, the increase in perception of effort over time during control, not associated with
increased EMG/Force, suggests there is an inherent increase in perception of effort which may be independent of CMD.

Evidence for Oxygen Conforming Effects in Exercising Human Forearm Muscles

The oxygen conforming response is characterized by a rapid restoration (within 20 s) of the EMG/Force relationship in response to changes in muscle oxygenation (32, 38, 51). Specifically, the OCR is bi-directional. When EMG is held constant via electrical stimulation, muscle force increases and decreases with increased and decreased muscle oxygenation, respectively (32, 38, 51). Importantly, upon restoration of muscle oxygenation, force production rapidly and always returns to pre-compromise levels (32, 38, 51). As opposed to the OCR, when skeletal muscle is fatigued the EMG/Force relationship is compromised (increased EMG per unit force or decreased force per unit EMG) and remains compromised for some time following exercise cessation independent of muscle O₂D (59, 82). Therefore, while skeletal muscle fatigue and the OCR are fundamentally different, it is essential, in order to distinguish the two, to demonstrate the rapid restoration of the EMG/Force relationship characteristic of the OCR.

The rapid restoration of the EMG/Force relationship characteristic of the OCR is well established in electrically stimulated muscle contractions in human (32, 51) and animal exercise (38–41). In contrast, little is known regarding the existence of an OCR in voluntary human exercise. Only one study has quantified changes in EMG during voluntary human exercise (38) under conditions where it was assumed that exercising muscle blood flow was compromised and then restored. However, the immediacy of the restoration of EMG/Force relationship was not identified. Hobbs and McCloskey (38) had participants perform plantar flexion exercise, during
which the exercising limb was moved from a heart level position to 30° and 60° above heart level and then back to heart level. An important limitation of Hobbs and McCloskey’s (38) design was that neither muscle blood flow nor muscle oxygenation were measured, therefore, the interpretation that muscle activation increased as a result of a decrease in muscle O2D was never directly tested. Recently, Bentley et al. (10) identified a vasodilator compensator phenotype, whereby when perfusion pressure is challenged by limb movement above heart level, some individuals will increase vasodilation and thereby defend exercising limb blood flow. Therefore, Hobbs et al.’s (38) design may not have been effective in reducing exercising limb blood flow.

In the present study we used brachial artery compression to reduce steady state FBF by ~50% and maintain that reduction regardless of whether an individual responded with compensatory vasodilation. The successful and consistent reduction in FBF with each bout of brachial artery compression created consistent reductions in muscle oxygenation (Fig. 3-4). By measuring exercising limb blood flow and muscle oxygenation in combination with force and muscle activation the existence of an OCR in voluntary human exercise could be established based on whether a rapid restoration of EMG/Force occurred with oxygenation restoration. During the first compromise to O2D, EMG/Force increased (Fig. 3-5). However, upon restoration of O2D (i.e. when brachial artery compression was discontinued), EMG/Force did not return to pre-compromise levels. These findings are not consistent with an OCR in which a rapid restoration of the EMG/Force relationship would be expected. Rather, the sustained compromise in EMG/Force, following the first compromise to muscle O2D, is consistent with the development of peripheral skeletal muscle fatigue (45, 82). Subsequently, upon the second compromise to O2D, EMG/Force increased once again. As would be expected, for the same compromise to FBF, C1 and C2 were not different from each other (see Table 3 and Figure 3-5). Therefore, if the first increase in EMG
RMS/Force during C1 was partially due to the OCR as well as skeletal muscle fatigue (per the sustained compromise to EMG RMS/Force) we would not expect the second compromise (same amount as first compromise) to contribute to more skeletal muscle fatigue. Then it may be that the second compromise to FBF did not create additional skeletal muscle fatigue. Thus, when O₂D was restored following C2 at NC2, EMG RMS/Force returned to pre-compromise levels. The restoration of EMG/Force upon the second restoration of O₂D is consistent with an OCR existing in voluntary human exercise. The sustained compromise in EMG RMS/Force following the first compromise to O₂D actually reinforces the interpretation that the rapid restoration of EMG RMS/Force following the second compromise is a fundamentally different phenomenon that is highly consistent with an OCR. To our knowledge, these findings are the first to support the existence of an OCR in voluntary human exercise.

**Oxygen Conforming Effects Contribute to Perception of Effort**

Skeletal muscle fatigue has important implications for exercise tolerance. As skeletal muscle fatigue becomes more severe, the amount of CMD required to maintain a given force output increases (6, 8, 55, 59). Importantly, when CMD increases, efferent sensations (conscious awareness of CMD activating motor neurons) also increase. This increase in efferent sensations is measured as an increase in an individual’s perception of effort (6, 8, 56, 59). While it is well established that skeletal muscle fatigue can act to increase the Effort/Force relationship, it was not known whether changes in muscle oxygenation can rapidly act to modify the Effort/Force relationship.

To measure efferent sensations during exercise, we chose to use the TEA scale developed by Swart et al. (71). The TEA scale was selected for its specific instructions (see methods) asking
participants to rate their perception of effort as the amount of attention, mental effort and difficulty experienced whilst continuing to exercise. In following the TEA scale instructions (71), we are confident that our participants were only rating their efferent sensations experienced during exercise and not afferent sensations of discomfort.

During the BAC protocol, perception of effort followed changes in EMG/Force (Fig. 3-5). Specifically, when O$_2$D was compromised (Fig. 3-4) both EMG/Force and perception of effort increased (Fig. 3-5). As mentioned previously, with the first restoration of O$_2$D the EMG/Force levels remained elevated. Consistent with TEA reflecting efferent perception of effort, the TEA score also remained elevated. In response to the second O$_2$D compromise and restoration sequence, TEA followed the compromise in EMG/Force with O$_2$D compromise and the restoration of EMG/Force when O$_2$D was restored. These observations that TEA mirrored EMG/Force when it remained compromised during the first O$_2$D restoration, and also mirrored EMG/Force when it was rapidly restored following the second O$_2$D restoration eliminates the possibility that TEA was simply a function of the physical act of brachial artery compression and strongly support that TEA reflects changes in muscle activation. We therefore conclude that whether muscle activation increases due to skeletal muscle fatigue or an OCR, perception of effort follows changes in muscle activation. These are, to our knowledge, the first findings to suggest that, similar to skeletal muscle fatigue, an OCR can also increase the Effort/Force relationship when O$_2$D is compromised.

During the control protocol, perception of effort increased over time despite no changes in EMG/Force relationship (see Fig. 3-5). Interestingly, perception of effort and EMG/Force were not different between the BAC and control conditions at either of the NC time points during exercise. We interpret these findings to suggest that there is an inherent increase in the amount of
attention, mental effort and difficulty experienced while continuing to exercise that is not associated with changes in muscle activation.

**Methodological Considerations**

There are several methodological considerations that warrant discussion. First, we were not able to conduct potentiated twitch force assessment of fatigue and we did not measure peripheral fatigue related metabolites during exercise. Therefore, we were unable to provide direct support that the sustained increase in EMG/Force during the first brachial artery compression was due to increases in fatigue related metabolites. Consequently, our interpretation that there are fatigue mechanisms involved in the first increase in EMG/Force is based off of the response of EMG/Force alone. However, the consistent experimental support documenting compromised EMG/Force at a given muscle oxygenation (59, 82) as characteristic of skeletal muscle fatigue is highly consistent with our interpretation that the maintained compromise in EMG/Force following the first BAC reflects fatigue.

Second, we did not ask participants to rate their P-RPE, therefore, we are unable to report whether participants would have rated their P-RPE to change any differently from TEA during exercise or whether participants are even able to distinguish between these hypothesized afferent (P-RPE) and efferent (TEA) sensations during voluntary handgrip exercise (71). Measurement of P-RPE was left out in order to ensure participants’ attention was exclusively directed to interpreting sensations of effort while completing voluntary handgrip exercise (67). Third, participants were not blinded to the brachial artery compression. Therefore, it may have been possible for participants to modify their subjective perceptions in response to the act of brachial artery palpation. However, because we see the lack of TEA decrease following the first
compression, when brachial artery compression is stopped (measurement NC1 following C1), we interpret these findings to argue strongly against the sensation of palpation leading to increased TEA.

Fourth, By design, the experimental protocol started with control in order to confirm that the work rate identified on day 1 was in fact non-fatiguing prior to continuing to the BAC protocol. Thus the issue of a possible order effect impacting assessment of differences between BAC and control must be considered. However, given that steady state measures of EMG RMS/Force were not different between control and BAC protocols, it is unlikely that there were any residual effects of control on BAC (see Figure 3-5).

Finally, the magnitude of reduction in perfusion and oxygenation employed was substantial in order to ensure an adequate deoxygenation effect on EMG/Force, and was only performed under non-fatiguing forearm exercise. Whether less of a change in oxygenation is required to evoke an OCR response at higher exercise intensities remains to be determined. Importantly, an exercise intensity dependence of the OCR has been demonstrated in stimulated exercise (32), therefore, while we chose to use a non-fatiguing work rate to minimize fatigue as a confound, it would seem important to explore this potential OCR exercise intensity dependence in voluntary human exercise. Therefore, our “proof of principle” finding would suggest pursuing such a question is a worthwhile next step.

CONCLUSIONS

This study is the first to simultaneously measure muscle oxygenation, EMG and Force during intermittent controlled alterations in oxygenation in order to determine the existence of an
oxygen conforming response in voluntary human exercise. Specifically, we have demonstrated that, for a given skeletal muscle force output, muscle activation increases when muscle oxygenation is decreased, and that this response is rapidly reversible upon muscle reoxygenation. Furthermore, we have demonstrated that perception of effort, as measured by the TEA scale, follows changes in muscle activation associated with changes in muscle oxygenation during voluntary small muscle mass exercise. In conclusion, similar to skeletal muscle fatigue, we interpret these findings to support the ability of an OCR to modify perception of effort during voluntary rhythmic small muscle mass exercise. This may have implications for OCR as a contributor to exercise tolerance.

Grants

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Disclosures

No Conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions

P.J.D and M.E.T. conceived and designed research; P.J.D., Z.I.K., O.K.M., M.J.T.L and A.M.F. performed experiments; P.J.D. analyzed data; P.J.D. and M.E.T. interpreted results of experiments; P.J.D. prepared figures; P.J.D. drafted manuscript; P.J.D., Z.I.K., O.K.M., M.J.T.L, A.M.F. and M.E.T. edited and revised manuscript; P.J.D., Z.I.K., O.K.M., M.J.T.L, A.M.F. and M.E.T. approved final version of manuscript.
Chapter 4 - General Discussion

Integrated Summary

The delivery of oxygen to the exercising muscle is essential for the production of energy required to sustain continuous exercise. Importantly, there is variability in O$_2$D between individuals where some people will have inherently better or worse O$_2$D to their exercising muscle for the same exercise intensity (9). This individual variability in O$_2$D is a particularly important observation because the OCR which has been established in stimulated animal (38) and human (32, 51) exercise has demonstrated that the amount of force produced for a given electrical stimulation decreases with decreased muscle O$_2$D. This compromise to force production is a product of attempting to prevent muscle fiber (myocyte) disturbance (42). However, while this adjustment in force production may benefit myocyte homeostasis it may have negative implications for our ability or willingness to complete exercise.

While exercising individuals are typically unable to objectively quantify the amount of electrical activity sent to exercising muscle during exercise, it is clear that they are aware of sensations of effort which are proportional to the amount of electrical activity required to continue exercising (20, 28, 44). Importantly, our sensation and perception of effort during exercise contributes to our willingness to continue exercising (8). Therefore, if electrical activity sent to the muscle increases as a result of decreased muscle oxygenation we would predict an increase in perception of effort required to maintain a given force of contraction. We would also expect these changes in perception of effort to rapidly follow the restoration of EMG/Force upon muscle reoxygenation consistent with the OCR. Consequently, while it may be predicted that our perceptions of exercise effort will follow muscle oxygenation dependent changes in electrical
activity sent to the muscle, such a response has not been demonstrated in voluntary human exercise. Therefore, to investigate the existence of an OCR and its ability to modify perception of effort in voluntary human exercise, brachial artery compression was used to reduce blood flow by 50% of steady state measurement during constant force non-fatiguing rhythmic voluntary handgrip exercise.

The manuscript within this thesis is the first to simultaneously measure muscle blood flow and oxygenation while providing support for the existence of an OCR and its ability to rapidly impact perception of effort in voluntary human exercise. These interpretations are drawn from the rapid restoration of the EMG/Force relationship seen when muscle oxygenation was restored following the second compromise to muscle O\textsubscript{2}D (i.e. C2 to NC2). This OCR response is fundamentally different from the sustained compromise in EMG/Force characteristic of skeletal muscle fatigue (59, 82) seen during the first compromise to O\textsubscript{2}D (i.e. C1 to NC1). Interestingly, the Effort/Force relationship follows the EMG/Force relationship. Specifically, when the EMG/Force relationship is not restored, neither is the Effort/Force relationship. However, when the EMG/Force relationship is restored so is the Effort/Force relationship. Therefore, the Effort/Force relationship follows the EMG/Force relationship whether the EMG/Force relationship is compromised due to OCR or skeletal muscle fatigue mechanisms. We interpret these findings to support the hypothesis that the OCR can rapidly modify perception of effort. In contrast, during the control exercise condition where no impairments to exercising muscle blood flow occurred, there was an increase in the Effort/Force relationship but not in the EMG/Force relationship. The EMG independent increase in the Effort/Force relationship may support the interpretation that there is an inherent increase in perception of effort which may be independent of CMD.
Strengths of the thesis work

As opposed to the only other study to have tested for the existence of an oxygen conforming response in voluntary human exercise (38), our design has several strengths. First, our design kept the arm in a consistent position throughout exercise. In keeping arm position consistent, we can be confident that any changes in the EMG/Force relationship with BAC is as a result of changes in our manipulation and not as a result of changes in muscle recruitment that may result from changes in joint angles. Second, in order to avoid the issue of the vasodilatory compensator phenotype (10), our design used brachial artery compression (BAC) rather than arm movement to control limb blood flow. The use of BAC ensures we are able to maintain the compromise in FBF despite any compensatory vasodilation which may occur during the compromise to FBF. Third, as opposed to Hobbs et al. (38), our design measured both muscle oxygenation and blood flow, therefore, we were able to directly test the for the existence of an OCR in voluntary human exercise. Fourth, extensive efforts to familiarize participants with the TEA scale used to measure perception of effort was completed. In doing this, we are confident that participants were accurately rating their perception of effort based off the intended sensations.

Limitations of the thesis work

Our design has several methodological limitations which warrant consideration. First, we did not measure peripheral fatigue related metabolites during exercise, nor were we able to conduct potentiated twitch force assessment of fatigue. Therefore, we were unable to provide direct support that the sustained compromise in EMG/Force, during and following the first brachial artery compression, was due to increases in fatigue related metabolites. Consequently, our interpretation
that there are fatigue mechanisms involved in the first compromise in EMG/Force is based off the maintenance of compromised EMG/Force despite restoration of exercising muscle oxygenation (59, 82). Second, we did not ask participants to rate their P-RPE, therefore, we are unable to report whether participants would have rated their P-RPE to change any differently during exercise or whether participants are even able to distinguish between these hypothesized afferent (P-RPE; pain in the contracting muscle) and efferent (TEA; sensation of CMD) sensations during voluntary handgrip exercise (71). Measurement of P-RPE was left out in order to ensure participants’ attention was exclusively directed to interpreting sensations of effort while completing voluntary handgrip exercise (67). Ensuring attention was directed to the assessment of effort was deemed important because our hypothesis required the quantification of changes in effort in response to changes in muscle oxygenation, therefore, sensations of pain and discomfort were not necessary in answering the present research question.

Fourth, participants were not blinded to the brachial artery compression. Therefore, the act of brachial artery compression may have led participants to increase their subjective perception of effort. However, because we do not see TEA decrease following the first compression, when brachial artery compression is stopped, we are confident that participants are not modifying their perception of effort based off of the sensation of palpation. Finally, brachial artery compression employed led to a substantial hypoperfusion (~50% reduction). This magnitude of hypoperfusion, though extreme, was used to assist in developing a proof of concept for the OCR response. This proof of concept is particularly relevant for individual differences in oxygen delivery during exercise. If an individual has an inherently lower oxygen delivery, our proof of concept study would support the idea that exercise would be perceived to be more difficult for this individual, and if we were able to increase oxygen delivery we could help make exercise feel easier. Therefore,
an important follow up to this study will be to test the ability of increased oxygen delivery to improve an individual’s perception of effort and make exercise feel easier, therefore improving exercise tolerance.

**Future directions**

This study is an important first step in identifying the existence of, and characterizing, an OCR in voluntary human exercise. This study has demonstrated that if muscle oxygenation is reduced, muscle activation and perception of effort increases for the same force output. What then would happen to force output if muscle activation was held steady while muscle blood flow is decreased? What would happen to muscle activation and perception of effort if muscle force output was held constant when muscle oxygenation is increased above normal levels? Alternatively, what would happen to force output and perception of effort if muscle oxygenation is increased above normal levels? Is there an exercise intensity dependent OCR? Are there undiscovered sex differences in the OCR? In answering these questions we will be able to develop a fundamental understanding of the OCR in voluntary human exercise.

**Summary**

We have interpreted the results from the present thesis manuscript to support the existence of an oxygen conforming response and its ability to rapidly modify perception of effort in voluntary human exercise. This is an important proof of concept study which will assist in the development of further studies to explore the oxygen conforming response in voluntary human exercise. Importantly, this proof of concept study should be reproduced and further studies should
be completed to develop our understanding of the OCR involvement in modifying CMD and exercise behaviour.
References


75. Walker KL, Saunders NR, Jensen D, Kuk JL, Wong S, Pyke KE, Dwyer EM, Tschakovsky


Appendix A: Subject Information Forms and Perceptual Scales
CSEP Get Active Questionnaire

Get Active Questionnaire
CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY – PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Certified Exercise Professional (CSEP) – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications or health care provider is advisable. This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.

- [ ] I am completing this questionnaire for myself.
- [ ] I am completing this questionnaire for my child/dependent as parent/guardian.

---

**PREPARE TO BECOME MORE ACTIVE**

The following questions will help to ensure that you have a safe physical activity experience. Please answer YES or NO to each question before you become more physically active. If you are unsure about any question, answer YES.

1. Have you experienced ANY of the following (A to F) within the past six months?
   - [ ] A diagnosis/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
   - [ ] B A diagnosis/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
   - [ ] C Dizziness or lightheadedness during physical activity?
   - [ ] D Shortness of breath at rest?
   - [ ] E Loss of consciousness/fainting for any reason?
   - [ ] F Concussion?

2. Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?

3. Has a health care provider told you that you should avoid or modify certain types of physical activity?

4. Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

- [ ] NO to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY
- [ ] YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE

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Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1. During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)?

2. On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity?

   For adults, please multiply your average number of days/week by the average number of minutes/day.

   Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do vigorous-intensity physical activity (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances. Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct. If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

I answered YES to any question on Page 1

Check the box below that applies to you:

☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.

☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Sign and date the Declaration below

Name (Name of Parent/Guardian if applicable) [Please print] Signature (or Signature of Parent/Guardian if applicable) Date of Birth

Date Email (optional) Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active. (This completed questionnaire will help the QEP get to know you and understand your needs.)

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PAGE 2 OF 2
You are invited to participate in a research study conducted under the “Peripheral Vascular Control in Humans” Research Program.

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Purpose of the Study:

The purpose of this study is to improve our understanding of how muscle oxygenation affects our perception of effort.

Benefits For You:

There are no direct benefits to you by participating in this study.
Description of Experiment and Risks:

What will happen? During this study, you will take part in some of the specific experimental procedures outlined below. These procedures have been checked. They may be performed at one or more of three sites: The Human Vascular Control Laboratory (HVCL) on the Queen’s University Campus, the Laboratory for Clinical Exercise Physiology (LACEP) of Dr. Alberto Neder in Kingston General Hospital, or at Hotel Dieu Hospital. Depending on the specific experimental protocol, the combination of these procedures will be different. The investigator will explain to you in detail how each of these procedures will be combined in the particular experiment involving your participation. Please initial by each bullet point that is marked.

- **HEART RATE MEASUREMENTS:** Heart rate is continuously monitored by an electrocardiogram (EKG) through 3 spot electrodes on the skin surface. The electrodes are normally placed in the lower portion of the chest and they can detect the electrical activity that makes your heart beat.

  RISKS: This procedure is entirely safe. In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.

- **BLOOD PRESSURE MEASUREMENTS:**
  1. A cuff that can be inflated with air is wrapped around your upper arm, just as would occur if you had your blood pressure measured at the doctor’s office. This cuff is inflated to a pressure higher than your systolic blood pressure (the pressure in your blood vessels when the heart beats), and gradually deflated over a number of seconds to measure systolic blood pressure and diastolic (the pressure in your blood vessels when the heart is relaxed) blood pressure. Meanwhile, your wrist is secured in a wrist brace and a small pressure sensor is placed over your radial artery at the wrist. This pressure sensor is able to detect the increases and decreases in size of your radial artery that occur with each heart beat, and what the pressure sensor measures is compared to the pressure that the upper arm cuff measures (this calibrates the sensor). From then on, the pressure sensor at the wrist measures blood pressure continuously, while the upper arm cuff may be inflated intermittently.
  OR
  2. A small cuff is fit around your finger. This cuff inflates to pressures that match the blood pressure in your finger, so you feel the cuff pulsing with your heart beat. It shines infrared light through your finger to measure changes in the size of your finger with each heartbeat.

  RISKS: These techniques are non-invasive and pose no risk.
**LIMB BLOOD FLOW AND BLOOD VESSEL DIAMETER MEASUREMENTS:**
The blood flowing through your brachial (above the elbow), radial (above the wrist), or femoral (above the groin) artery can be detected and your artery diameter measured using Doppler and imaging ultrasound. A probe will be placed on the skin over your artery and adjustments in its position controlled by hand by the investigator. Measurement of femoral artery flow takes place on the lower abdomen just above the groin. Shorts will be tied up at the site of measurement to expose the skin in this region. High frequency sound (ultrasound) will penetrate your skin. The returning sound provides information on blood vessel size and blood flow.

**RISKS:** This technique is non-invasive and poses no risk.

**ELECTROMYOGRAPHY (EMG):** This measures the electrical activity of your muscles. Electrodes will be placed on muscles of interest for a given study.

**RISKS:** This procedure is entirely safe. In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.

**GAS EXCHANGE:** This measures your breathing and the changes in oxygen and carbon dioxide as a result of your body utilizing oxygen and producing carbon dioxide. It involves breathing through a mouthpiece attached to a one way valve system, and wearing nose clips.

**RISKS:** This procedure is entirely safe. There are no known risks.

**MULTIPLE INERT GAS REBREATHE:** This technique measures the amount of blood pumped by your heart. You will breathe through a mouthpiece connected to an inflatable bag. This bag will contain two gases (nitrous oxide, sulfur hexafluoride) that are completely inert, which means that they do not react with anything in your body and are completely safe to breathe. When measurements are taken, you will be asked to breathe at a certain rhythm for up to 10 seconds and to inhale enough to empty the bag with each breath in.

**RISKS:** The inert gases are completely safe to breathe. There are no known risks to this procedure.

**NEAR INFRARED SPECTROSCOPY:** This technique is used to measure the oxygen in your muscle. It consists of an infrared light emitter and sensor block that is positioned on the skin surface of your leg or arm and secured by wrapping with bands that prevent penetration of outside light. The infrared light that shines into your tissue is partially absorbed and partially reflected back to the sensor and this provides information on how much oxygen there is in the blood in your muscle.

**RISKS:** There are no risks to this non-invasive procedure.
• **FEMORAL NERVE MAGNETIC STIMULATION:** This technique involves placing a magnetic coil over your femoral nerve just above your thigh. This magnetic coil is activated in order to cause your femoral nerve to fire. This will result in the thigh muscles on the front of your thigh to contract.

**RISKS:** The sensation of nerve stimulated muscle contraction can be strange and even uncomfortable. However, this technique is well established and has minimal risk, other than the typical risk of muscle strain that occurs with any everyday activity involving voluntary muscle contraction.

• **VENOUS BLOOD SAMPLING:** Blood samples from veins are used to measure the amount of lactic acid and oxygen in your blood. We need to take a blood sample from a vein on the back of your hand, after we have increased blood flow to that hand by having you hold it in tolerably hot water until blood flow is maximized. For this, a researcher trained and certified in venipuncture (needle or catheter placement into a vein) will use sterile technique to draw a blood sample of ~1 ml into a syringe. We also need to take multiple 1 ml samples of blood from a vein at the elbow. In this instance, the researcher will place a teflon catheter into your vein using sterile technique. The catheter will be secured to your skin with tape and a self-sealing access attached to allow for drawing blood from the vein. We will take a volume of blood that is in total no more than ~120 ml. This represents approximately 1/3 of the volume of blood taken when you donate blood (370-400 ml). Periodically, the researcher may, after drawing some blood, inject (flush) sterile saline through the catheter into your vein. When the study is over, we will remove the catheter and secure sterile gauze over the puncture site.

**RISKS:** The most common complications of inserting a small catheter in the arm is a small bruise and pain at the site of catheter insertion. This might last several days after removal of the catheter. It is also possible that this pain may refer down the arm (a “shooting” pain sensation), if there has been nerve irritation in the catheterization process. When the catheter is removed pressure must be applied to the vein to prevent internal bleeding. If adequate pressure is not applied a bruise and some discomfort might result for a short period of time. The puncture site should be kept clean and covered with a sterile gauze pad while stopping the bleeding after catheter removal to prevent infection. There is very little risk of infection or injury to the vein. The amount of blood taken can result in at most a 2% reduction in the hemoglobin content in your blood (hemoglobin carries oxygen in your blood), in comparison to ~7.5% reductions experienced when you donate blood. Nevertheless, this 2% does constitute a very mild anemia, and in the case of a person with chronic hemoglobin disorders it could increase the risk of adverse health consequences.

• **FOREARM AND LEG VOLUME MEASUREMENTS:** The volume of your forearm or calf can be measured by a thin, stretchable rubber band placed around your respective limb that is filled with mercury. A very small electrical current runs through this gauge and changes in the length of this mercury-filled rubber band
are detected by changes in this current that occur in proportion to changes in the length of the rubber band.

**RISKS:** This technique is non-invasive and poses no risk.

- **BLOOD OXYGEN CONTENT:** A plastic clip is placed over your left index finger. This clip aims light through your finger, and the absorption of that light by the blood provides information on how much oxygen the blood contains.

  **RISKS:** This technique poses no risks.

- **MUSCLE MASS:** Circumference and length measurements of segments of your arm or leg will be taken via manual placement of a tape measure on your limbs by the investigator. OR

  At Kingston General Hospital, you will lay on a table and a scan of your body will be performed using a technique called “dual-energy x-ray absorptiometry” (DXA). This technique uses a small amount of x-ray energy to scan a “picture” of your body and identify how much muscle there is on your arms and legs.

  **RISKS:** Radiation levels with DXA are considered trivial by radiation regulatory agencies. The technique uses less radiation than a dental X-ray, roughly equivalent to the background amount a person would be exposed to when flying from Cincinnati to the West Coast. This is a mere fraction of the radiation dose we are all exposed to every week, from just being alive.

- **FOREARM OR LEG OCCLUSION:** In order to completely block the blood flow through your forearm or leg, a pressure cuff will be inflated around your arm or around your upper or lower leg for 1-10 min or inflated and deflated rhythmically depending on the protocol. You may feel a strong pressure and some mild tingling with cuff inflation but it should not be uncomfortable. If there is pain, immediately notify the investigator and the cuff will be deflated and repositioned. Upon cuff release there will be a large rush of blood into your forearm or leg. This may feel warm and you may experience mild tingling but no discomfort.

  **RISKS:** This technique is non-invasive and poses no risk.

- **FOREARM COMPRESSION:** A stylus will be positioned over your artery pulse to control the amount of flow through the artery. The arterial compression provided by the stylus will be varied to create different blood flow profiles. Increases in stylus downward pressure with result in decreases in blood flow, while controlled release of stylus downward pressure will result in increases in blood flow. The blood flow to your limb will never be completely occluded by the arterial compression. In some cases, manual finger pressure will be used instead of the stylus.
OR

A cuff will be positioned around your forearm or leg, and can be inflated and deflated at will to increase and decrease blood flow to your limb.

**RISKS:** The brachial artery and nerve run close together, thus the compression of this particular artery may result in a tingling sensation and some temporary numbness in the forearm. The compression of the artery can also become somewhat uncomfortable over time. These symptoms will subside within 5 minutes of compression release. There are no risks to your forearm from temporarily stopping blood flow to the forearm.

- **FOREARM OR HAND HEATING:** In order to increase the blood flow through your brachial artery and/or radial artery, your forearm or hand will be enclosed in a water bath that is circulated with warm water. The warm water will result in the dilation of your skin blood vessels. The water bath consists of a cylinder that is circulated with heated water. Your arm will rest inside the tube enclosed in a plastic glove that prevents your skin from being in direct contact with the water. A temperature sensor will be fixed to your skin and your skin temperature will be maintained between 41 and 42°Celsius. The water for the bath is heated remotely to a temperature not exceeding 45°Celsius and is circulated into the bath via a water pump. The water in the bath will feel quite warm, but not too hot. If at any time you feel discomfort the warm water inflow will be stopped and replaced with cooler water to allow the bath temperature to drop to a more comfortable level. Your forearm may be heated for a total of one to two hours.

**RISKS:** When the skin blood vessels fill with blood for an extended period while undergoing arterial compression it causes a temporary swelling as some fluid escapes from the blood vessels into the surrounding tissue. This minor swelling should resolve itself within 24 hours. Elevation of the arm will help to speed up the process. Your skin may appear red after removal from the bath. This is due to the increased skin circulation. The redness should resolve within 24-36 hours.

- **CONGESTION OF YOUR FOREARM OR LEG VEINS:** One inflatable cuff will be placed around your upper arm or above the knee and another may be placed around your wrist or ankle. The wrist cuff will be inflated to a pressure that prevents blood flow to your hand for a period of 10-15 minutes at a time. This should not be uncomfortable. If it is, notify the investigator and the position of the cuff will be adjusted until inflation without discomfort is achieved. These cuffs will be inflated to pressures that feel like a mild to moderate squeeze. This will prevent blood from flowing out of your limb back to the heart, but allow blood to flow in to your arm. Your limb will fill with blood and if the cuff inflation is maintained for a number of minutes, you may feel a sensation of swelling. This is because some of the plasma (water portion of your blood) will leak out of the small blood vessels and into the space between other cells in your limb. This is similar to when you stand up in
the morning and stay upright during the day. In that case, gravity makes it difficult for blood to flow back to the heart from the legs, and they slowly swell over the course of the day as plasma leaves the blood vessels. When the cuff is released, the limb will slowly return to normal as the plasma moves back into the blood vessels.

**RISKS:** The movement of fluid out of the blood vessels into your limb may in extreme cases cause discomfort. This discomfort should resolve itself within minutes of deflating the cuff, and the swelling should subside within 24 hrs. Elevating the arm above the heart for 15 minutes should speed this process.

- **INTERMITTENT COMPRESSION OF THE FOREARM OR LEG:** You will have an inflatable cuff placed around your forearm or leg. We can rapidly inflate and deflate this cuff to different pressures that are able to squeeze the blood out of the veins in your limb. Inflation is maintained for only a brief period of time (a few seconds). The sensation of limb compression will feel like a strong grip, but should not be painful. If it is uncomfortable, notify the investigator and the position of the cuff can be adjusted.

**RISKS:** There are no risks associated with this procedure.

- **ALTERNATING FOREARM SUCTION AND COMPRESSION:** Your forearm will be enclosed in a plexiglass box and sealed with a neoprene sleeve around the upper arm. Suction or compression of your forearm can be created by rapidly adding or removing air in the box via a connected automated air compressor. The sensation of suction and compression should not be painful. Notify the investigator if there are any feelings of discomfort.

**RISKS:** There are no risks associated with this procedure.

- **EXERCISE MANEUVERS THAT ALTER BLOOD PRESSURE:** You may be asked to perform one of the following MANEUVERS to temporarily increase your blood pressure: 1) squeezing a handgripper with your forearm for a few minutes with or without blood flow to your forearm being prevented 2) contracting your leg muscles with or without blood flow to your leg being prevented.

**RISKS:** When muscle contractions are performed while the blood flow to the limb is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no risk to your muscles in performing this exercise.

- **STROOP TEST:** In order to create a mental stress a “STROOP” test will be performed. A series of words for colours will be displayed such as “RED”. However, the word will be displayed in a different colour, perhaps the colour green. You must read out the colour in which the word is written in, not the word itself. Therefore, upon seeing “RED” (written in green text) you will respond by saying “green”. You
will be asked to perform the task as fast as you can. Part of the study evaluates the score you achieve on the test and it is very important that your score achieves the normal range for persons of your age and education. Your performance will be measured by how much of the list you read through in two minutes time, as well as how many mistakes you make.

**RISKS:** There are no risks posed by this procedure.

- **ANGER TEST:** In order to create emotional stress an anger test will be performed. Prior to the testing day, you will have been asked to fill out an anger questionnaire in order to recall a past event that made you very angry. We will use the questionnaire to elicit momentary anger. You will be asked to describe the event while re-experiencing the event in your imagination, as well as report on thoughts, feelings, and physical aspirations about the situation. The test will last two minutes.

  **RISKS:** You will feel momentary anger that will subside following the interview. It is possible that this anger interview might contribute to renewing problems between yourself and this individual. If you believe that this might in any way be problematic, you are encouraged to withdraw from participation in this study.

- **CONTROL TEST:** A control test will be performed in order to understand if verbalization is contributing to the blood vessel response. You will simply count from ‘one’ in Mississippi’s. Your verbalization will start as “one Mississippi, two Mississippi, three Mississippi...” and will continue for two minutes.

  **RISKS:** There are no risks posed by this procedure.

- **LOWER BODY NEGATIVE PRESSURE:** You will lay on your back and your lower body will be enclosed in an air-tight box. Various levels of suction will then be applied to the box to simulate how the blood normally shifts in the body during activities like standing up. This will cause your heart rate to increase and your blood vessels to constrict to maintain blood pressure. This is a normal response that you experience every morning when you get up out of bed.

  **RISKS:** There is a small chance that you may begin to faint with this procedure. We will be monitoring your blood pressure continuously. If you experience any of the following symptoms, notify the investigator immediately: nausea, narrowing field of vision, sweating. Changes in your blood pressure that we detect will most likely indicate that fainting is imminent well before you experience any of these symptoms. By shutting off the suction, blood will rapidly return to your heart and symptoms of fainting will be reversed. You may feel nauseous for a few hours after this procedure if you came close to fainting. This should resolve itself without any complications.

- **COLD PRESSOR TEST:** In this test, you will place your hand or foot in an ice
water bath for a few (1-3) minutes. This will cause your heart rate to increase and your blood vessels to constrict as the cold will activate your sympathetic nervous system (the part of your nervous system involved in the “fight or flight” response).

RISKS: There are no risks posed by this procedure. However, it can be quite painful. You have the right at any time to withdraw your hand or foot from the ice water bath if you feel unable to continue.

- **CHEST WALL STRAPPING:** You will have either a tensor bandage or a custom strapping device applied to your chest and abdomen. You are asked to breathe out as much air from your lungs as you can, and then hold that as the strapping is tightened around your chest and abdomen. You can indicate the need to breathe during this procedure at any time. After catching your breath you will again empty your lungs and strapping will continue. This will be repeated until the strapping is complete. The purpose of this is to restrict how much you can expand your chest and abdomen in the effort of breathing in and to reduce the amount of air left in your lungs at the end of a normal expiration. This mimics “restrictive” lung disease.

RISKS: The strapping can feel uncomfortable but should not be painful. If it is painful notify the investigator immediately and strapping will be adjusted. There is a small chance that you may begin to faint with this procedure. We will be monitoring your blood pressure continuously. If you experience any of the following symptoms, notify the investigator immediately: nausea, narrowing field of vision, sweating. Changes in your blood pressure that we detect will most likely indicate that fainting is imminent well before you experience any of these symptoms. These are reversed by rapidly removing the strapping and having you rest laying on your back with your legs raised.

- **HANDGRIP EXERCISE:** You will be asked to perform handgrip squeezing exercise. The duration of this exercise can vary from a few seconds to 10-20 minutes, and at an intensity that can vary from very mild to maximal contraction force. Exercise may take place in combination with any of the above-mentioned techniques which can control the blood flow to your limbs, congest the limbs, and which can alter your blood pressure.

RISKS: When forearm muscle contractions are performed while the blood flow to the forearm is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no risk to your muscles in performing this exercise. You may experience muscle soreness in the muscles of your forearm for 24-72 hours after performing the handgrip exercise, much as you would if you had been lifting weights.

- **LEG EXERCISE:** You will be asked to contract your leg muscles, either continuously or intermittently. The duration of this exercise can vary from a few seconds to 10-20 minutes, and at an intensity that can range from very mild to maximal contraction force. Exercise may take place in combination with any of the
above-mentioned techniques which can control the blood flow to your limbs, congest the limbs, and which can alter your blood pressure.

**RISKS:** When leg muscle contractions are performed while the blood flow to the forearm is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no damage or risk to your leg from this. You may experience muscle soreness in the muscles of your leg for 24-72 hours after performing the leg exercise, much as you would if you had been lifting weights.

- **DIETARY NITRATE CONSUMPTION:** Nitrate is a compound that is present in several foods, including plant foods (vegetables and a few fruits), processed meats, baked goods, cereals, and drinking water. Beets are an excellent source of nitrate. You will be randomly assigned to a “nitrate” or “placebo” group, and this will be done in a double-blind fashion (meaning that neither you nor the experimenters involved in data collection and analysis will know which group you are in until the completion of the study). You will be asked to either: 1. consume a specified amount each day for a few days prior to your testing day, and to consume your final amount a few hours before coming to the lab for your testing session, 2. Consume a specified amount a few hours before coming for your testing. Beetroot juice comes in small “shots” which are commercially available (Beet It Beetroot Products Co.). The quantity of nitrate in the beetroot juice is an amount achievable through normal dietary intake by making appropriate selections of high-nitrate-containing foods as part of the Dietary approaches to Stop Hypertension (DASH) diet, and is similar to the dose of dietary nitrate provided in several recent studies. You may experience red urine and red stools during this time period (due to the red beet colour), and this is a normal response to consuming beetroot juice.

**RISKS:** There are no known risks of acute dietary nitrate supplementation. Dietary nitrate can interact with certain medications (proton pump inhibitors, phosphodiesterase type 5 inhibitors, nitroglycerine or other “nitric oxide donor” drugs); if you are taking any of these medications, you will be excluded from the study.

How long will it take?

On an initial visit we will use ultrasound to get an image of the blood vessels in your limbs in order to determine whether you are eligible to participate in the main study.

For the main study: preparing all of the techniques for measuring your response and creating the correct experiment conditions usually takes ~45 minutes. The actual experiment will take ~1-3 hours.

Talking and Movements:

Talking or moving during the times that we are taking measurements will cause
variations in the measurements we are making. If you have any discomfort, please let us know immediately and we can temporarily break from data collection. However, if everything is comfortable, please maintain a very quiet posture. Even very slight movements interfere with our experiments.

Special Instructions:
Participants are asked to not drink alcohol or caffeine during the 12 hours prior to the study. Also, we ask that you do not consume any food during the 4 hours preceding the experiments. You should empty your bladder immediately prior to starting the test. When the study is finished, we will have you sit in the laboratory for a short time to allow you to readjust to the upright posture. These precautions should be enough to prevent any sensations of dizziness. Please be aware that sensations of dizziness are not normal and you should let us know if you experience any discomfort before you leave the laboratory.

Attached Medical Screening Form:
This questionnaire asks some simple questions about your health. This information is used to guide us with your entry into the study. Current health problems indicated on this form which are related to cardiovascular diseases (including high blood pressure) and liver or kidney problems will exclude you from the study only if the particular experiment in question requires healthy participants.

Safety Precautions:
Safety precautions for the study will include the following:

- **Participants** who enter the study will be identified as either healthy men and women, insulin resistant, or type II diabetic.

- Before entering the study you will be screened using a medical screening form. You will not be able to enter the study if anything is found which indicates that it is dangerous for you to participate.

- We will continuously monitor your heart rate and blood pressure, and you will be laying on your back or seated upright. These precautions allow us to quickly identify if you are becoming faint and simply stopping the experimental manipulation will allow you to quickly recover.

Confidentiality:

All information obtained during the course of the study is strictly confidential and will not be released in a form traceable to you, except to you and your personal physician. Your data will be kept in locked files which are available only to the investigators and research assistants who will perform statistical analysis of the
data. There is a possibility that your data file, including identifying information, may be inspected by officials from the Health Protection Branch in Canada in the course of carrying out regular government functions. The study results will be used as anonymous data for scientific publications and presentations, or for the education of students in the School of Physical and Health Education at Queen’s University.

Study Compensation

A monetary compensation for your time will be identified by a research team member during the explanation of the study. This will include any expenses you incur and reflect imposition on your time by your participation in this study.

Freedom to Withdraw from the Study

Your participation in this study is voluntary. You may refuse to participate or you may discontinue participation at any time during the duration of the study without penalty and without affecting your future medical care.

Participant Statement and Signature Section

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I will receive a copy of this consent form for my information.

If at any time I have further questions, problems or adverse events, I will contact:

Michael E. Tschakovsky, Ph.D.
(Principal Investigator)
KHS 306, Kinesiology and Health Studies Building
Queen’s University, Kingston, ON, K7L 3N6
Tel: (613) 533-6000, ext, 74697

Jean Cote, Ph.D.
Director, School of Kinesiology and Health Studies
KHS 206, Kinesiology and Health Studies Building
Queen’s University, Kingston, ON, K7L 3N6
Tel: (613) 533-3054

If I have any questions concerning research participant’s rights, I will contact:

Dr. Albert F. Clark, Chair
Office of Research Services
Fleming Hall, Jemmett Wing 301
Queen’s University, Kingston, ON, K7L 3N6
By signing this consent form, I am indicating that I agree to participate in this study.

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<thead>
<tr>
<th>Participant Signature</th>
<th>Signature of Person Obtaining Consent</th>
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<tr>
<td>Participant Name (please print)</td>
<td>Name of Person Obtaining Consent (please print)</td>
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<td>Date (day/month/year)</td>
<td>Date (day/month/year)</td>
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Using the Borg Scale

While doing physical activity, we want you to rate your perception of exertion. **This feeling should reflect how heavy and strenuous the exercise feels to you, combining all sensations and feelings of physical stress and fatigue.** Do not concern yourself with any one factor such as leg pain or shortness of breath, but try to focus on your total feeling of exertion.

Look at the rating scale below while you are engaging in the activity; it ranges from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion." Choose the number from below that best describes your level of exertion which is based only on the physical sensations which you feel as a result of the exercise and NOT the mental and psychological effort required to continue the task.

Try to appraise your feeling of exertion as honestly as possible, without thinking about what the actual physical load is. Your own feeling of physical effort and exertion is important, not how it compares to other people. Look at the scales and the expressions and indicate a number.
<table>
<thead>
<tr>
<th>RPE</th>
<th>Description</th>
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<tbody>
<tr>
<td>6</td>
<td>No exertion</td>
</tr>
<tr>
<td>7</td>
<td>Very, very light</td>
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<tr>
<td>8</td>
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<td>9</td>
<td>Very light</td>
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<td>10</td>
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<td>11</td>
<td>Fairly light</td>
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<td>13</td>
<td>Somewhat hard</td>
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<td>Very hard</td>
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<tr>
<td>19</td>
<td>Very, very hard</td>
</tr>
<tr>
<td>20</td>
<td>Maximum exertion</td>
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</table>
Using the Task Effort Awareness Scale

We want you to rate the psychological and mental effort required to perform this exercise bout at the intensity you have chosen. **The feeling / emotion that you report should reflect how much attention, mental effort and difficulty you experience whilst continuing to exercise at the present intensity.** Although your physical sensations will determine the mental effort required to continue, they should not be included in the TEA value that you report. In addition, the scale includes a component to measure how often you are conscious of the required effort.

Look at the rating scale below while you are engaging in an activity; it ranges from -4 to 10, where -4 means that you are unaware of any mental effort required to continue and therefore have no sensations telling you to “slow down” and 10 means that you are constantly aware of a severe effort required to continue at the current pace and will need to “slow down”. Choose the number from below that best describes your level of exertion.

Try to appraise your feelings as honestly as possible, without thinking about what the actual physical load is. Your own feeling of effort and exertion is important, not how it compares to other people. Look at the scales and the expressions and indicate a number. You can also use a decimal point to describe your value e.g. 6.5 or “six and a half”.

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Task Effort and Awareness (TEA) Scale

-4
  -2
  0
  2
  4
  6
  8
  10

- Unaware / None
  - Aware when asked / Mild
  - Intermittent / Moderate
  - Constant / Severe
**TEA score / RPE score test**

**Name**…………………………………………..

**Answer true (T) or False (F) to the following questions:**

1) The TEA score incorporates physical symptoms as well as effort and fatigue symptoms  (T / F)  

2) The TEA score is a direct measure of how hard you are pushing on the pedals (T / F)  

3) The TEA score is only going to be high when you are really tired. (T / F)  

4) If you ride at an easy intensity to complete a distance, TEA score will probably be low. (T / F)  

5) The TEA score does not have a physical component. (T / F)  

6) The sensations measured by the TEA score are always present. (T / F)  

7) A very high TEA score will mean that I can't continue at that pace for too long if I want to finish. (T / F)  

8) A TEA score of 9 means that I am constantly aware of the need to slow down. (T / F)  

9) I can feel really fresh yet have a high TEA score (T / F)  

10) The TEA score is only concerned with mental effort and not physical effort (T / F)  

TEA and P-RPE scales and descriptors are the work of Swart et al. (2012)
Appendix B: Individual Brachial Artery Compression Response Data
**Figure 4-1.** Individual electromyography root mean square (EMG RMS) divided by force response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-2. Individual percent oxyhemoglobin (%O\textsubscript{2}Hb) response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
**Figure 4-3.** Individual percent deoxyhemoglobin (%HHb) response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-4. Individual forearm blood flow (FBF) response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-5. Individual percent electromyography root mean square (EMG RMS) divided by force response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-6. Individual median frequency (mF) response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-7. Individual task effort awareness (TEA) response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
**Figure 4-8.** Individual heart rate (HR) response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-9. Individual mean arterial pressure (MAP) response data. Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-10. Individual force output data as a percentage of pre-exercise maximal voluntary contraction (%MVC). Data recorded from the brachial artery compression protocol. SS, Steady state; C, compression; NC, no compression.
Appendix C: Individual Control Response Data
Figure 4-11. Individual electromyography root mean square (EMG RMS) divided by force response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-12. Individual percent oxyhemoglobin (%O$_2$Hb) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-13. Individual percent deoxyhemoglobin (%HHb) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-14. Individual forearm blood flow (FBF) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-15. Individual electromyography root mean square (EMG RMS) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-16. Individual median frequency (mF) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-17. Individual task effort awareness (TEA) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
**Figure 4-18.** Individual heart rate (HR) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Figure 4-19. Individual mean arterial pressure (MAP) response data. Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
**Figure 4-20.** Individual force output data as a percentage of pre-exercise maximal voluntary contraction (%MVC). Data recorded from the control protocol. SS, Steady state; C, compression; NC, no compression.
Appendix D: Intraclass Coefficient Data
**Figure 4-21.** Intraclass correlation coefficient (ICC) for percent oxyhemoglobin (%O₂Hb) compared between compression (C) 1 and 2 of the brachial artery compression protocol.
Figure 4-22. Intraclass correlation coefficient (ICC) for percent deoxyhemoglobin (%HHb) compared between compression (C) 1 and 2 of the brachial artery compression protocol.
Figure 4-23. Intraclass correlation coefficient (ICC) for forearm blood flow (FBF) compared between compression (C) 1 and 2 of the brachial artery compression protocol.