Prolonged static stretching causes acute, non-metabolic fatigue and impairs exercise tolerance during severe intensity cycling.

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Running head: Non-Metabolic Fatigue and Exercise Tolerance
Abstract:

We tested the hypothesis that static stretching, an acute, non-metabolic fatiguing intervention, reduces exercise tolerance by increasing muscle activation and affecting muscle bioenergetics during cycling in the “severe” intensity domain. Ten active men (24±2 years, 74±11 kg, 176±8 cm) repeated an identical constant load cycling test, two tests were done in control conditions and two after stretching, that caused a 5% reduction of maximal isokinetic sprinting power output. We measured: i) oxygen consumption (VO₂); ii) electromyography: iii) deoxyhemoglobin iv) blood lactate ([La⁻]); v) time to exhaustion (TTE) vi) perception of effort. Finally, VO₂ and deoxyhemoglobin kinetics were determined. Force reduction following stretching was accompanied by augmented muscle excitation at a given workload (p=0.025), and a significant reduction in TTE (p=0.002). The time to peak of VO₂ was reduced by stretching (p=0.034), suggesting an influence of the increased muscle excitation on the VO₂ kinetics. Moreover, stretching was associated with a mismatch between O₂ delivery and utilization during the on-kinetic, increased perception of effort and [La⁻], that are all compatible with an increased contribution of the glycolytic energy system to sustain the same absolute intensity. These results suggest a link between exercise intolerance and the decreased ability to produce force.

Novelty bullets:

- We provided the first characterization of the effects of prolonged stretching on the metabolic response during severe cycling.
- Stretching reduced maximal force, augmented muscle activation in turn increasing the metabolic response to sustain exercise.

Keywords: Oxygen Consumption; VO₂ slow component; exercise tolerance; stretching; muscle stretching; loss of efficiency; muscle fatigue; VO₂ kinetics; NIRS; EMG.
**Introduction:**

During whole-body exercise at constant load in the moderate domain, oxygen consumption (VO\(_2\)) adapts to the energetic demands of locomotor and ventilatory muscles within 3 minutes (Poole and Jones 2012). If relative intensity rises above the gas exchange threshold (GET), approximately after the third minute of exercise VO\(_2\) displays a “slow component” (VO\(_{2sc}\)) that is typically interpreted as an increased cost of locomotion for a given exercise intensity (Poole and Jones 2012). In particular, when exercise is performed between the metabolic rates associated to the GET and the respiratory compensation point (RCP; i.e. heavy intensity domain) (Keir et al. 2015, 2018) VO\(_{2sc}\) tends to a steady-state; however, when effort rises above RCP (i.e. severe exercise domain) a steady-state is no longer achievable and VO\(_2\) increases over time tending to the maximum oxygen consumption (VO\(_{2max}\)) (Jones et al. 2011). The magnitude of VO\(_{2sc}\) is considered linked with exercise intolerance and fatigue (Grassi et al. 2015). Therefore, during the past forty years many researchers have focused their attention on clarifying its physiological bases (Jones et al. 2011). Two main theories have been proposed to explain the physiological origin of VO\(_{2sc}\): i) decreased metabolic stability of type I muscle fibres associated with increased O\(_2\) cost of ATP resynthesis and/or increased ATP cost of contraction (Jones et al. 2011; Grassi et al. 2015) and/or ii) recruitment of fast-fatigable intrinsically inefficient type II muscle fibres to obtain/maintain the external power output above a certain intensity threshold (e.g. RCP) (Jones et al. 2011; Poole and Jones 2012; Grassi et al. 2015). However, the exact physiological mechanisms underpinning VO\(_{2sc}\) remain elusive, one of the reasons being the difficulty to selectively affect either metabolic stability or type II fibre recruitment in human models. In fact, the different manipulations used in interventional studies (e.g. speed of movement, intensity modulation, aerobic training, priming exercise, nutritional interventions) affect to some extent both metabolic stability and fibre recruitment (Jones et al. 2011).

An interesting approach to selectively augment fibre recruitment while trying to avoid the perturbation of metabolic stability is acute, non-metabolic fatigue that reduces the ability of muscles to produce force. Among the interventions able to cause acute, non-metabolic fatigue, a promising model could be static stretching, that can impair force production as result of prolonged nervous stimulation (Trajano et al. 2017). It was broadly documented that stretching, particularly when positions are maintained for more than 60 sec, can impair maximal force in many different tasks and conditions for a period lasting up to 1 hour (Behm et al. 2016). Given that no effort is required to perform stretching, and that force impairment after stretching is mostly caused by neural mechanisms (Trajano et al. 2017), this would be a particularly convenient model to acutely reduce force and investigate the link between muscle activity and metabolism. Indeed, recent studies (Esposito et al. 2012)
documented that when maximal force was acutely reduced by stretching, the oxygen cost of locomotion increases both during ramp incremental (exercise modality in which the VO\textsubscript{2sc} is defined as “excess VO\textsubscript{2}” (Grassi et al. 2015)) and constant load exercises (Esposito et al. 2012; Limonta et al. 2015). However, the above studies did not specifically investigate the underpinnings of VO\textsubscript{2sc} and were, therefore, lacking measures to investigate the link between muscle activation and increased VO\textsubscript{2} (e.g. electromyography, EMG). In a recent study from our group, the effects of stretching on the VO\textsubscript{2} response during ramp incremental cycling were described while also implementing measures of muscle excitation (Colosio et al. 2019). We found that when muscle force is acutely impaired by stretching also muscles excitation increases, at unison with an increased cost of locomotion (i.e. VO\textsubscript{2} at a given absolute workload).

The above findings, in an incremental exercise paradigm, support the existence of a sequence of events, i.e. acute fatigue, increased muscle activation, loss of metabolic efficiency, causing the VO\textsubscript{2sc} with increasing exercise intensity. In this context, constant load exercise represents the ideal model to determine the possible role of increased muscular activation over time (necessary to maintain the same workload when fatigued) in the genesis of the VO\textsubscript{2sc}. In fact, only under prolonged, constant load conditions, the increased cost of locomotion (i.e. VO\textsubscript{2sc}) at a given intensity has the time to fully manifest itself. The confirmation of a connection between fatigue/increased muscle activation and the loss of metabolic efficiency over time during a constant load exercise paradigm would further support the existence of a causative link.

Accordingly, this study investigated the effects of acute, non-metabolic fatigue induced by stretching on central and peripheral physiological measures (VO\textsubscript{2}, blood lactate accumulation [LA\textsuperscript{-}], EMG, Near-Infrared Spectroscopy (NIRS)) during constant load cycling in the severe exercise domain. We hypothesis that stretching \textit{i}) will reduce maximal muscle force; \textit{ii}) in turn, force loss will translate in increased muscle excitation at a given absolute workload \textit{iii}) increased muscle excitation will reduce exercise tolerance and increase the VO\textsubscript{2} cost of locomotion. Finally, this study will provide the first comprehensive investigation on the effects of static stretching on high-intensity constant-load cycling.
Materials and methods:

Participants:

Ten active men gave written informed consent to participate in the study (age: 24±2 years, body mass: 74±11 kg, stature: 176±8 cm). Inclusion criteria were male sex and age between 20 and 35 years; exclusion criteria were smoking and any condition that could influence the physiological responses during testing. The study was approved by Departmental Ethics Committee and adhered to the principles of the declaration of Helsinki. All participants were instructed to avoid physical activity for at least 24 h before each testing session and followed a standard and individualised food intake prescription before all the testing sessions to minimise variability of glycogen stores and glucose oxidation (i.e. 2 g of low glycemic index carbohydrates per kg of body weight, 2 hours before testing; 0.5 L of water in the 90 min before testing; restriction from caffeine during the 8 h before testing).

Experimental Protocol:

After medical clearance, participants visited the laboratory on eight occasions within a maximum of three weeks. On the first two visits, subjects familiarized with a test consisting of isokinetic sprints for the determination of the maximal cycling power output. On the third appointment, isokinetic sprints were performed pre and post either the control condition (i.e. 40 min of seated rest, control) or 40 min of stretching, to determine the effect of stretching on the maximal cycling power output. On the fourth visit, subjects performed a ramp incremental test to exhaustion for the determination of the GET, the RCP and the VO$_{2\text{max}}$. Then, during the last four visits participants repeated four identical constant load trials in the severe exercise intensity domain (at a power output corresponding to ∆60% between GET and VO$_{2\text{max}}$). Randomly, 2 of the constant load trials were done in control conditions and 2 after 40 min of stretching. A schematic representation of the protocol is provided in fig. 1.

All the tests were conducted at the same time of the day in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity), on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, Netherlands). Ergometer position was chosen during the first familiarization visit and recorded for the successive appointments.

Stretching procedure

Control consisted in 40 minutes of resting in a sitting position under the examiner’s surveillance.
Six cycles of stretching, were used to maximise acute force reduction (Behm et al. 2016). The standardised stretching cycle sequentially involved i) the quadriceps of the right leg, ii) the right hamstrings, iii) left quadriceps, iv) left hamstrings. Each position was maintained for 80 sec with no recovery between positions. Subjects were continuously encouraged to stretch muscles to the point of discomfort. The total duration of the stretching intervention was about 40 minutes. Stretching effectiveness in increasing flexibility was measured pre and post stretching and control using a sit-and-reach test (Limonta et al. 2015).

**Isokinetic maximal sprints**

To assess force reduction after stretching, isokinetic maximal sprints were performed on an electromagnetically braked cycle-ergometer in isokinetic mode equipped with a pedal force sensor (Sport Excalibur PFM, Lode, Groningen, NL) as previously described (Colosio et al. 2019). In brief, two pedalling frequency (60 and 120 rpm) were used to measure velocity-specific peak power as proposed by Cannon et al. (Cannon et al. 2011). Each sprints session was composed of 4, five-seconds maximal sprints alternating between 60-120-60-120 rpm. The 4 maximal sprints were separated by a 2-min passive rest, to maximise recovery while limiting the total duration of the sprints session.

**Ramp incremental test**

The ramp incremental test consisted of a 4-min baseline cycling at 20 W, followed by increases in power output (PO) ranging from 17.5 to 25 W/min according to individuals’ predicted fitness level with the aim of obtaining a time to exhaustion around 8-12 minutes (American College of Sports Medicine 2017) using a method extensively described elsewhere (Pogliaghi et al. 2014). Participants were asked to pick a self-selected cadence in the range of 70-90 rpm and to maintain it throughout all tests. Failure to maintain the indicated cadence within 5 rpm (for longer than 5 sec) during testing despite strong verbal encouragement was considered as the criterion for exhaustion. Breath-by-breath pulmonary gas exchange, ventilation and heart rate were continuously measured using a metabolic cart (Quark B², Cosmed, Italy) as previously described (De Roia et al. 2012).

**Constant load trials**
After the preliminary ramp incremental test, subjects completed 4 constant load trials at the power output corresponding to the 60%Δ between GET and VO2max. Two of the constant load trials were performed after stretching and 2 after control in a randomized order. In each condition, one constant load trial lasted 10 min while the other one was performed to exhaustion to allow recording of time to exhaustion (TTE) after control and stretching. Constant load trials were preceded by a 4-min warm-up at 20 W in which cycling cadence was limited to 30 rpm to minimize any metabolic activation that could influence the effects of stretching and the physiological response at the onset of exercise. Throughout the test, subjects kept the same, constant rpm selected during the ramp incremental test and the same bike position selected during the sprints test.

Surface EMG of the right vastus lateralis and biceps femoris muscles were continuously recorded by means of a wireless system (Wave wireless EMG, Cometa, Milan, Italy). A pair of surface Ag/AgCl electrodes (Blue sensor, Ambu®, Ballerup, Denmark) was attached to the skin with a 2-cm inter-electrode distance. The electrodes were placed longitudinally with respect to the underlying muscle fibres arrangement, according to the recommendations by Surface EMG for Non-Invasive Assessment of Muscles (Hermens et al. 2000). Before electrode application, the skin was shaved, scratched with sand-paper and cleaned with alcohol in order to minimize impedance. Semi-permanent ink marks allowed consistent re-positioning of the electrodes between sessions. The EMG transmitter connected to the electrodes was well secured with adhesive tape to avoid movement-induced artifacts.

VO2, ventilation (VE), respiratory exchange ratio (RER), and heart rate (HR) data were measured with the same method described for the ramp incremental test. During each constant load trial, capillary blood samples (20 μl) were drawn from the earlobe in the last 30 sec of warm-up, during the 1st, 3rd, 5th, 7th, 10th and then every 5 min of the trial to exhaustion. Moreover, blood samples were drawn at the 1st, 3rd, 5th and 7th min after exhaustion. Samples were immediately analysed to measure [La−] (Biosen C-Line, EKF Diagnostics, Barleben, Germany).

Deoxygenation of the left vastus lateralis was evaluated in microcirculation using a quantitative near-infrared spectroscopy system (Oxiplex TSTM, ISS, Champaign, USA) that provided continuous measurement (sampling frequency 1 Hz) of the absolute concentrations (μM) of deoxyhemoglobin ([HHb]). After shaving, cleaning and drying of the skin area, the NIRS probe was positioned longitudinally on the belly of the vastus lateralis muscle ~15 cm above the patella, attached to the skin with a bi-adhesive tape and secured with elastic bandages around the thigh. The device was calibrated before each test after a warm-up of at least 30 minutes as per manufacturer recommendations.
Finally, perceptual responses to exercise was monitored using a 0-100 rating perceived exertion (RPE) scale (Borg and Kaijser 2006). The scale was displayed to the participants during baseline, every five minutes during the constant load trials and immediately after exhaustion.

**Data analysis**

**Isokinetic Sprints test**: Crank torque was measured independently from the two crank arms by strain gauge transducers (maximal recordable force 2000 N, <0.5 N resolution and measurement uncertainty of <3%). Angular velocity of the crank was recorded every 2 degrees using three independent sensors sampling in series with uncertainty of measurement <1%. Overall power for each pedaling cycle was calculated as the sum of the left and the right crank as resulted by the pedal force measurement analysis software. The initial and the last pedaling cycles of each sprint were excluded from computation. Then, maximal power expressed during each pedaling cycle was detected and cycles were averaged to obtain a mean peak power output for every sprint. Finally, mean peak power output of the two repetitions of the 60 and 120 rpm sprints performed either pre- or post-intervention were averaged and the relative % change between pre and post conditions were calculated.

**Ramp incremental test**: For the gas exchange variables, aberrant data-points that lay 3 SD from the local mean were removed, and trials were linearly interpolated on a 1-sec basis and then averaged every 5 sec. $\text{VO}_{2\text{max}}$ was determined as the highest $\text{VO}_2$ obtained over a 10-sec interval (Fontana et al. 2015). GET and RCP were determined with the standard technique from gas exchange variables by three blinded expert reviewers as detailed elsewhere (Fontana et al. 2015). Briefly, GET was determined by visual inspection as the $\text{VO}_2$ at which $\text{CO}_2$ output began to increase out of proportion in relation to $\text{VO}_2$, with a systematic rise in the VE-to-$\text{VO}_2$ relation and end-tidal $\text{PO}_2$ whereas the ventilatory equivalent of $\text{VCO}_2$ (VE/$\text{VCO}_2$) and end-tidal $\text{PCO}_2$ is stable (Beaver et al. 1986). RCP was determined as the point where end-tidal $\text{PCO}_2$ began to fall after a period of isocapnic buffering (Whipp et al. 1989). This point was confirmed by examining VE/$\text{VCO}_2$ plotted against $\text{VO}_2$ and by identifying the sec breakpoint in the VE-to-$\text{VO}_2$ relation. $\text{VO}_{2\text{max}}$ was determined as the highest $\text{VO}_2$ obtained over a 10-sec interval. Finally, we determined the constant workload equivalent to the specific severe (60%Δ between GET and $\text{VO}_{2\text{max}}$) $\text{VO}_2$ target. To this aim, the $\text{VO}_2/W$ relationship identified with the incremental test was left-shifted to
account for the individual mean response time. Briefly, the mean response time was determined as the time interval
between the onset of the incremental portion of the exercise (time = 0) and the increase of the VO₂ signal above
baseline. It was determined as the x coordinate of the intersection of the forward extrapolation of the baseline VO₂
and the back-wards extrapolation of the linear VO₂–time relationship below the GET (Fontana et al. 2015).

Constant Load Trials

The raw EMG signal was rectified and smoothed using a fourth-order band-pass Butterworth digital filter with a
frequency range set between 20 and 500 Hz. Root mean square (RMS) was calculated every second and averaged
at 30 sec intervals from the raw signal and was used as an index of the total muscle excitation for vastus lateralis
(RMS\textsubscript{VL}) and biceps femoris (RMS\textsubscript{BF}) (Moritani et al., 1986; Ryan & Gregor, 1992). Thereafter, the RMS recorded
during the last 2 minutes of 20 W baseline for each test was used to normalize the constant load trials and expressed
as multiples of baseline.

Time to exhaustion was calculated as the total duration of exercise from workload onset to failure.

VO₂ during constant load trials was cleaned and interpolated using the same procedure described for the ramp
incremental test. Then, data of the two constant load trials performed in each condition were mediated in order to
reduce breath-by-breath signals variability. Finally, 30 sec means were calculated.

Net [La\textsuperscript{-}] accumulation during constant load trials was calculated as the difference between [La\textsuperscript{-}] at a specific
timepoint and the [La\textsuperscript{-}] during cycling at 20 W. The highest value after exercise end was considered as the peak
of blood lactate concentration.

NIRS derived [HHb] response during constant load trials was time aligned with the onset of exercise transition,
treated by subtracting the steady-state value measured during the last 2 min of warm-up, and then averaged at 30
sec bins.

VO₂, [HHb] kinetics and VO₂\textsuperscript{sc}:

Using 1 sec bins data, the on-transient responses to exercise of VO₂ was modelled as follows: first, the VO₂
response from -60 up to 180 seconds (time 0 being exercise onset) was preliminarily characterized with a two-
component model (linear + exponential), integrated by a Heaviside function, after the exclusion of the data-points
of the initial 20 sec of exercise that correspond to the cardiodynamic phase (Murias et al. 2011a)). With this
approach, we derived the initial parameters for the primary component. Then, the complete on-transient responses to exercise of VO\(_2\) were modelled from the onset of workload to the end of the 10\(^{th}\) min (or to exhaustion for tests that lasted less than 10 min after stretching) using the following two-component exponential equation integrated by a Heaviside function (De Roia et al. 2012):

\[
Y(t) = Y_{\text{blsn}} + \text{AMP}_p \left( 1 - e^{-\left(1 - TD_p\right)/\tau_p} \right) + \text{AMP}_s \left( 1 - e^{-\left(1 - TD_s\right)/\tau_s} \right)
\]

Where \(Y(t)\) represents the increase in VO\(_2\) at the onset of exercise, \(Y_{\text{blsn}}\) is the baseline VO\(_2\) value recorder during the 4 min 20 W cycling, \(\text{AMP}_p\) and \(\text{AMP}_s\) represent the amplitude of the VO\(_2\) response above the baseline value of the primary and the slow component respectively; \(\tau_p\) and \(\tau_s\) and \(TD_p\) and \(TD_s\) are the time constant and the time delay of the response for each component. The mean response time (MRT) was then calculated as the sum of \(\tau + TD\). Furthermore, we calculated the time requested to reach VO\(_{2\text{max}}\) during constant load trials by resolving on the individual fitting of VO\(_2\) data for the time coordinate corresponding to VO\(_{2\text{max}}\).

[HHb] signal was fitted on a time window of -60 to 180 sec (time 0 being exercise onset) using a two-component model (linear + exponential), integrated by a Heaviside function, as previously described (De Roia et al. 2012).

Finally, [HHb] and VO\(_2\) data were normalized with 0\% corresponding to the value recorded while cycling at 20 W baseline and 100\% reflecting the maximal response in the 180 sec window and expressed as \(\Delta[\text{HHb}]\) and \(\Delta\text{VO}_2\).

Individualized 1 sec \(\Delta[\text{HHb}]\) and \(\Delta\text{VO}_2\) were time-aligned by left-shifting the VO\(_2\) data by 20 sec (i.e. the typical duration of the cardiodynamic phase in young individuals (Murias et al. 2011b). Then, the ratio between \(\Delta[\text{HHb}] / \Delta\text{VO}_2\) was calculated during the first 180 sec of exercise to express the fractional muscle O\(_2\) extraction required to sustain a given net increment of VO\(_2\) (De Roia et al. 2012). Finally, the following indexes were calculated: \(\Delta[\text{HHb}] / \Delta\text{VO}_2\) AUC, as the integral of the total mismatch between O\(_2\) delivery and utilization (i.e. index values > 1); \(\Delta[\text{HHb}] / \Delta\text{VO}_2\) peak, as the maximal value reached within the 180 sec; \(\Delta[\text{HHb}] / \Delta\text{VO}_2\) time to peak, as the time requested to reach the peak in \(\Delta[\text{HHb}] / \Delta\text{VO}_2\). Moreover, given that these time-resolved values are typically implemented during steady-state condition, an overall quantification of the increase in fractional muscle O\(_2\) extraction required to sustain a given net increment in VO\(_2\) during the primary phase of exercise was calculated by dividing the amplitudes of the response in VO\(_2\) and [HHb] between the onset of exercise and the onset of the slow component: overall \(\Delta[\text{HHb}] / \Delta\text{VO}_2\) (Tam et al. 2018).
Statistics

After assumptions verification (i.e., normality, homogeneity of variance), two-way repeated measures ANOVA was applied to compare flexibility values (pre and post sit-and-reach after control/stretching). Pre and post peak power output measured during isokinetic sprints at 60 and 120 rpm were compared pre and post between stretching, using a two-way repeated measures ANOVA (time x pedalling frequency).

For constant load trials, two-way repeated measures ANOVAs were performed to compare VO\textsubscript{2}, net [La\textsuperscript{-}], RPE, [HHB], RMS\textsubscript{VL} and RMS\textsubscript{BF} between conditions over time (time x condition). Post-hoc analyses were performed using Holm-Sidak test. Student’s t-test was applied to compare between conditions the time to exhaustion, parameters of VO\textsubscript{2} and [HHb] kinetics (τ, TD and MRT), time to VO\textsubscript{2max}, Δ[HHb]/ΔVO\textsubscript{2} AUC, Δ[HHb]/ΔVO\textsubscript{2} peak, Δ[HHb]/ΔVO\textsubscript{2} time to peak, overall Δ[HHb]/ΔVO\textsubscript{2}.

Data are presented as means ± SD. α was set in advance at the 0.05 level and significance was accepted when \( p < \alpha \). The 95% confidence intervals of the TD\textsubscript{p} and TD\textsubscript{ac}, τ\textsubscript{p} and τ\textsubscript{ac} of VO\textsubscript{2} kinetics and of TD and τ of [HHb] kinetics were calculated based on the asymptotic intervals of the non-linear parameters resulting from the fitting (Field et al. 2012). Effect sizes of the differences between control and stretching were also reported (Cohen’s \( d \), ranked as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79) and large (≥ 0.80)) as objective and standardized measures to quantifying the magnitude of difference after stretching vs control (Cumming 2014). In Cohen’s effect size calculation, the SD in the control condition was used to standardize the mean difference for each contrast (Field et al. 2012). Moreover, generalized eta squared (\( \eta^2_G \)) were calculated to quantify the effects sizes of different independent variables during the constant load trials (Olejnik and Algina 2003; Bakeman 2005). Based on an expected standard deviation of breath-by-breath VO\textsubscript{2} measurements for steady-state exercise equal to 2.5%, and a minimum detectable change in VO\textsubscript{2} of 100-170 ml·min\textsuperscript{-1} at a VO\textsubscript{2} of 2.1 to 3.5 L·min\textsuperscript{-1} (Keir et al. 2015), the minimum sample size to obtain a power of 0.8 was 6 individuals. All statistical analyses were performed using Sigmaplot version 12.
Results:

Flexibility, as measured by sit-and-reach test, was not significantly different at baseline between stretching and control and significantly improved only after stretching (+0.3±6.5 cm pre vs +6.1±5.9 cm post stretching, p<0.001, \( d=+0.89 \)). The peak power output measured during isokinetic sprints pre stretching was reduced, after the intervention, of \( \approx 5\% \) (table 1). ANOVA revealed a significant main effect of “time” (p≤0.001) and “pedaling frequency” (p≤0.001), with no interaction (p=0.885). Post-hoc analysis confirmed that peak power output was significantly reduced by stretching both during the 60 RPM and the 120 RPM sprints (table 1).

Subjects mean VO\(_{2}\max\) and peak PO measured at the end of the ramp incremental test were respectively 3505±375 ml/min and 315±26 W. GET and RCP were detected at a VO\(_{2}\) of 2155±355 ml/min and 2900±472 ml/min. The calculated target VO\(_{2}\) and PO for the Δ60% constant load trials were 3030±411 ml/min and 232±29 W (74±7% of the peak PO) respectively. As expected under the non-steady-state conditions of the severe intensity domain, the contribution of the VO\(_{2}\) slow component raised the actual experimental VO\(_{2}\) above the initially predicted target so that values close to VO\(_{2}\max\) were measured in the last 20 sec of the 10-min trials. In one subject only, the target intensity turned out to fall clearly below the desired severe domain (i.e. both VO\(_{2}\) and [La\(^{-}\)] were stable over time after the 10th min of exercise and time to exhaustion exceeded 40 min). Therefore, for this subject the constant load trials performed until that moment were repeated at +20 W after a wash-out period of three weeks in order to assure a metabolic intensity corresponding to the desired severe intensity domain.

The time to exhaustion of the control constant load trials was 839±200 sec (14’19’’±3’20’’). stretching significantly affected this parameter, that was reduced to 743±166 sec (12’23’’±2’46’’), p=0.002, \( d=0.48 \). Reduced TTE was associated with increased levels of RMS\(_{VL}\), [HHb], peak net [La\(^{-}\)], and perceived exertion at exhaustion (fig. 2). In fact, RMS\(_{VL}\), [HHb], peak net [La\(^{-}\)], and perceived exertion were significantly higher in the stretching vs control condition (main effect of “time” RMS\(_{VL}\): p<0.00, \( \eta\text{G}^2\): 0.30, [HHb]: p<0.00, \( \eta\text{G}^2\): 0.65, peak net [La\(^{-}\)]: p<0.00, \( \eta\text{G}^2\): 0.99, perceived exertion: p<0.00, \( \eta\text{G}^2\): 0.990, main effect of “condition” RMS\(_{VL}\): p=0.025, \( \eta\text{G}^2\): 0.44, [HHb]: p=0.011, \( \eta\text{G}^2\): 0.53, net [La\(^{-}\)]: p=0.023, \( \eta\text{G}^2\): 0.49, perceived exertion: p=0.003, \( \eta\text{G}^2\): 0.50; “time” x “condition” interactions for RMS\(_{VL}\): p<0.001, \( \eta\text{G}^2\): 0.70, [HHb]: p=0.007, \( \eta\text{G}^2\): 0.34, net [La\(^{-}\)]: p=0.221, \( \eta\text{G}^2\): 0.01 and perceived exertion: p=0.044, \( \eta\text{G}^2\): 0.01). On the contrary, there were no changes in VO\(_{2}\), and RMS\(_{BF}\) in stretching vs control (main effect of “time” VO\(_{2}\): p<0.001, \( \eta\text{G}^2\): 0.99, RMS\(_{BF}\): p<0.001, \( \eta\text{G}^2\): 0.77; no main effect of “condition” VO\(_{2}\): p=0.864, \( \eta\text{G}^2\): 0.01, RMS\(_{BF}\): p=0.362, \( \eta\text{G}^2\): 0.09). VO\(_{2}\) kinetics analysis revealed that the time
required to reach the VO$_{2\text{max}}$ during constant load trials was reduced by stretching (control= 709±183 vs stretching 639±197, p=0.034, $d$=-0.38).

Other parameters regarding VO$_2$ and [HHb] kinetics (e.g. $\tau$, TD etc.) are presented in table 2 and fig. 3 displays the mean signals during the transient phase after control and stretching. VO$_2$ showed no changes between control and stretching in time delay (p=0.874, $d$=+0.04), and in $\tau$ (p=0.066, $d$=-0.31, table 2). On the contrary, stretching reduced the time delay of [HHb] (p=0.023, $d$=-0.61, table 2 and fig. 3), but no difference was detected in $\tau$ (p=0.690, $d$=+0.19). Finally, during the transient phase (first 180 sec of exercise), $\Delta$[HHb]/$\Delta$VO$_2$ revealed an increased mismatch in oxygen delivery and utilization at the peripheral level after stretching (fig. 3), corroborated by a significant difference between conditions in $\Delta$[HHb]/$\Delta$VO$_2$ peak (p=0.038, $d$=+1.01) and a larger, yet not significant of $\Delta$[HHb]/$\Delta$VO$_2$ AUC (p=0.099, $d$=+0.59 table 2 and fig. 3).
**Discussion:**

This investigation evaluated the physiological effects of acute non-metabolic fatigue induced by static stretching on high-intensity constant-load cycling performance. We had hypothesized that stretching, through a sequence of events going from a reduction of maximal muscle force, to an increase in muscle excitation at a given absolute workload, to an increase the VO$_2$ cost of locomotion would ultimately reduce exercise tolerance. In agreement with our hypothesis, stretching caused a significant fatigue, as indicated by the reduction of the maximal power output during isokinetic sprints. In turn, force reduction was accompanied by an augmented muscle excitation at a given workload. Finally, the above sequence of events was associated with a significant reduction in time to exhaustion. Contrary to our hypothesis, no changes were detected between control and stretching in the magnitude of VO$_2$sc in the time window in which VO$_2$sc is typically investigated (from the $\approx 2^{nd}$-$3^{rd}$ to the $9^{th}$-$10^{th}$ min). However, the time required to reach VO$_{2\text{max}}$ was reduced by stretching, suggesting an influence of the increased muscle excitation on the dynamics of VO$_2$ during constant-load exercise in the severe intensity domain. In support of this, stretching was associated with an increased metabolic instability/mismatch between O$_2$ delivery and O$_2$ utilization during the on-kinetic, increased perception of effort and an increased blood lactate accumulation, that are all compatible with an increased contribution of the glycolytic energy system to sustain the same absolute intensity.

The effectiveness of stretching in acutely impairing maximal force/power was previously proven during cycling-specific (Colosio et al. 2019) and non-specific tasks (Behm et al. 2016). In agreement with the above findings, our study confirmed a reduction of the maximal cycling PO following stretching intervention of a similar order of magnitude (i.e. $\approx 5\%$) (table 1) of that previously described. The physiological causes of the impairment of maximal PO documented after repeated bouts of prolonged stretching remain to be elucidated and supraspinal, spinal or muscle-related mechanisms have all been proposed as possible explanatory causes of reduced maximal force (Trajano et al. 2017). Independently from the cause, a reduction of maximal force may translate in the requirement of higher levels of relative force to maintain the same absolute workload. In turn, this could augment the recruitment of high-order, fast fatiguing, motor units (progressive recruitment theory (Henneman et al. 1965)) or the frequency of activation of motor units, at the same absolute power output. Both mechanisms are likely to cause an increase in the overall electrical activity of the muscle, as evaluated by surface EMG (Vigotsky et al. 2018).
To our knowledge, our study was the first to examine EMG after stretching during cycling. According to our hypothesis, the impairment of maximal PO documented after stretching translated in augmented muscular excitation of the vastus lateralis (fig. 2); this finding supports the hypothesis that either the recruitment of higher order motor units or an increased activation frequency were necessary to sustain exercise at the same absolute workload compared to the control condition (fig. 2). The increase in muscle activation (vs control) manifested clearly within the first minute after the onset of exercise, with no further effect over time. This would support the idea that the increased muscle excitation following stretching was a result of the acute loss in maximal PO rather than to progressive fatigue. Interestingly, our intervention affected the muscle excitation of the vastus lateralis to a larger extent than that of the biceps femoris (fig. 2). We speculate that this difference may be due to a larger effectiveness of stretching on the extensor of the knee compared to the flexor (i.e. mostly for the different anatomical insertion of these muscles). Moreover, a smaller contribution of the biceps femoris than the vastus lateralis during cycling, particularly in a sample of non-cyclist subjects, may have influenced these results.

Contrary to our hypothesis, increased muscle excitation did not influence the VO\(_{2sc}\). Previous studies on the VO\(_{2sc}\) response following fatigue have led to inconsistent results: the VO\(_{2sc}\) was augmented (Colosio et al. 2019), unaffected (Hopker et al. 2016), or even diminished (Deley et al. 2006). This may be explained by the heterogeneity of the fatiguing protocols adopted in the different studies (e.g. stretching, dropjumps, electrical stimulation, etc.), by the exercise domain investigated (heavy/steady-state vs severe/non-steady-state) and by the time window considered and the analysis strategy (e.g. fitting, integral, etc.). Furthermore, in the non-steady-state, severe-intensity exercise used in our study, VO\(_2\) rapidly projects to VO\(_{2\text{max}}\) (fig. 2) reaching this upper ceiling within ~10 min. Under these conditions the potential for an increase in the VO\(_{2sc}\) (i.e. the difference in VO\(_2\) between the \(\approx 2^{nd}-3^{rd}\) and the \(9^{th}-10^{th}\) min) in response to stretching may have been too small to be measurable. In support of this view, the magnitude of the stretching effect measured in our study is consistent with the rather small increase in VO\(_2\) (around 100-150 ml/min) reported by Esposito et al. (Esposito et al. 2012) following stretching. Interestingly however, our data showed a reduction in the time necessary to reach VO\(_{2\text{max}}\) (of \(\approx 70\) sec, \(\approx 10\)% and augmented peak [La\(^-\)] and peak RPE (fig. 2) following stretching compared to the control condition. These findings appear compatible with a faster projection of VO\(_2\) towards VO\(_{2\text{max}}\) following stretching, possibly driven by the increased amount of muscle fibres necessary to sustain the same absolute workload, as indicated by the increased EMG\(_{VL}\) in the fatigued condition.

Finally, a mirror intervention of acute-fatigue that could provide complementary information on the link between muscle recruitment and VO\(_{2sc}\) is represented by strength training. In this context, Tam et al. trained for two months...
a group of older people with either interval training or resistance training (Tam et al. 2018). A direct comparison with this study is not fully applicable due to the lack of EMG measures and to the fact that anatomical and functional adaptations other than an isolated improvement of muscle strength occurred in Tam’s study. Still, the increased muscle strength reported together with a slight decrease of the VO\textsubscript{2sc} amplitude seem to corroborate a possible role of muscle activation in the genesis of the VO\textsubscript{2sc} (Tam et al. 2018).

Regarding the metabolic impact of stretching documented with NIRS (fig. 2), a reduced time delay of the [HHb] kinetics at the onset of exercise (fig. 3) and an augmented peak [HHb]/VO\textsubscript{2} ratio (fig. 3, table 2) were observed. These indexes indicate an earlier mismatch between oxygen delivery and oxygen utilization within the working muscles and a larger mismatch during the on-kinetics, respectively (Grassi et al. 2003). Both findings are compatible with either an increase in oxygen extraction and/or a lower oxygen delivery in the working muscles. While this is the first study that measured tissues oxygenation levels during cycling after stretching, recent studies showed that stretching can induced temporary ischemia followed by reactive hyperaemia and possibly enhance O\textsubscript{2} delivery at the macro- (Venturelli et al. 2019) and micro- (Trajano et al. 2014) circulatory levels. Therefore, while a reduction in muscle perfusion cannot be completely ruled out, it seems unlikely that oxygen availability in the working muscles would be reduced post stretching. Alternatively, stretching may be associated with a faster oxygen extraction caused by a faster and larger disturbance of intracellular homeostasis. The higher deoxygenation during the fatigued condition may be explained by the greater proportion of glycolytic muscle fibres being involved into exercise. In fact, the glycolytic fibres seem to display higher levels of O\textsubscript{2} extraction compared to the aerobic fibres at the onset of exercise (Ferreira et al. 2006; Koga et al. 2014), possibly due to a local Bohr effect (Jensen 2004). Interestingly, these speculations appear consistent with the finding of a faster projection of VO\textsubscript{2} towards VO\textsubscript{2max} following stretching.

In conclusion, prolonged stretching caused acute fatigue as indicated by a reduced the maximal power output in isokinetic sprints. Stretching-induced fatigue, in turn, caused augmented levels of muscle excitation at a given workload when cycling in the severe exercise domain, and a significant reduction in time to exhaustion. No changes were detected between control and stretching in the magnitude of VO\textsubscript{2sc} “per-se”; however, the time required to reach VO\textsubscript{2max} was reduced by stretching, suggesting an influence of the increased muscle excitation on the kinetics of the slow component of VO\textsubscript{2}. Finally, stretching was associated with an increased metabolic instability/mismatch between O\textsubscript{2} delivery and O\textsubscript{2} utilization during the on-kinetic, increased perception of effort and an increased blood lactate accumulation, all these phenomena are compatible with an increased contribution of the glycolytic energy system to sustain the same absolute intensity.
Acknowledgments:

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Conflict of interest Statement:

Authors declare no conflict of interest.
References:


Ferreira, L.F., McDonough, P., Behnke, B.J., Musch, T.I., and Poole, D.C. 2006. Blood flow and O2 extraction...


### Table 1, Mean ± SD peak power output during isokinetic sprints pre and post static stretching

<table>
<thead>
<tr>
<th></th>
<th>Pre stretching (W)</th>
<th>Post stretching (W)</th>
<th>Δ%</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 RPM</td>
<td>670 ± 132</td>
<td>626 ± 107 *</td>
<td>-6.0 ± 5.3</td>
<td>0.009</td>
<td>-0.33</td>
</tr>
<tr>
<td>120 RPM</td>
<td>903 ± 86</td>
<td>863 ± 112 *</td>
<td>-4.6 ± 4.8</td>
<td>0.014</td>
<td>-0.46</td>
</tr>
</tbody>
</table>

Isokinetic sprints peak power output is presented for the 60 and 120 RPM pedalling frequencies pre and post stretching with p-values and effect sizes (d). * represents significant differences between pre and post values.
### Table 2, Mean ± SD kinetics parameters for oxygen consumption and deoxyhemoglobin

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stretching</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (ml/min)</td>
<td>787.1 ± 174.6</td>
<td>790.7 ± 105.9</td>
<td>0.938</td>
<td>+ 0.02</td>
</tr>
<tr>
<td>AMPₚ (ml/min)</td>
<td>2015.4 ± 480.1</td>
<td>2045.2 ± 509.9</td>
<td>0.607</td>
<td>+ 0.06</td>
</tr>
<tr>
<td>TDₚ (sec)</td>
<td>14.7 ± 4.8</td>
<td>14.9 ± 10.7</td>
<td>0.873</td>
<td>+ 0.04</td>
</tr>
<tr>
<td>TDₚ 95% CI (sec)</td>
<td>13.2 – 16.2</td>
<td>13.6 – 16.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>τₚ (sec)</td>
<td>32.6 ± 10.5</td>
<td>29.3 ± 11.2</td>
<td>0.066</td>
<td>- 0.31</td>
</tr>
<tr>
<td>τₚ 95% CI (sec)</td>
<td>29.8 – 35.4</td>
<td>26.9 – 31.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRTₚ (sec)</td>
<td>47.3 ± 10.7</td>
<td>44.2 ± 12.3</td>
<td>0.116</td>
<td>- 0.29</td>
</tr>
<tr>
<td>AMPₛₑ (ml/min)</td>
<td>752.1 ± 386.9</td>
<td>756.1 ± 272.8</td>
<td>0.207</td>
<td>+ 0.01</td>
</tr>
<tr>
<td>TDₛₑ (sec)</td>
<td>152.6 ± 37.5</td>
<td>155.3 ± 25.6</td>
<td>0.803</td>
<td>+ 0.05</td>
</tr>
<tr>
<td>TDₛₑ 95% CI (sec)</td>
<td>146.5 – 158.7</td>
<td>147.1 – 163.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>τₛₑ (sec)</td>
<td>321.9 ± 149.7</td>
<td>282.9 ± 111.5</td>
<td>0.333</td>
<td>- 0.26</td>
</tr>
<tr>
<td>τₛₑ 95% CI (sec)</td>
<td>251.9 – 392.0</td>
<td>147.1 – 163.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>[HHb]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µM)</td>
<td>24.7 ± 9.4</td>
<td>27.3 ± 10.3</td>
<td>0.138</td>
<td>+ 0.38</td>
</tr>
<tr>
<td>AMP (µM)</td>
<td>14.8 ± 9.4</td>
<td>17.3 ± 12.2</td>
<td>0.359</td>
<td>+ 0.26</td>
</tr>
<tr>
<td>TD (sec)</td>
<td>6.9 ± 3.8</td>
<td>4.6 ± 3.0</td>
<td>0.023</td>
<td>- 0.61</td>
</tr>
<tr>
<td>TD 95% CI (sec)</td>
<td>6.2 – 7.7</td>
<td>4.0 – 5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>τ (sec)</td>
<td>16.8 ± 6.5</td>
<td>18.0 ± 4.7</td>
<td>0.689</td>
<td>+ 0.19</td>
</tr>
<tr>
<td>τ 95% CI (sec)</td>
<td>15.7 – 17.9</td>
<td>16.8 – 19.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRT (sec)</td>
<td>23.7 ± 9.0</td>
<td>22.6 ± 7.5</td>
<td>0.767</td>
<td>- 0.12</td>
</tr>
<tr>
<td><strong>Δ[HHb]/ΔVO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>30.4 ± 15.8</td>
<td>41.1 ± 16.1</td>
<td>0.099</td>
<td>+ 0.75</td>
</tr>
<tr>
<td>Peak</td>
<td>1.9 ± 0.5</td>
<td>2.4 ± 0.4</td>
<td>0.038</td>
<td>+ 1.01</td>
</tr>
<tr>
<td>Time to Peak (sec)</td>
<td>18.9 ± 5.8</td>
<td>18.4 ± 8.5</td>
<td>0.891</td>
<td>- 0.09</td>
</tr>
<tr>
<td>Overall Δ[HHb]/ΔVO₂ ratio (µM/ml/min)</td>
<td>0.011 ± 0.008</td>
<td>0.014 ± 0.012</td>
<td>0.275</td>
<td>+ 0.28</td>
</tr>
</tbody>
</table>

Values for VO₂, [HHb] and Δ[HHb]/ΔVO₂ kinetics are presented. Acronyms represent: AMP = amplitude, TD = time delay, τ = time constant of response, MRT = mean response time, AUC = area under the curve. Subscripts in VO₂ kinetics represent: p = primary component, sc = slow component.
Fig. 1,
Schematic representation of the overall protocol (above), and of the single testing sessions (below).

Fig. 2,
The physiological responses during control (black dots) and stretching (white dots) conditions are presented in 30 sec means ±SD. * represent statistical difference between control and stretching for a given timepoint.

Fig. 3,
The VO\textsubscript{2} (top panel), [HHb] (medium panel), and ∆[HHb]/∆VO\textsubscript{2} (bottom panel) kinetics during the first 180 sec after exercise onset are presented during control (black dots) and stretching (white dots). * represent statistical difference between control and stretching for a given value of the kinetics. Acronyms represent: TD = time delay, τ = time constant of response, MRT = mean response time, AUC = area under the curve.
Schematic representation of the overall protocol (above), and of the single testing sessions (below).
The physiological responses during control (black dots) and stretching (white dots) conditions are presented in 30 sec means ±SD. * represent statistical difference between control and stretching for a given timepoint.
The VO2 (top panel), [HHb] (medium panel), and Δ[HHb]/ΔVO2 (bottom panel) kinetics during the first 180 sec after exercise onset are presented during control (black dots) and stretching (white dots). * represent statistical difference between control and stretching for a given value of the kinetics. Acronyms represent: TD = time delay, \( \tau \) = time constant of response, MRT = mean response time, AUC = area under the curve.