Quantification of the anaerobic contribution in low to moderate intensity exercise with fluctuations

Background:

Several studies have quantified the anaerobic contribution in high intensity exercises with or without intervals. However, the anaerobic contribution during lower intensities have not been investigated sufficiently.

Aim:

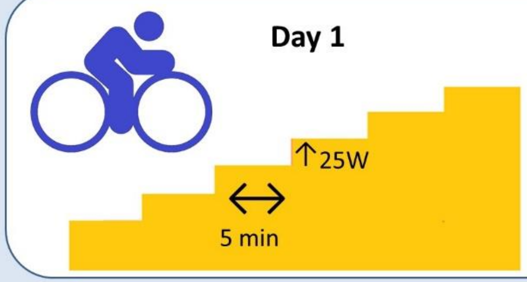
Quantification of the anaerobic contribution in the low to moderate intensity range when including intensity fluctuations, and to investigate the effects of fluctuations with different amplitudes.

Methods:

15

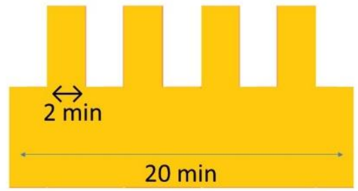
sedentary to well-trained

Day 1



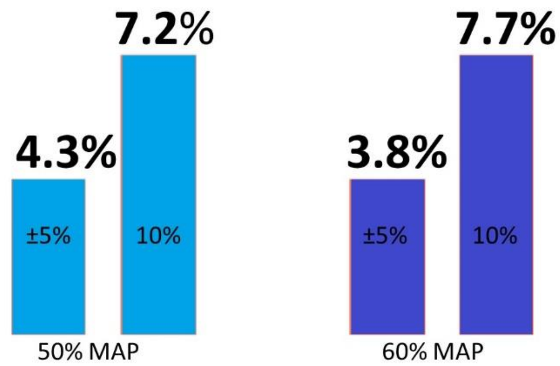
Day 2 and 3

4 modalities
50%±5% MAP
50%±10% MAP
60%±5% MAP
60%±10% MAP



Results:

Relative anaerobic contribution (when negative values of AnaMR is converted to zero)



- Greater amplitude of fluctuations elicits higher AnaMR when negative values of AnaMR is converted to zero.
- Intensity has no effect on AnaMR.
- No interaction effect of intensity and amplitude on AnaMR.

Abstract

There are many studies quantifying the anaerobic contribution in high intensity exercises with or without intervals. However, the anaerobic contribution during lower intensities have not been investigated sufficiently. The aim of this study was to quantify the anaerobic contribution in the low to moderate intensity range when including intensity fluctuations, and to investigate the effects of fluctuations with different amplitudes during stationary bicycle pedaling exercise.

Fifteen participants ranging from sedentary to well-trained performed three tests. Thie first was a maximal test and was performed to obtain the maximal aerobic power (MAP) of the participants, with 5-minute incremental stages of 25 W until fatigue. The second and third tests were the main tests for quantification of the anaerobic contribution and included four modalities of low to moderate intensity fluctuations around 50% or 60% MAP with the amplitude of ±5% or ±10%MAP, where each modality was composed of 10 bouts of 2 minutes. Participants performed two of the four modalities on each of the main test days in a randomized cross-over order.

When instances of negative values for anaerobic metabolic rate were converted to zero (pos.AnaMR), the relative anaerobic contribution was around 4% and 7.5% for amplitudes of ±5% MAP and ±10% MAP respectively, no matter if the baseline intensity was at 50% or 60% MAP. There was statistically significant effect of amplitude on AnaMR ($p<0.001$) but not for effect of intensity ($p=0.226$) and the interaction effect ($p=0.429$). The relative anaerobic contribution when those negative instances of AnaMR were taken into consideration (net AnaMR) was around 1.5% for all the four modalities with no statistically significant effect of intensity ($p=0.236$), amplitude ($p=0.719$) or interaction effect ($p=0.784$) on AnaMR.

When taking the pos.AnaMR into account, there is a substantial increase in the relative AnaMR at each intensity from amplitude of ±5% MAP to ±10 MAP. This denotes that the effect of amplitude on the relative AnaMR is much bigger than that of intensity of low to moderate range, considering that the changes in intensity did not have significant effect on AnaMR by itself.

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List of Abbreviations:

AeroMR	Aerobic metabolic rate
AnaMR	Anaerobic metabolic rate
BLA	Blood lactate
HIIT	High intensity interval training
MAP	Maximal Aerobic Power
MR	Matabolic rate
Net AnaMR	Net anaerobic metabolic rate
Pos.AnaMR	Positive anaerobic metabolic rate
ReqMR	Required metabolic rate
RPE	Rate of perceived excertion

1. Introduction

Our muscles need energy in the form of ATP for any sort of physical activity. There are three pathways through which our muscle cells provide the required energy: Anaerobic a-lactic and lactic systems, and Aerobic system. Which system is used when has been widely investigated in the last decades and there are recent findings that show it is mainly dependent on the intensity and duration of the exercise (Losnegard, 2019).

Anaerobic system can provide energy very quickly but over a shorter period of intense exercise. It is involved in short bursts of explosive muscle power, at transitions to higher intensity breakthroughs or from resting to a more demanding activity, as well as at high intensities over the anaerobic threshold. On the other hand, the aerobic system can provide energy for a much longer period of lighter exercise but at a slower rate, which is best suited for endurance activities. It is also more efficient, meaning that it produces a much higher amount of ATP per molecule of substrate. In aerobic metabolism, a molecule of glucose breaks down via cellular respiration and yields up to a net of 36–38 molecules of ATP. The three steps of cellular respiration are glycolysis, the Krebs cycle, and oxidative phosphorylation in electron transport chain. In the cytoplasm of the cell, two ATP molecules are generated through glycolysis of one glucose molecule into two pyruvate molecules. In the mitochondria, the Krebs cycle results in the production of two ATP and in the mitochondria's inner membrane. The energy produced in Krebs cycle, also known as the citric acid cycle or the tricarboxylic acid (TCA) cycle, fuels oxidative phosphorylation process which generates 32–34 ATP molecules. In anaerobic metabolism, on the other hand, which happens in two steps of glycolysis and fermentation, only two net ATP molecules are generated from one glucose molecule and those are from the glycolysis step not the fermentation.

Overall, aerobic metabolism produces significantly more ATP compared to anaerobic metabolism when we take the utilization of fatty acids into consideration as well. During aerobic metabolism, Acetyl-CoA, which is created by the beta-oxidation of fatty acids enters the Krebs cycle and then the electron transport chain. 1 molecule of palmitic acid, a common 16-carbon saturated fatty acid can produce a net of approximately 106 molecules of ATP. However, this number can vary depending on the type of fatty acid and the metabolic pathway involved.

With anaerobic energy expenditure, glycolysis happens at an accelerated rate. The increased rate of glycolysis generates more H+ ions, leading to a buildup of acidity in the blood. To buffer the acidity, the body bonds the H+ ions to pyruvate and produces lactate. Thus, production of blood lactate helps maintain the pH balance in the body. Lactate, being able to be converted back into pyruvate, then gets cleared from the blood into the liver and other organs such as heart and brain to be used again as a source of energy.

During rest, the body's energy demands are relatively low, and the body can meet these demands through the efficient and sustainable process of aerobic metabolism. This process provides the majority of the energy needed for basic physiological functions, such as maintaining body temperature and organ function. At the start of moderate intensity continuous training, the resting balance is disrupted and there is a transitional phase in equilibrium from rest to the applied higher intensity. VO2 increases gradually and reaches a constant value in about 2 - 3 minutes while the mechanical load increases suddenly. In this transient phase, the body is incapable of maintaining total ATP resynthesis rate through aerobic system per se, at the same rate of ATP hydrolysis during muscle contraction. It is when the anaerobic metabolism gets engaged to fill the gap (Ferretti et al., 2022).

At high intensities above a certain limit, so-called the anaerobic threshold, our body needs more energy than the aerobic metabolism can produce. The body's oxygen supply may be limited during activities such as sprinting, weightlifting, or other high-intensity exercises. During these activities, the muscles require a large amount of energy in a short amount of time, and aerobic metabolism cannot provide energy quickly enough to meet this demand. In response, the body activates the anaerobic energy system, which can provide energy quickly, but only for a short period before fatigue sets in.

The amount of aerobic energy release during exercise can be easily quantified by measuring the oxygen consumption (VO2). However, it is more complicated to quantify the anaerobic energy provision. Although the direct method of one-legged dynamic knee extension, introduced by Bangsbo et al. (1990) including vastus lateralis muscle biopsies for local muscular lactate, ATP, creatine phosphate and inosine monophosphate measurements, and sampling arterial and venous blood through catheters for local VO2 and blood lactate measurements is probably the most accurate method, it is disadvantageous for being invasive as well as being not applicable for whole-body training in which many large muscle groups are engaged and the active muscle mass in unknown (Noordhof et al., 2010).Therefore, several indirect methods which are based on estimations have been introduced and used such as the maximal accumulated oxygen deficit method (MAOD), the gross efficiency method (GE) and the critical power method (CP) first described by Medbø et al. (1988), Seresse et al. (1988) and Monod and Scherrer (1965) respectively. The MAOD method is based on the estimation of oxygen demand and by calculating the linear relationship between submaximal intensity/speed and oxygen uptake, and then extrapolating oxygen demand at supramaximal intensities, therefore calculating the maximal accumulated O2 deficit which is being used as a direct quantitative expression of the anaerobic capacity. However, a linear regression method based on external power output (PO) and metabolic rate (MR) has been suggested to be more suitable than the conventional MAOD method (Andersson & McGawley, 2018). In the GE method, GE is calculated by dividing the mechanical power output by the metabolic rate and the highest GE during a submaximal exercise and is assumed to remain unchanged in supramaximal intensities, at which the MR will be calculated through dividing PO by GE (GE=PO/MR, MR=PO/GE). In the CP method, CP is defined as the highest power that is produced mainly by aerobic energy system and can be sustained for an indefinite duration, and W' is defined as the amount of work than can be done above CP, possibly representing the anaerobic work capacity (AnWC). There is a variety of methods to calculate CP and W' with a range of one to seven maximal tests and various test duration and several studies suggest that estimates of CP can vary and are influenced by the test protocol design (Maturana et al., 2018).

Spencer and Gastin (2001) quantified the anaerobic energy contribution during 200-m, 400-m, 800-m and 1500-m running which took on average 22s, 49s, 1:53 and 3:55 respectively, to be 71%, 57%, 34% and 16% respectively. These numbers demonstrate that the longer the run is performed, thus with lower speed (intensity), the lower anaerobic contribution is present. Noordhof et al. (2021) measured the overall anaerobic energy contribution during the periods of active propulsion of a 21-min simulated cross-country skiing mass-start competition including ~13 minutes of high intensity effort, to be on average between 10-15% depending on the method used. We can observe that the anaerobic contribution is slightly lower than the 1500-m run in the previous study, although it consisted of 7 bouts, each of which including less than two minutes of high intensity work, for which a higher anaerobic metabolic contribution is expected. The similar or even less anaerobic contribution in the latter study is happening because of the effects of intensity fluctuations which will be discussed later. Gastin (2001) found ~20% of the total energy required for four consecutive bouts of 3-4 minutes skiing on undulating terrain, contributed by anaerobic energy production. Again, compared to 1500-m run in the first mentioned study, we can see here that the anaerobic contribution is only slightly higher although there are four bouts of 3-4 minutes compared to one bout of almost 4 minutes. This is also happening because of undulating terrain which resembles intensity fluctuations.

Andersson et al. (2021) found 11-16% of anaerobic contribution during a 4-minute treadmill running time trial, in spite of using three different methods other than Spencer and Gastin (2001)

It is known that the physiological adaptations associated with endurance training happen in response to increased energy demands of muscle cells (Coyle, 2000). Submaximal training in sedentary individuals and beginners can improve the aerobic energy system and physical work capacity through central and peripheral adaptations (Green et al., 1990). It means that even with submaximal training, there is an increased oxygen delivery to the working muscle as well as increased utilization of delivered oxygen by the muscle cells in this population, thus increased VO2 at any given intensity, if the training is continued for several weeks.

Interval training with intensity fluctuations is a highly effective form of exercise, which involves alternating periods of high and low-intensity activity. This type of workout closely mimics the demands of outdoor activities or sports that take place on undulating terrain such as road cycling, cross-country skiing and trail running. On the other hand, training within low intensity range with intensity fluctuations could resemble the above-mentioned outdoor activities on undulating terrain, but with moderate steepness.

Several studies have investigated the immediate metabolic and physiological responses to high intensity interval training, and it is well established that at the high intensity bouts, the anaerobic contribution increases dramatically and the anaerobic energy system becomes the main supplier of body's required energy (Almquist et al., 2021; Ziemann et al., 2011), and with lower intensity bouts with almost entirely aerobic contribution, the anaerobic reserves get the chance to be restored to allow longer duration of exercise at high intensity. However, to my knowledge, there is no study attempting to quantify the anaerobic contribution in a low to moderate intensity exercise and to investigate the immediate metabolic and physiological responses of intensity fluctuation within this intensity range.

The aim of this study is to quantify the anaerobic contribution in the low to moderate intensity range when including intensity fluctuations, and to investigate the effects of fluctuations with different amplitudes.

2. Methods

2.1 Participants

Eighteen subjects participated in this study. The final data analysis was carried out with fifteen participants consisting of twelve males and three females. Three datasets were excluded due to errors in measurement. Participants' characteristics are shown in **Table1**. Participants had different levels of daily physical activity, ranging from sedentary to well-trained. All participants got informed and instructed about the study, provided written consent prior to the study and were asked to refrain from intense exercise 24 hours before the start of each test day. The study was approved by the Norwegian Social Science Data Services (NSD) and conducted in agreement with the Helsinki Declaration of 1976.

Table 1 – Participants' characteristics	
Age (years)	28 ± 10
Height (cm)	179.4 ± 8.3
Body mass (kg)	74 ± 8.6
VO2max (mL/min)	3766 ± 868
Maximal Aerobic Power (Watts)	278.4 ± 77.3

2.2 Tests and protocols

A total number of three tests were performed by each participant. The first test was a maximal test to measure their VO2 max. The 2nd and 3rd tests were designed with different low to moderate interval intensity and amplitude. Participants were asked to refrain from strength training or intense physical activity 24 hours prior to test time. The second test was conducted at least 48 hours after the first test. The third test was conducted at least 24 hours after the second test. The time-of-day was set to be not more than 4 hours apart.

2.2.1 Preliminary test

Preliminary test was conducted to obtain the maximal aerobic power (MAP) of the participants. Weight and resting lactate level of the subjects were measured before the actual test started. Participants could decide to have a few minutes of warmup if they wanted before the test started. The initial power output of the actual test was 75W or 100W which was chosen according to the participants' performance on the first day, followed by stages of five minutes with 25W increase in each. A blood lactate (BLA) measurement was taken 60-30 seconds before the end of each stage. The test stopped when the subject was fully fatigued. 1 minute after the test cessation, a last lactate measurement was taken. **Figure 1** illustrates the protocol for the preliminary test.

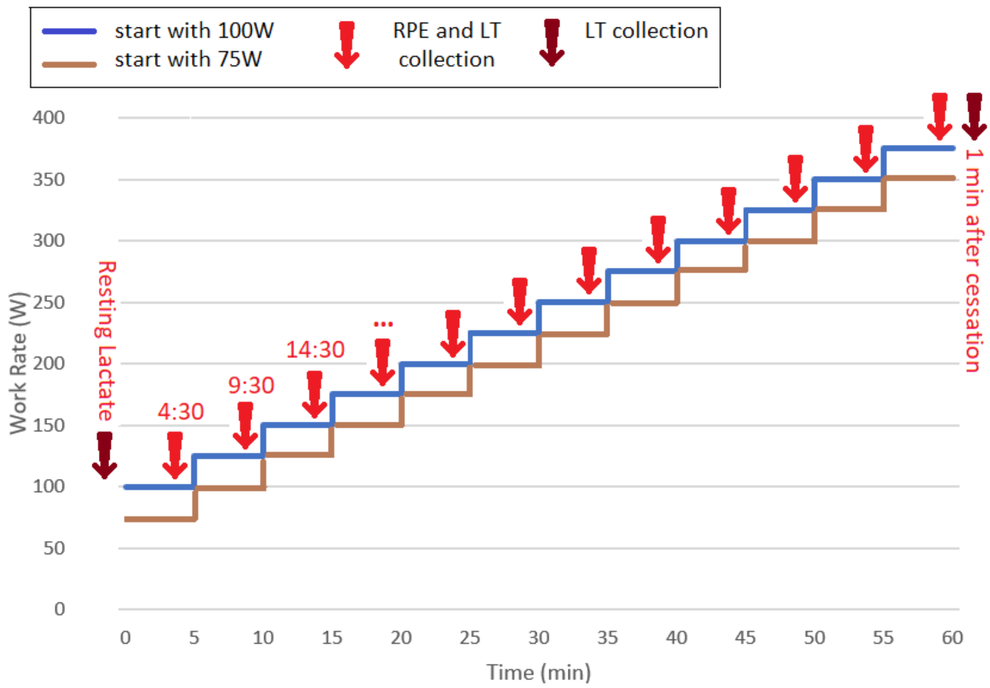

Figure 1 - the protocol for the preliminary test. Participants started at 75W or 100W according to their fitness level, with 25W incremental stages of 5 minutes.

2.2.2 Main Protocol

The main protocol was designed for quantification of the anaerobic contribution during low to moderate intensity exercise with fluctuations and was composed of two testing days each consisting of three phases; The first and second tests were manipulated around 50% and 60% of MAP respectively.

Test phases:

1. **Warmup:** for 24 minutes, including 3 consecutive stages of 8 minutes, at 40%, 50% and 60% of MAP for the first test and at 50%, 60% and 70% of MAP for the second test.

2. **Bout A:** First phase of fluctuations for 25 minutes, starting with 5 minutes at 50% of MAP for the first test, followed by 20 minutes of fluctuations, composed of 10 bouts of 2 minutes at either ±5% MAP or ±10% MAP, decided randomly. The same pattern was applied for the second test with 10% higher intensity (60% of MAP) and a randomized crossover model which denotes application of the amplitude other than the first test.

3. **Bout B:** Second phase of fluctuations for 25 minutes, starting with 5 minutes at 50% of MAP for the first test, followed by 20 minutes of fluctuations, composed of 10 bouts of 2 minutes at the amplitude other than that of previous phase. The same pattern was applied for the second test with 10% higher intensity.

Figure 2 illustrates the protocol for the two tests of the main protocol.

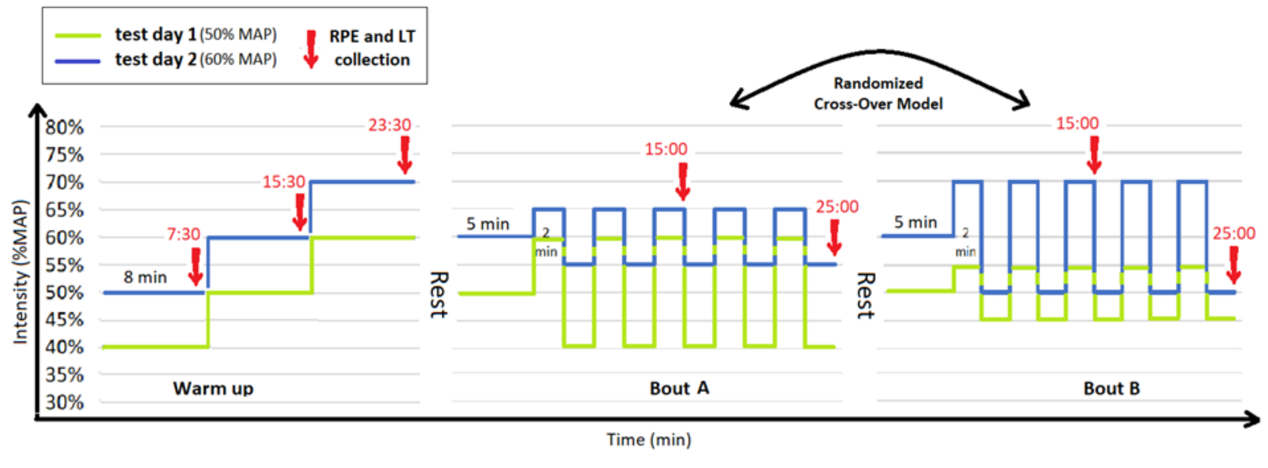

Figure 2 - the protocol for the two main tests, each composed of three phases: warmup, bout A and bout B. After warmup, participants randomly performed bout A then bout B protocols, or vice versa.

In both tests of the main protocol:

Participants were weighed first, after their arrival.

There were no initial, out of protocol warmup like the first two sessions.

After the 24-minute warmup as well as the 25-minute first phase of fluctuations, the participant could take off the mask, drink some water and rest for a few minutes until they felt they were ready for the next phase of the test.

BLA was measured 30 seconds before the end of each 8-minute stage in warmup phase (three measurements), as well as in the middle and at the end of the 20-minute fluctuations in both bout A and bout B (4 measurements). A total number of 7 BLA measurements was recorded for each participant for each of the test days. Rate of perceived exertion (RPE) was collected using the Borg Scale 6-20 at the same timepoints as the LT measurements.

2.3 Measurements and devices

All three tests were performed on a LODE Excalibur Sport Cycle ergometer (LODE B.V., Groningen, Netherlands) and the test protocols were automatically applied to the device by its software. Participants could freely adjust their seat position and sitting posture on the first day and the setting was replicated on the two subsequent tests. Ventilatory parameters and pulmonary gas exchange data were collected using a mask through breath-by-breath measure provided by a spirometry system, Vyntus CPX, Vyaire medical GmBH, Hoechberg, Germany, showing respiratory data averaged over every 10 seconds. Before each test, the system would go through automated flow and gas calibration to ensure a proper flow as well as gas concentrations of 15% O2 and 5.85% CO2. Gas from Riessner-Gase GmbH & Co, lichtenfels, Germany). Capillary blood samples were taken from fingertips at timepoints illustrated on the figures 1-4, to measure blood lactate with Biosen C-Line, EKF diagnostics, Barleben /Magdeburg, Germany). Rate of perceived exertion (RPE) was collected using the Borg Scale 6-20.

2.4 Calculations and Data Analysis

VO2max(ml.min-1) was considered as the highest registered value of one-minute averaged VO2 in the preliminary test.

Maximal Aerobic Power (MAP) was calculated by a linear regression of the average VO2 of the last minute of each stage on Load, based on the preliminary test data. For this regression, only the data of the completed 5-minute stages were used to attain the best fit to the regression line and to avoid any extrapolation for MAP which could lead to higher power output than real at each of the intensities, thus attainment of excessive AnaMR. MAP = A x VO2max + B

Metabolic rate (MR_{mes}) was measured from the average VO2 and VCO2 of the last two minutes (minutes 6 to 8) of each constant intensity at warmup and was calculated according to the Péronnet and Massicotte (1991) equation: Metabolic Rate = 281.67 x VO2 + 80.65 x VCO2

Required metabolic rate (ReqMR) was calculated as: ReqMR = A x Load + B, from the warmup and was used during the whole protocol of the main tests.

Where A and B were calculated by a linear regression of MR_{mes} on Load at warm up.

The possible lag between changes in load and the subsequent change in metabolic rate was corrected through cross correlation. The data was transformed to second-by-second data by adding 10 replications of each time sample (of 10 seconds) and a cross correlation was performed with power output and metabolic rate, with a max of 30 samples.

Aerobic metabolic rate (AeroMR) was calculated using Péronnet and Massicotte equation:

AeroMR = 281.67 x VO2 + 80.65 x VCO2

Net anaerobic metabolic rate was calculated: net AnaMR = ReqMR - AeroMR

Positive AnaMR was calculated by conversion of instances of negative values of net AnaMR to zero.

2.5 Statistical Analysis

Two-way ANOVA analysis was performed using SPSS, 28^{th} version to investigate the interaction effect and main effects of intensity and amplitude on AnaMR, AeroMR, BLA and RPE.

A linear regression of the average VO2 of the last minute of each stage on Load was performed for calculation of MAP.

For investigation of the effect of MAP on AnaMR, Pearson correlations were performed on SPSS 28.

3. Results

The required, aerobic and anaerobic metabolic rates for each of the four bouts of our test are visualized in **Figure 3**. **Figure 4** shows the relationship between metabolic rate and work rate (intensity) during the warmup in the two tests of the main protocol. The ReqMR for the fluctuations in this study has been calculated based on this relationship.

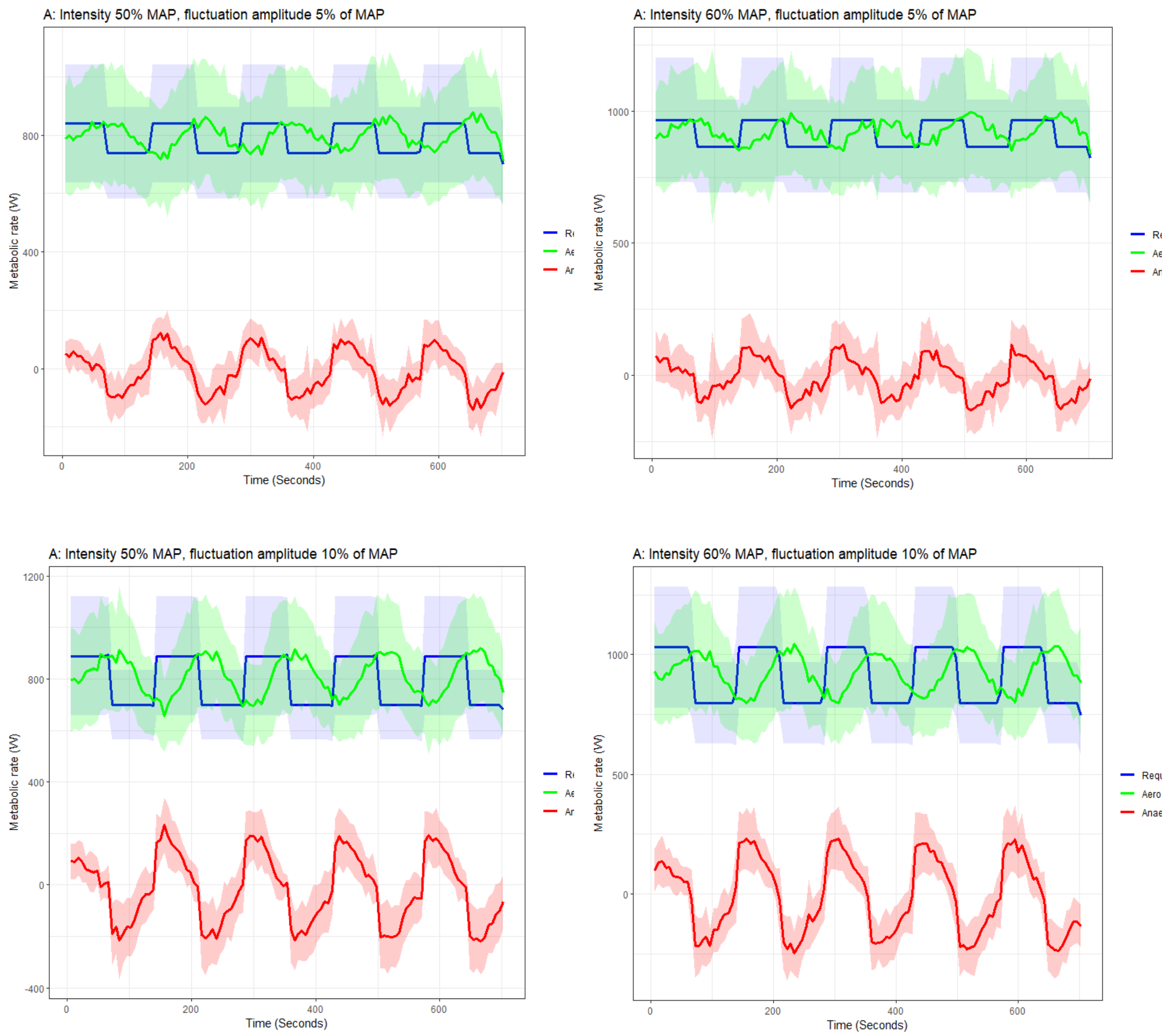

Figure 3 – The shadows around the lines illustrates the standard deviation.

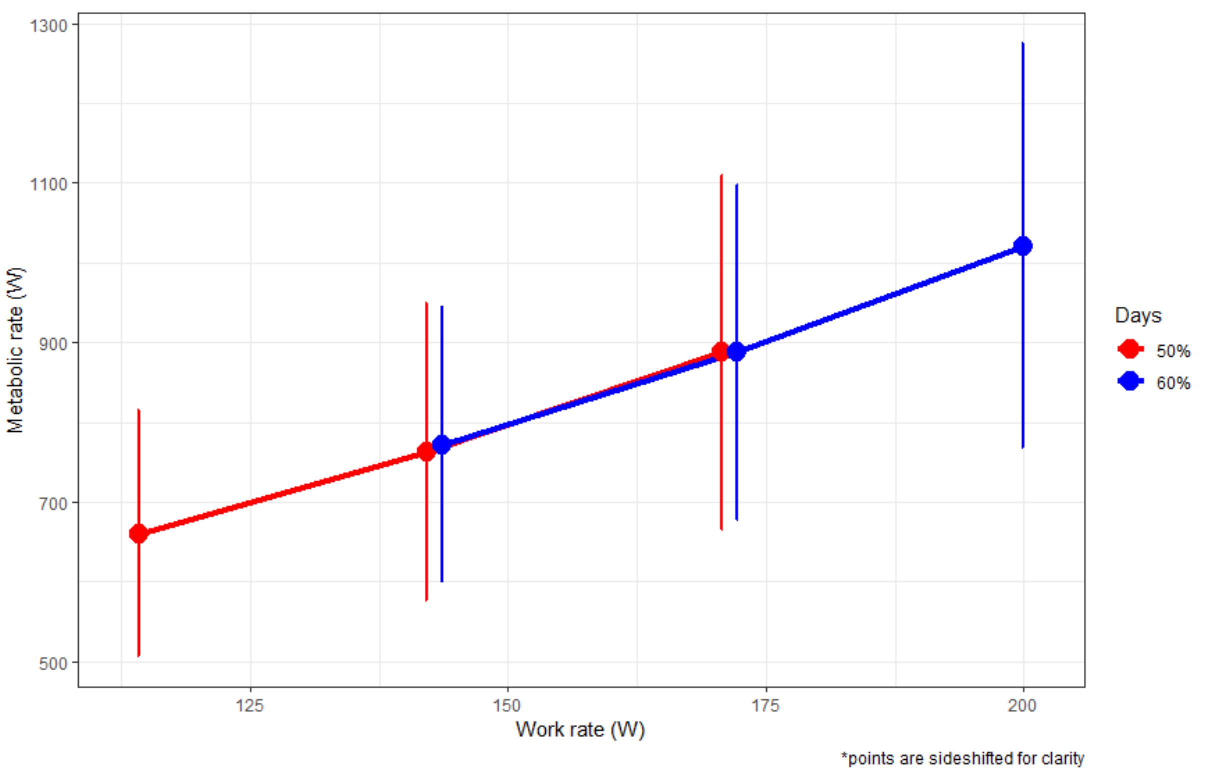

Figure 4 - Changes in the metabolic rate during warmup for both of the main tests.

Table 2 contains the Mean ± SD for required, aerobic and anaerobic metabolic rates as well as the relative anaerobic contribution in each of the four modalities of our test for 16 participants who completed the test. In this table, instances of negative values for anaerobic metabolic rate have been converted to zero, while in **table 3**, those negative values have been taken into consideration and have remained unchanged.

Table 2 – Mean ± SD for required, aerobic and positive anaerobic metabolic rates during each of the four modalities.

Modality	50% ± 5%	50% ± 10%	60% ± 5%	60% ± 10%
Required metabolic rate (J)	91942 ± 19995	92590 ± 19558	106680 ± 22836	106616 ± 23034
Aerobic metabolic rate (J)	93167 ± 20671	94640 ± 19871	107713 ± 21559	107744 ± 21750
Anaerobic metabolic rate (J)	3996 ± 1637	6795 ± 2471	4353 ± 3769	8479 ± 3989
Relative positive anaerobic contribution (%)	4.3 ± 1.6	7.2 ± 2.0	3.8 ± 2.4	7.7 ± 2.2

Table 3 – Mean ± SD for required, aerobic and net anaerobic metabolic rates during each of the four modalities.

Modality	**50% ± 5%**	**50% ± 10%**	**60% ± 5%**	**60% ± 10%**
Required metabolic rate (J)	91942 ± 19995	92590 ± 19558	106680 ± 22836	106616 ± 23034
Aerobic metabolic rate (J)	93167 ± 20671	94640 ± 19871	107713 ± 21559	107744 ± 21750
Anaerobic metabolic rate (J)	1271 ± 1543	1329 ± 1191	1887 ± 3432	2313 ± 3073
Relative net anaerobic contribution (%)	1.4 ± 1.8	1.4 ± 1.3	1.5 ± 1.3	1.9 ± 2.1

Figure 5 and 6 illustrate the anaerobic contribution during the four different modalities of our tests. Two-way ANOVA statistical analysis show insufficient evidence to reject the null hypothesis of no interaction effect ($p=0.784$) Since there is no statistical interaction effect, we can conclude that the effect of intensity on the anaerobic metabolic rate is independent of the amplitude or vice versa. The sole effect of amplitude ($p=0.719$) or intensity ($p=0.236$) on the anaerobic metabolic rate are also statistically insignificant.

When disregarding the instances of negative values for anaerobic metabolic rate, the effect of amplitude on the anaerobic metabolic rate becomes statistically significant ($p<0.001$) while the interaction effect ($p=0.429$) and the effect of intensity ($p=0.226$) remain statistically insignificant.

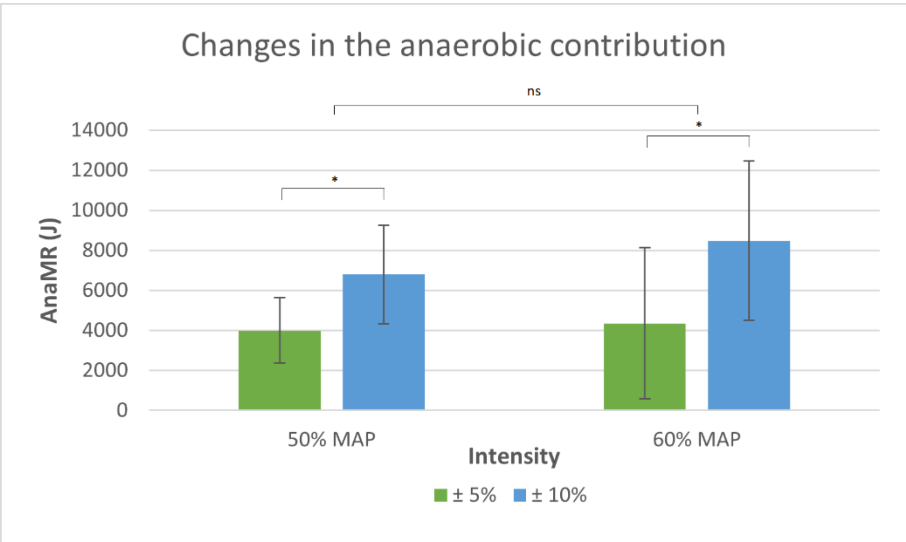

Figure 5 - Instances of negative values for anaerobic metabolic rate have been converted to zero. "*" indicates statistical significance. "ns" indicates no statistical significance.

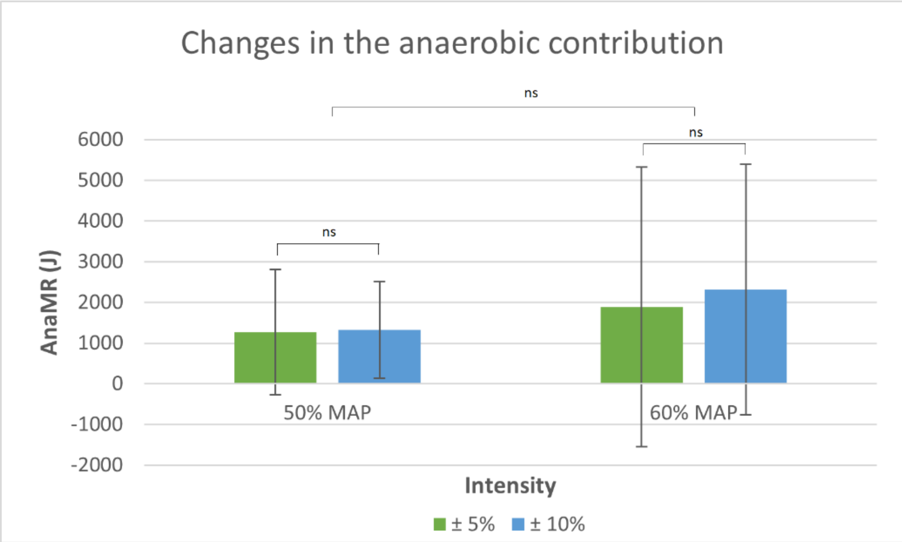

Figure 6 - Instances of negative values have been taken into consideration.
"ns" indicates no statistical significance.

Figure 7 visualizes the aerobic contribution. The analysis of the aerobic contribution indicates that the sole effect of intensity on the AeroMR is significant (p=0.017) while the effect of amplitude (p=0.894) or the interaction effect (p=0.898) are not statistically significant. The changes in the blood lactate and the scores of RPE are depicted in **Figures 8 and 9** respectively. Intensity has significant effect on both BLA (p<0.001) and RPE (p<0.001) while there is no significant effect of amplitude (p=0.29 for BLA, p=0.85 for RPE) or interaction effect (p=0.6 for BLA, p=0.85 for RPE) on any of them.

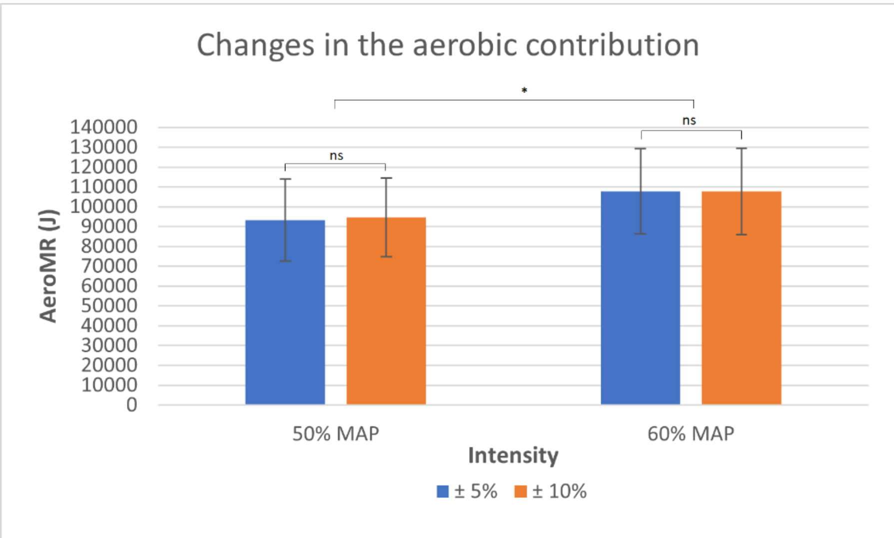

Figure 7 - "*" indicates statistical significance. "ns" indicates no statistical significance.

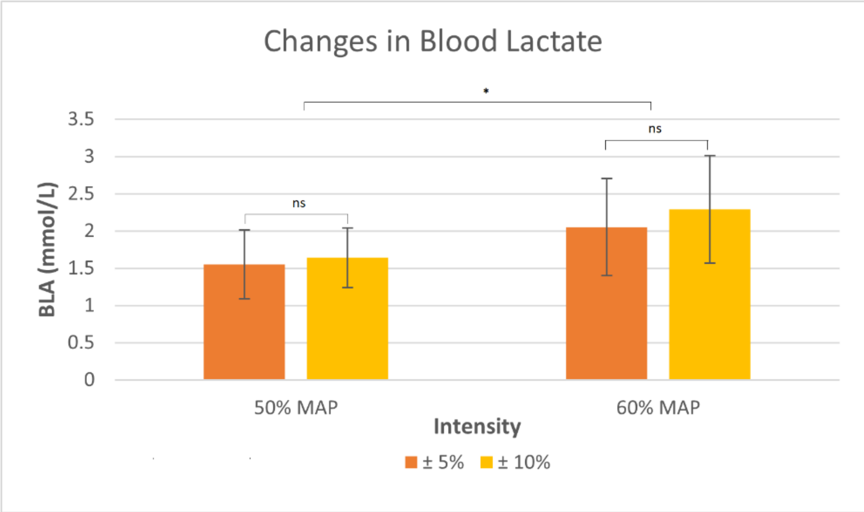

Figure 8 - “*” indicates statistical significance. “ns” indicates no statistical significance.

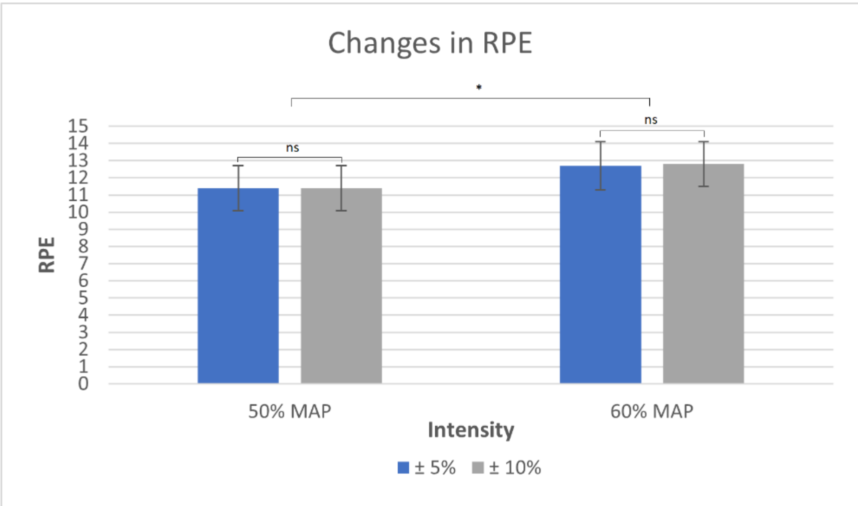

Figure 9 – “*” indicates statistical significance. “ns” indicates no statistical significance.

Figure 10 shows the relationship between MAP and AnaMR. The Pearson’s correlation coefficient was 0.917 ($p<0.001$), 0.592 ($p=0.02$) and 0.728 ($p=0.002$) for relationship between MAP and pos.AnaMR, net AnaMR and relative AnaMR respectively.

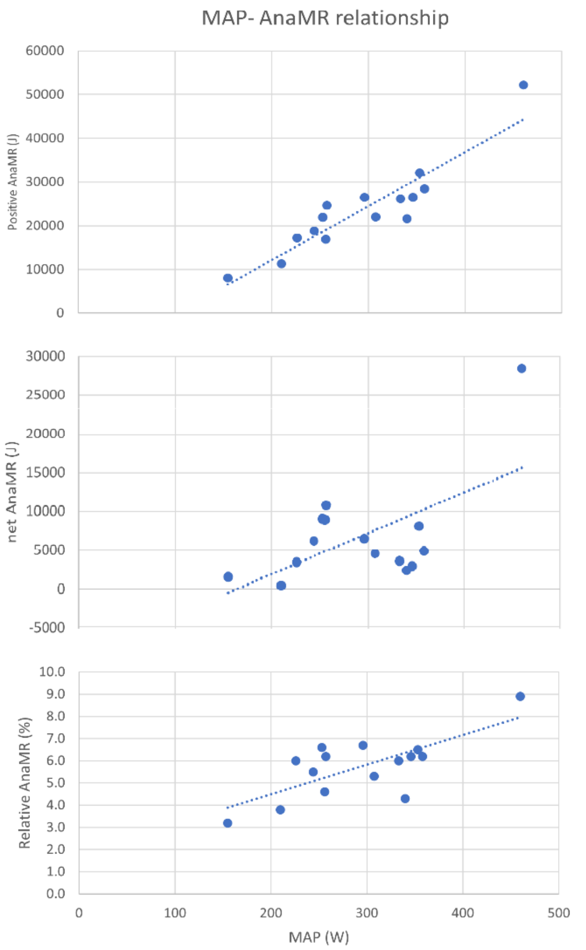

Figure 10 – the MAP – AnaMR relationship. AnaMR presented is for the sum of all 4 modalities. Relative AnaMR presented is the pos.AnaMR/ReqMR.

4. Discussion

The purpose of this study was to quantify the anaerobic energy contribution during stationary bicycle pedaling exercise with fluctuations at two different low to moderate intensities, each at two amplitudes, and investigate the effect of intensity and fluctuations on AnaMR. I found that in average, when instances of negative values for anaerobic metabolic rate were converted to zero (pos.AnaMR), around 4 percent of the total energy expenditure comes from the AnaMR for fluctuations of ±5% MAP no matter if the baseline intensity is at 50% or 60% MAP (4.3% at 50% ±5% MAP and 3.8% at 50% ±10% MAP), and this relative contribution is around 7.5% MAP for fluctuations of ±10% MAP (7.2% at 50% ±10% MAP and 7.7% at 60% ±10% MAP) when those negative instances of AnaMR have been taken into consideration (net AnaMR). The mean for absolute pos. AnaMR, ranged from 3996 for 50% ±5% MAP to 8479 J for 60% ±10% MAP, and for absolute net AnaMR, from 1271 to 2313 J.

The net AnaMR was considerably lower than pos.AnaMR at any of the four different modalities. The aim of this study was not to measure the anaerobic capacity, so there is no certainty that the excess of oxygen uptake than required gets fully allocated to the recovery of the anaerobic reserves. For this reason, the negative AnaMR values which were taken into consideration in net AnaMR might not equal the actual AnaMR. Since the recovery bouts of 2 minutes give a decent chance for recovery of the anaerobic energy reserves, bearing in mind that the intensity of the higher-load bouts were not too high, therefore it is more informative to also use the pos.AnaMR values when discussing the anaerobic contribution in this study because they might better represent the true anaerobic energy expenditure.

When taking the pos.AnaMR into account, we can observe that there is a substantial increase in the relative AnaMR at each intensity from amplitude of ±5% MAP to ±10 MAP. This denotes that the effect of amplitude on the relative AnaMR is much bigger than that of intensity of low to moderate range, bearing in mind that the changes in intensity did not have significant effect on AnaMR by itself. This can be a factor to be investigated in future studies, incorporating bigger amplitudes to examine whether the relative AnaMR would be higher.

The mean BLA levels at the intensity of 60% MAP was higher than that of 50% MAP as expected, but similar for the two amplitudes at each intensity. Therefore, we can hypothesize that performing exercise at higher intensities within the moderate intensity range, with bigger amplitudes which demands higher anaerobic energy contribution, might enhance buffer capacity thus increase the anaerobic capacity, with much lower RPE compared to high intensity (interval) training.

A crucial indicator of a cell's energy status during training is the AMP/ATP ratio. High ratios suggest an ATP shortage and the need for energy synthesis. Low ratios, on the other hand, indicate adequate energy and a lack of energy demand. Rapid ATP breakdown during anaerobic exercise causes a high concentration of AMP and a high AMP/ATP ratio. The body's different cellular functions and signaling pathways can be impacted by this ratio. The increase in the AMP/ATP ratio can activate AMP-activated protein kinase (AMPK), a key enzyme in the regulation of energy metabolism, drive the absorption of glucose and the oxidation of fatty acids to make more ATP (Liu et al., 2010), which can better satisfy the increased energy requirements during exercise. There is a potential for enhancement of this signaling pathway by application of fluctuations with greater amplitudes, which was shown to elicit greater AnaMR in this study.

The highest registered mean RPE score in this study was below 13 Borg scale, which means that the exercise was perceived as light. There is no doubt that high intensity interval training (HIIT) can utilize higher amounts of anaerobic energy. However, it is critical for exercise programs to be perceived as enjoyable, because even if they are effective and time-efficient, they might be unlikely to be sustained for a long enough period of time to yield worthy health and fitness benefits. Due to the discomfort associated with many of the HIIT protocols, long-term adherence is questionable, at least for some individuals (Foster et al., 2015). On the other hand, HIIT might sometimes be contraindicated, for example for diabetic patients whose blood glucose levels are poorly controlled, patients with retinopathy for whom HIIT may increase the risk of retinal detachment, patients with severe unstable heart failure or patients who have experienced a progressive decline in exercise tolerance or dyspnea at rest in the last 3-5 days (Taylor et al., 2019).

Knowing that incorporating fluctuations within low to moderate intensity training can increase the AnaMR significantly, and the potential for expenditure of higher AnaMR than what I found if the amplitudes were bigger, the exercise program in this study can be a pleasant program with considerably higher comfort and adherence than HIIT, and comparable with the traditional low intensity steady-state (LISS) program, especially for those who have newly started training, and with lower potential risks for the above-mentioned patients and may have greater efficacy than LISS. Additionally, this program might have some potential to get some of the health benefits of HIIT, even to a lesser extent, which could be investigated in future studies. Benefits such as improvement of both aerobic and anaerobic fitness (Ziemann et al., 2011), improvement of the enzymatic activities of the energetic pathways (Rodas et al., 2000), insulin sensitivity enhancement, especially in those with type 2 diabetes or metabolic syndrome (Jelleyman et al., 2015), stimulation of human growth hormone production as well as increased body's ability to burn fat, leading to a better body composition (Miguet et al., 2020; Wahl et al., 2013; Wahl et al., 2010), improvement of cardiovascular health by improving cholesterol levels, lowering blood pressure and the risk of heart disease (Kessler et al., 2012; Martland et al., 2020; Musa et al., 2009), reduction in body inflammation for patients with chronic disease (Ross et al., 2016), and etc.

Several studies have shown that during high intensity interval training, anaerobic power decreases with time as the anaerobic energy reserves get depleted (Almquist et al. 2021). However, performing moderate intensity interval training, even for longer periods of time could be an efficient strategy of relatively low strain for maintaining endurance performance.

4.1 Methodological considerations

Day-to-day variations can affect physical performance and training. Factors such as sleep, stress level, nutrition etc. In this study, by incorporating the 24-minute warmup in each of the two tests of the main protocol, the relationship between metabolic rate and work rate was reestablished each day. This strengthens my calculations for the ReqMR for the fluctuations. In addition, since the domain of the warmup intensities has fully covered the intensities that were used later during the fluctuations, another methodological strength of this study is that there was no need to extrapolate the ReqMR, which is what has been being done in other studies investigating AnaMR in high intensity interval training. Therefore, the numbers for AnaMR in this study are likely to be more robust.

The bulk of studies suggest that steady state can be reached after 2-3 minutes of exercise at constant intensity and that was the reason for the choice of 2-minute fluctuation bouts in this study. However, due to differences in the fitness level of the participants, in order to obtain trustworthy values for ReqMR, we allocated 8 minutes of constant intensity to each of the three intensity levels at warmup.

Due to difficulties in recruiting only well-trained individuals for me, a mixture of sedentary, trained and well-trained individuals was recruited. As we can observe in **Figure 10**, the correlation coefficents of MAP-AnaMR relationship, especially that of 0.917 for MR-pos.AnaMR relationship, show that those with higher MAP may have a higher AnaMR as expected, mainly because a greater MAP enduces higher power output during fluctuations, thus greater AnaMR. This could suggest that the AnaMR measured in this study could have been higher if the study was conducted with well-trained participants only

4.2 Conclusion

Increasing intensity fluctuation amplitudes during low to moderate intensity exercise, induce a greater amount of anaerobic contribution with similar perceived strain. This would potentially induce greater stimulus for training adaptation and could potentially make it superior to traditional low intensity steady state (LISS) exercise in terms of efficacy or HIIT in terms of adherence to the training program.

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