The Difference in Peripheral Chemosensitivity Between Trained and Untrained Individuals During Exercise

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

ABSTRACT

Hypercapnic chemosensitivity is the ventilatory response to increased partial pressure of CO_2 and is the result of central and peripheral chemosensor stimulation. The hypercapnic chemosensitivity of the peripheral chemoreceptors is potentially impacted by exercise, fitness, and sex, but this is not conclusive. This thesis sought to determine the difference of the peripheral chemoresponse to a transient hypercapnic test at rest and during exercise in individuals of various fitness. We hypothesized that 1. People that are trained will have a lower hypercapnic chemosensitivity compared to those that are untrained. 2. Individuals without mechanical limitations, with a lower $\dot{V}_E$ than a participant of similar $\dot{V}O_2$, will have a lower hypercapnic chemosensitivity. Twenty-nine healthy participants (n=16 males) participated in one test day involving transient hypercapnic chemosensitivity testing and a maximal exercise test. Chemosensitivity testing involved two breaths of 10% CO_2 repeated five times (30-45 sec between repeats) at rest and during the first two stages of exercise. Stage 1 of exercise started at 60W and 80W for females and males respectively, and both increased by 20W for stage 2. After stage 2, intensity increased in 20W increments every 1.5 minutes for both sexes. Overall, there was no significant difference between males and females, flow limited and nonflow limited, or high and low fitness groups during each stage of chemosensitivity testing ($p>0.05$). We did however see an effect of acute exercise, where there was a significant increase in the hypercapnic response during exercise compared to the response at rest ($p<0.05$). When the male and female participants were compared at an iso-$\dot{V}CO_2$, at 80W, the male participants had a significantly higher hypercapnic chemoresponse compared to females ($p<0.05$); however, this difference was absent when the response was scaled for BSA. These results suggest that fitness and sex do not influence hypercapnic chemosensitivity. These results also demonstrate the importance of taking

these measurements both at rest and during exercise. Finally, the results of this study suggest that the mechanisms that lead to different hypercapnic chemosensitivities are not affected by fitness and that differences in the hypercapnic chemosensitivity between individuals do not have implications for those that develop flow limitations.

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LIST OF ABBREVIATIONS

$AaDO_2$	Alveolar arterial oxygen difference
ANOVA	Analysis of variance
BSA	Body surface area
BTPS	Body Temperature Pressure Saturated
CO_2	Carbon Dioxide
EELV	End expiratory lung volume
EFL	Expiratory flow limitation
EIAH	Exercise induced arterial hypoxemia
EILV	End inspiratory lung volume
Fb	Breathing frequency
FVC	Forced vital capacity
HCVR	Hypercapnic ventilatory response
HVR	Hypoxic ventilatory response
MEFV	Maximal expiratory flow volume
O_2	Oxygen
$PaCO_2$	Arterial partial pressure of carbon dioxide
PAO_2	Alveolar partial pressure of oxygen
PaO_2	Arterial partial pressure of oxygen
PCO_2	Partial pressure of carbon dioxide
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
$P_{ET}O_2$	Partial pressure of end-tidal oxygen
PO_2	Partial pressure of oxygen
SpO_2	Oxyhemoglobin saturation
STPD	Standard Temperature Pressure Dry
$\dot{V}_A$	Alveolar ventilation
$\dot{V}CO_2$	Cardon dioxide production
$\dot{V}_E$	Expiratory ventilation
$\dot{V}_{Ecap}$	Ventilatory capacity
$\dot{V}_I$	Inspiratory ventilation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
V_T	Tidal volume
W_b	Work of breathing

1.0 LITERATURE REVIEW

The primary goal of the respiratory system is to maintain blood gas homeostasis through exchanging oxygen (O_2) and carbon dioxide (CO_2). This homeostasis is accomplished through matching the metabolic demand and alveolar ventilation ($\dot{V}_A$) in order to maintain a precise partial pressure of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$). The arterial partial pressure of oxygen (PaO_2) is held constant between 90-100 mmHg. While the arterial partial pressure of carbon dioxide ($PaCO_2$) is maintained at 40 mmHg through changing the $\dot{V}_A$ in proportion to carbon dioxide produced ($\dot{V}CO_2$) (77).

Alveolar ventilation equation: $\dot{V}_A = (\dot{V}CO_2 \times 0.863) / PaCO_2$

Alveolar ventilation maintains the alveolar partial pressure of oxygen (PAO_2) and thus a high-pressure gradient between the alveoli and the deoxygenated blood in the capillaries, allowing for oxygen to diffuse into the blood (77). Increasing $\dot{V}_A$ during exercise, typically below an oxygen uptake ($\dot{V}O_2$) of 50mL/kg/min, offsets the alveolar-to-arterial oxygen difference ($AaDO_2$) that naturally widens (69), allowing for PaO_2 to remain relatively unchanged. During high intensity exercise $\dot{V}_A$ will increase out of proportion to the $\dot{V}CO_2$, causing $PaCO_2$ to drop below resting levels, while the ventilatory system helps to buffer the metabolic acidosis.

The precise mechanism of increasing ventilation ($\dot{V}_E$) during exercise is still not completely understood, but one component is the control of breathing from the central and peripheral chemoreceptors. These receptors respond to a variety of chemical stimuli including the partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2). Maintaining adequate $\dot{V}_A$ can

also be limited by mechanical factors at or near maximal exercise intensities. The interaction between mechanical ventilatory limitations and control of breathing during exercise and how the mechanics and chemosensitivity could be limiting both the trained and untrained populations will be the focus of this thesis.

1.1 Hypercapnic Chemosensitivity

1.1.1 Central and Peripheral chemoreceptors

Changes in blood gas levels, such as CO_2, are sensed by the central and peripheral chemoreceptors. The central chemoreceptors are located in the medulla and control $\dot{V}_E$ in response to changes in the pH of the cerebral spinal fluid (77). As the concentration of CO_2 increases or decreases in the arterial blood, the cerebral spinal fluid pH will also change and stimulate changes in $\dot{V}_E$ accordingly. For example, if the CO_2 concentration is too high, $\dot{V}_E$ will increase to remove excess CO_2. While the cerebral spinal fluid has a higher PCO_2 than the arterial blood, allowing for normal metabolic CO_2 to be removed, when there is excess $PaCO_2$ from hypoventilation or a hypercapnic gas, there will be an increase the cerebral spinal fluid PCO_2. The diffusion time of the PCO_2 into the cerebral spinal fluid is approximately 60s (41), causing the central chemoreceptor response time to be lower than the peripheral chemoreceptors. The central chemoreceptors are also slower in response due to the decreased permeability to HCO3- and H+; these take much longer to equilibrate with changes in PCO_2, causing a delayed response to prolonged periods of hyper or hypocapnia (41).

The peripheral chemoreceptors, located in the carotid arteries and the aortic arch, respond to changes in partial pressure of both CO_2 and O_2 (PCO_2 and PO_2, respectively) as well as other stimuli such as pH and K+ (77). Increases in PCO_2 will cause a very rapid response in order to

change the $\dot{V}_E$ and return the PCO_2 back to homeostasis. The rapid response of the peripheral chemoreceptors is in part due to their proximity to the heart and direct exposure to arterial blood that was very recently in the lungs for gas exchange. Changes in the stimuli are sensed by glomus cells and release neurotransmitters that send an impulse to the central nervous system (24). The response to changes in PCO_2 is also dependent on the PO_2 level. Decreases in PO_2 will cause a greater change in $\dot{V}_E$ for the same PCO_2 level (53). Hypercapnic chemosensitivity is the magnitude of the response that individuals have to increased PCO_2. The stronger their sensitivity to the change in CO_2 the greater their ventilatory response.

1.1.2 Tests for chemosensitivity

There are several types of tests for determining the hypercapnic chemosensitivity, including steady state where the participant is held at a constant end-tidal CO_2 ($P_{ET}CO_2$) for several minutes, CO_2 rebreathing, and transient CO_2 tests.

Steady state hypercapnic tests are used to increase a participant's $P_{ET}CO_2$ by a specific amount, typically between 5-15 mmHg, and held there for several minutes. As there is minimal (if any) end-tidal to arterial CO_2 gradient, increasing the $P_{ET}CO_2$ also increases arterial CO_2. The greater $PaCO_2$ stimulates a greater $\dot{V}_E$ in participants, and the magnitude of the response of the respiratory system to the specific change in $P_{ET}CO_2$ can be measured to determine the hypercapnic chemosensitivity. To bring the participants to the desired steady state value the researcher will manually titrate in 100% CO_2 with the inspired air, this value is then monitored, and the amount of CO_2 given is adjusted as needed to maintain steady state (8, 79).

Alternatively, the participant might be given CO_2 at different flows such as 0.2 L/min, for several minutes, and then repeated with different flows to determine the impact of the increasing

CO_2 (13, 36). Both these methods stimulate both the central and peripheral chemoreceptors; thus, one is unable to determine the contribution of each chemoreceptor individually and the test provides a more general overview of the response CO_2 to a longer exposure compared to the transient test (described below).

Several studies have used the steady state method to test this hypercapnic response in a variety of settings. This test has been used both at rest and during exercise as well as with different types of participants ranging from sedentary to athletic lifestyles. Using the steady state method, the response to carbon dioxide increases as exercise intensity increases (13, 35, 76). The increase in response to carbon dioxide during exercise is suggested to be due to an increase in the amplitude of oscillations in blood gases as ventilation increases (6, 24, 80).

When comparing athletic to nonathletic participants there are very few studies, however one has found that the hypercapnic response is lower in the athletic participants (8). Steady state CO_2 has also been used to try to stimulate a higher level of $\dot{V}_E$ in athletes during exercise to determine if they have reached their maximum $\dot{V}_E$ (38). Overall, these results show that there are some differences during exercise and suggest that there are potential differences within athletes and their response.

The rebreathing test for chemosensitivity typically uses a rebreathing bag containing 7% CO_2 and 50% O_2 that the participant breathes typically from for around 4 minutes based on a standardized technique (61). This allows for their $\dot{V}_E$ to continually increase in response to the CO_2 and from there the participants hypercapnic ventilatory response (HCVR) can be determined. The 50% oxygen prevents the participant from becoming hypoxic during the test, which if not controlled for would introduce an additive stimulus that could alter the results. A caveat of preventing the participants from becoming hypoxic is that high amounts of oxygen can

also depress the respiratory response, which might reduce the response to CO_2. Animal models that have been used to look at the relationship of the peripheral chemoreceptors to the central chemoreceptors have shown that changes in activity in the peripheral receptors, from either hypercapnia, hypocapnia or hypoxia, has a hyperadditive effect when the central receptors are also exposed to the same stimulus (7, 67). If the response of the peripheral chemoreceptors was decreased then the central chemoreceptors had a subsequently decreased response, and vice versa for increased peripheral chemoreceptor activity.

Similar to the steady state CO_2 tests, the rebreathing test takes several minutes so both the central and peripheral chemoreceptors are being tested. Rebreathing tests, unlike steady state, allow for the response to increasing levels of CO_2 to be tested quickly as the concentration will continue increasing until the test is complete, but they do not allow for prolonged testing periods at each concentration of CO_2.

Results from studies that utilize the rebreathing method have shown conflicting results to the steady state method. Some studies that have compared the response at rest to the response during exercise have found there was no difference in the HCVR (23, 51). One study specifically used a 25W work rate which they believed might have been too low of a stimulus to cause a difference between rest and exercise (23). Miyamura et al. however, believed that the rebreathing test during exercise did not last long enough for a similar steady state response to occur, and perhaps that this short test did not allow for the equilibration that was required for the same level of stimulus as the steady state testing (51). The rebreathing method has been used to compare athletes to those that are sedentary and similar to the steady state tests they found that those with endurance training had a lower response to the hypercapnia (51, 52).

The last test for the hypercapnic response is a transient hypercapnic test. This method uses approximately 10-13% CO_2, instead of the 100% CO_2 used during the steady state tests, and the participant is given a few breaths of the gas mixture. The goal of this testing method is to target only the peripheral chemoreceptors by using only a few breaths, which will avoid the change in cerebral spinal fluid pH and prevent involvement of the central receptors. A single breath of 13% CO_2 has been shown to increase the alveolar CO_2 by 8-12 torr depending on the participant (49). This single breath of 13% of CO_2 was a strong enough stimulus to increase the $\dot{V}_E$ by 3-4L/min in these same participants (49).

The transient test is an excellent option to look at peripheral chemosensitivity because of the relative ease of administration and minimal risk of being exposed to only a few breaths of high CO_2. This type of test also allows for participants to be tested multiple times within one session because the washout time in between is very short. As a result, an average response is gathered allowing it to better account for within-day variability. Unfortunately, using this high concentration of CO_2 the participant can be aware when they are given a breath which may inadvertently change their response. There is also the potential for some CO_2 to be trapped within the anatomical deadspace, causing the $P_{ET}CO_2$ to be higher than the true $PaCO_2$, however the higher ventilations during exercise should sufficiently clear this deadspace to provide accurate $P_{ET}CO_2$.

Fewer studies around the transient testing have been completed, specifically during exercise. A study looking at the change from rest to exercise found an increase in the peripheral chemosensitivity during steady state exercise (59). Other studies have looked at the chemosensitivity in combination with other factors or stimuli such as hypoxia (25) or in those with exercise induced arterial hypoxemia (EIAH) (27).

1.1.3 Chemosensitivity and exercise

At the onset of exercise, $\dot{V}_E$ has an immediate increase followed by a slower rise to steady state for that specific work rate (24). During exercise there are a variety of stimuli that are believed to be responsible for exercise hyperpnea, one of which is the volume or pressure of CO_2. For example, studies conducted in animals have shown that the rate of CO_2 production is critical for $\dot{V}_E$ (57, 58). The HCVR during rest is believed to have a relationship to the ventilatory response to $\dot{V}CO_2$ at maximal exercise, those with a lower HCVR at rest also have a lower $\dot{V}_E/\dot{V}CO_2$ than those with a higher HCVR at rest (29, 47).

As previously mentioned, hypercapnic chemosensitivity shows some differences with exercise compared to rest. Some research has shown two key changes with chemosensitivity and exercise. The first is that the onset of exercise appears to increase the sensitivity to CO_2 (59, 76). A study comparing the hypercapnic response to a steady state test and a transient test found that both the steady state and transient had an increased response during exercise compared to rest (36). One theory for the increased response during exercise is that as $\dot{V}_E$ increases, the oscillations in blood gases also increase and this increased amplitude adds a further stimulus to the chemoreceptors (5, 24, 70, 80). Along with animal studies (70, 80), human research has shown that oscillations in arterial pH, caused by changes in $PaCO_2$, become larger during exercise (6). Though not all research agrees that oscillations increase during exercise (12), Cross et al. 1982 found both a decrease in amplitude of the oscillations and found that there was an increase in oscillation slope, suggesting that the slope along with the amplitude could be a contributing factor in the $\dot{V}_E$ increase.

The second key change is that with endurance training the overall chemosensitivity of an individual may decrease (8, 51). A multi-year study looked at the change in chemosensitivity of several participants over the course of a training period and detraining period (51). The results showed that the hypercapnic response, determined with the rebreathing method, was the lowest after the training period, and was the highest after the detraining period (51). However, not all research agrees with this. A study conducted in rats suggested that there was no change in the ventilatory response to CO_2 following aquatic endurance training (34).

Similar to the HCVR, the hypoxic ventilatory response (HVR) in regards to exercise also has a lot of conflicting data. A lower HVR has been shown to be related to a lower $\dot{V}_E$ during exercise which could be caused by a difference in the response of the chemoreceptors (47). The response has also shown to be lower in athletes compared to non-athletes (8). When participants were exposed to hypoxia during maximal exercise tests, the HVR was shown to be significantly correlated with the decrease in maximal oxygen uptake ($\dot{V}O_{2max}$), suggesting participants that had a smaller decrease in their $\dot{V}O_{2max}$ also had a higher sensitivity, further supporting a relationship between exercise and the HVR (54). Other research, however, has found that the HVR is not related to an individual's $\dot{V}O_{2max}$, suggesting that other factors have a stronger influence on HVR than exercise alone (66).

1.1.4 Chemosensitivity and genetics

A link has been proposed between genetics and the ventilatory response to hypercapnia (72). The role of genetics has been suggested in several diseases that are characterized by hypoventilation such as chronic obstructive pulmonary disease and sleep apnea (72). Research in twins has shown the HVR in identical twins to be similar, whereas nonidentical twins do not

have a similar response; however this study saw no relationship in the HCVR (11). Similarly, a study in adult female twins found a relationship to the peripheral response to O_2 suggesting a genetic component, but did not see a relationship to hypercapnia (3). There are several possible genetic abnormalities that could be leading to this altered ventilatory response. A study demonstrated that a gene called Phox2b is involved in the control of breathing, where knock-out studies in mice show no response to increased CO_2 (22). Goldberg et al. 2017 did a large genetic study looking at the hypoxic and hypercapnic responses and found a genetic link to the HCVR as well as a strong correlation between the HVR and gender but not the HCVR and gender (26). Lastly, a study examined the familial relationship between the HVR and the HCVR in athletes and found that the decreased HVR in runners had a strong familial link (64).

1.2 Respiratory Mechanics

One aspect of respiratory mechanics relevant to mechanical ventilatory constraints is each individual's maximal expiratory flow volume (MEFV) curve that outlines the highest flow an individual can generate at a given volume. These flows become pressure independent when approaching (5) or meeting the curve, so any further increase in pressure will only lead to compression of the airways (28). Another aspect of respiratory mechanics related to ventilatory constraints are the operational lung volumes. The total lung capacity is broken down into components such as the end expiratory lung volume (EELV) and end inspiratory lung volume (EILV). During exercise, EELV initially decreases from rest (or functional residual capacity) in order to allow for tidal volume expansion (31). As exercise intensity progresses, EELV may approach resting values again, but does not often meet or exceed functional residual capacity in

healthy young individuals (65). On the other hand, EILV continually increases throughout exercise and typically approaches ~85% of total lung capacity.

While at rest breathing is primarily accomplished by the diaphragm. Inspiratory work is done by the diaphragm while expiration is a passive relaxation of the diaphragm. At rest maintaining blood gas homeostasis requires relatively minimal effort and is not mechanically constrained in healthy individuals. At maximal exercise participants will generate high expiratory flow causing a decrease in $P_{ET}CO_2$ as $\dot{V}_E$ increases out of proportion of the production of CO_2 (24). The production of metabolic by-products (CO_2) and other stimuli will increase the hyperpnea as much as the pulmonary mechanics will allow. During exercise the inspiratory and expiratory work increase substantially from the greater $\dot{V}_E$ and so does the oxygen requirement of respiration (1). $\dot{V}_E$ is increased initially by tidal volume followed by an increase in breathing frequency in order to maximize $\dot{V}_A$ and maintain a lower deadspace ventilation (20). The goal while increasing $\dot{V}_E$ is to do this at the lowest energetic cost so that the increased oxygen is not being wasted on the active respiratory muscles (55).

As exercise intensity increases work of breathing (W_b), $\dot{V}_E$ will also increase through muscle recruitment in the attempt to compensate for the increased O_2 demand (19). The high flow rates of $\dot{V}_E$ cause increased turbulence in central airways, ultimately leading to further increases in the work of breathing. Those that reach the maximal expiratory flows and $\dot{V}_E$ allowed by their respiratory mechanics are said to be flow limited. Flow limitation is thought to occur in approximately 50% of people at maximal exercise (18).

Expiratory flow limitation (EFL) occurs when the increased intrapleural pressure during expiration does not lead to an increase in expiratory flow (20). When comparing the flow produced by the same amount of pressure at various lung volumes using isovolume pressure-

flow curves, we can see that at lower lung volumes the maximum expiratory flow will be reached at a lower pressure than higher lung volumes (77). When this maximum flow is reached no increase in pressure will change this. The flow has become pressure independent because the airways begin to be compressed by any increased pressure, further limiting any increase in flow (28).

The increase in pressure causing compression of the airways, narrowing the area for air to pass through, causes increased resistance (5). As the participant becomes flow limited their EELV will increase. Increased EELV requires the EILV to also increase towards the total lung capacity, bringing the tidal volume (V_T) of each breath towards its total lung capacity. As the V_T plateaus, $\dot{V}_E$ can only be increased through increasing breathing frequency and higher flows, which requires greater use of both the inspiratory and expiratory muscles causing a greater increase in the W_b (20). The increase in EELV also puts the respiratory muscles in a poor energetic orientation which results in a reduced efficiency and a further increase in work of breathing.

EFL is determined by the degree of overlap a participant's tidal volume breath has with their maximum expiratory flow volume loop (see methods section for further explanation). To test for the presence of EFL studies have often utilized heliox (~21% O_2: balance helium) to reduce turbulent flow, which allows for higher flows to be generated via an expansion of the MEFV curve (44). This method allows for flow limited participants to reach a higher $\dot{V}_E$ than previously achieved breathing room air (4, 17). Studies also compare the $\dot{V}_E$ achieved while breathing helium to the $\dot{V}_E$ achieved while breathing CO_2 which stimulates a higher $\dot{V}_E$ (4, 17). Participants that are able to achieve a higher $\dot{V}_E$ on heliox but unable to on CO_2 further support the development of a flow limitation at maximal exercise. Finally, to look for mechanical

constraints, trained participants were given CO_2 to breathe while exercising to determine if they were able to increase their $\dot{V}_E$ any further (38).

1.3 Exercise Induced Arterial Hypoxemia (EIAH)

Exercise induced arterial hypoxemia (EIAH) is a naturally occurring phenomenon where a decrease in arterial partial pressure of oxygen (PaO_2) causes the oxygen saturation (SpO_2) to decreases below resting levels during exercise. Typically, this phenomenon happens in highly trained individuals but has been seen in untrained females (29). There are several different mechanisms that lead to EIAH including: ventilation-perfusion mismatch, shunt, diffusion limitation, and relative alveolar hypoventilation (15). In most healthy individuals the difference between the alveolar partial pressure of oxygen (PAO_2) and the PaO_2, $AaDO_2$, changes minimally during exercise (69). Individuals are able to maintain a small $AaDO_2$ by increasing PAO_2 in order to maintain PaO_2 near rest. However, those that develop EIAH often developed an excessive widening of their $AaDO_2$, which reduces their PaO_2 causing a reduction in oxyhemoglobin saturation (15, 21).

Ventilation-perfusion mismatch occurs either when areas of the lung have more blood flow than $\dot{V}_A$ or when there is $\dot{V}_A$ to an area of the lung that has little to no blood flow, causing an increase in the $AaDO_2$ (33). As the PaO_2 decreases due to the increase in $AaDO_2$, the oxygen saturation will decrease causing the EIAH. A shunt occurs when blood bypasses the gas exchanging area of the lungs allowing for deoxygenated blood to be recirculated through the arterial system and reducing the volume of oxygenated blood (77).

Diffusion limitation reduces the amount of oxygen that is able to cross from the alveoli into the pulmonary capillaries, causing an increase in the $AaDO_2$ which ultimately lowers the

SpO_2 leading to EIAH (15). Diffusion limitation itself has a few different potential causes. An increase in transit time of the red blood cells could reduce the amount of arterial oxygen due to the limited time available to reach equilibrium between the alveoli and the blood cells. Alternatively, an individual with a high oxygen uptake ($\dot{V}O_2$) has a significantly higher volume of oxygen that they must move into the blood with the same pressure gradient of someone who has a lower $\dot{V}O_2$, increasing the likelihood that their saturation will decrease.

Relative alveolar hypoventilation causes a reduction in the PAO_2 which lowers the PaO_2, lowering the SpO_2 causing EIAH (15, 60). The hypoventilation could have a few different causes including a mechanical limitation, EFL, or a reduction in the drive to breathe from reduced chemosensitivity. Mechanical limitation has been confirmed in some cases of EIAH by using heliox (~21% O_2; balance helium) to reduce turbulence and increase $\dot{V}_E$. Studies that have utilized heliox have shown that compared to trials on room air, participants were able to increase their $\dot{V}_E$ and partially reverse the hypoxemia (14, 17). Alternatively, it has also been suggested that those that develop EIAH have a reduced ventilatory drive and sensitivity to CO_2. By having a lower sensitivity these individuals might develop EIAH simply because they do not have the same response to similar levels of CO_2 and as a result their $\dot{V}_E$ is not high enough to prevent them from desaturating. Studies that have looked at the HCVR in those that develop EIAH have found that at rest they do have a lower response to increased CO_2 (27, 30).

2.0 STUDY RATIONALE

The research around hypercapnic chemosensitivity, though often conflicting, has covered a wide range of topics to understand how it impacts the control of $\dot{V}_E$. One of the main areas that this research still has uncertainty is during exercise. As discussed previously, the majority of existing work has been at rest before and after exercise or using methods that do not differentiate between the response of the central and peripheral chemoreceptors. Thus, it is unknown if the response specifically of the peripheral chemoreceptors during exercise is different compared to rest. Another area that is under-researched is comparing the response of peripheral chemoreceptors in those with endurance training to those without. Based on the current gaps within this research field, the purpose of my thesis is to investigate differences between trained and untrained individuals and their response to a hypercapnic stimulus during exercise, and if there also differences between those that do and do not have mechanical limitations.

3.0 RESEARCH QUESTIONS AND HYPOTHESES

Research Questions:

1. Is peripheral chemosensitivity during exercise different between trained and untrained individuals?
2. Does hypercapnic chemosensitivity differ between individuals that develop expiratory flow limitations compared to those that do not?

Hypotheses:

1. People that are trained will have a lower hypercapnic chemosensitivity compared to those that are untrained.
2. Individuals without mechanical limitations, with a lower VE than a participant of similar $\dot{V}O_2$, will have a lower hypercapnic chemosensitivity.

4.0 METHODS

4.1 Ethics

The experimental procedures for this study were approved by the Office of Research Ethics at the University of Waterloo (ORE #42227). The research methods and protocols adhere to the recommendations outlined by the *Declaration of Helsinki* concerned with the use of human participants, except for registration in a database.

4.2 Participants

The sample size of this study was 29; 16 male and 13 female with a mix of trained and untrained participants for each sex. Sample size was calculated prior to testing using a two-tailed t-test between two independent groups using G*Power 3.1. A sample size of n=20, divided into two groups: 10 trained and 10 untrained, was determined based on an effect size of 2.02. The effect size was determined from a study that recorded a difference in the CO_2 response between athletic and sedentary participants (8). For this calculation the parameters were set as follows: α=0.05, power=0.8, and allocation ration=1.

Participants were healthy young adults with no conditions that could impact their response to exercise. Female participants were excluded if they were pregnant, nursing, or using a progesterone-only birth control, to minimize hormonal effects on their response. Progesterone-only birth controls were excluded from this study because of research suggesting that synthetic progesterone may alter the response to hypercapnia (68). The females were tested during the active phase of their birth control, or, if not using hormonal birth control, during primarily their self-reported low hormonal phase. Table 1 outlines the specific inclusion and exclusion criteria for the participants.

Table 1: Participant inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
• Age: 18-40 • Active: 2+ days per week or endurance trained	• Obesity • Cardiovascular disease • Respiratory disease • Diabetes • Cancer • Arthritis • Smoker • Females: pregnant, nursing, progesterone-only birth control

4.3 Experimental Overview

The testing procedures occurred on one day that included both hypercapnic chemosensitivity testing and a maximal exercise test performed on a cycle ergometer. After instrumentation, testing began with a 5 minute-rest period sitting in a chair followed by three 5-minute stages during which hypercapnic chemosensitivity was determined. The stages were as follows: (0) resting in the chair, (i) 60W or 80W for females and males respectively, and (ii) 80W and 100W for females and males respectively. Following the chemosensitivity testing the participants continued with a maximal incremental exercise test until exhaustion.

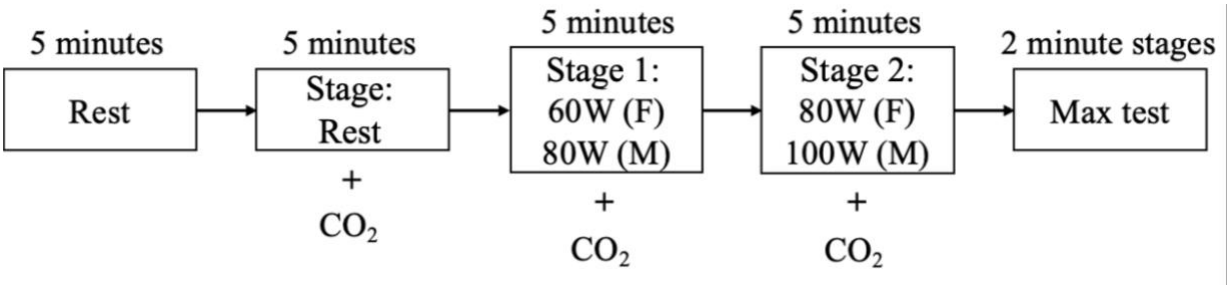

Figure 1: **Outline of test day protocol.**

4.4 Hypercapnic chemosensitivity testing

The chemosensitivity tests were completed by giving the participants 2 breaths of a 10% CO_2 (O_2~20.9%) gas mixture 5 separate times throughout the rest stage and the initial two 5-minute exercise stages. Prior to the first breath of CO_2 for each stage, roughly a minute and a half of data was collected to determine the pre-stimulus values for each participant. Between each set of hypercapnic breaths there was approximately 45 seconds to allow for $P_{ET}CO_2$ and $\dot{V}_I$ to return to pre-stimulus levels determined prior to the start of the hypercapnic trials (figure 2). Pre-stimulus $P_{ET}CO_2$ and $\dot{V}_I$ was confirmed visually before administering the next set of hypercapnic breaths. We elected to administer the participants two breaths of hypercapnic gas because of the space associated with the length of tubing between mouthpiece and the Douglas bag of CO_2. Specifically, large bore tubing connects the Douglas bag to the non-rebreathing valve the participant is breathing through. Bars on either side of the participant are used to suspend the large bore tubing to keep the weight off the participant and to reduce noise. The sample line was secured with tape to the tubing. Throughout the hypercapnic testing stages, end-tidal gases were measured at the mouth and during the last 20 seconds of each stage the sample line was connected to the mixing chamber to allow us to measure the $\dot{V}O_2$ for each of those stages.

We are testing the hypercapnic chemosensitivity at rest and at low intensity exercise to determine if there is a change in the ventilatory response during exercise. Low levels of exercise were chosen to minimize the influence of other factors known to impact ventilatory sensitivity such as pH, body temperature, etc., that are more prevalent as exercise intensity increases. The hypercapnic breaths were given using a 3-way valve to switch the participants between the hypercapnic mixture and room air. The valve made no audible noise and did not alert the participant to when they were given the breaths. Timing of the breaths was ‘marked’ using a

handheld piezoelectric pressure sensor interfaced with the data collection system, as shown in figure 3 in pink. The above allowed for the administration and time-marking without identifying noise or cues to alert the participants.

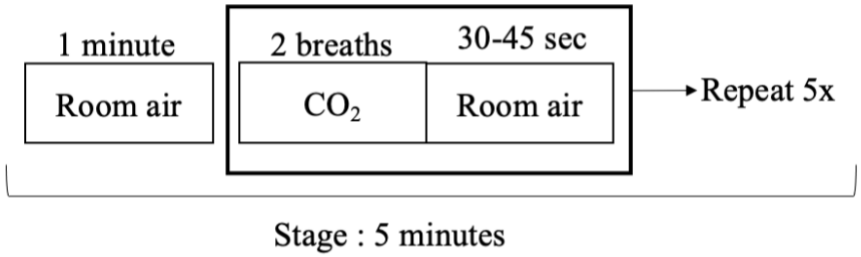

Figure 2: Outline of hypercapnic trials.

4.5 Maximal Exercise testing

The maximal exercise test was conducted on a cycle ergometer with 1.5-minute stages after the completion of stages 1 and 2, which are for hypercapnic chemosensitivity measures. The 1.5-minute stages allowed for the respiratory variables to stabilize before having the participant perform an inspiratory capacity maneuver in the last 30-seconds. The workload was increase by 20W every 1.5 minutes till the participant reached volitional fatigue. During the maximal exercise test the sample line was connected to the mixing chamber for the remainder of the exercise test for continuous measures of mixed expired gases. A second sample line connected to a separate CO_2 analyzer allowed for continuous measurement of $P_{ET}CO_2$ at the mouth.

4.6 Hypercapnic gas mixture

To make the 10% hypercapnic gas mixture, software interfaced with valves and a control box, known as 'Gas Mixer' (Pneumologix), allowed for the creation of different gas concentrations. Briefly, a pneumotach is calibrated using a 3-L syringe before being attached to a

small mixing chamber that is connected with tubing to tanks of pure nitrogen, oxygen, and carbon dioxide. Gas flow out of the tank is controlled by single-step solenoid valves. Opening each valve independently when connected to a pneumotach allows for the gas flow from each of these tanks to be determined. Using these values, the gas mixer program determines how long to leave each valve open to create the desired volume and concentration for the hypercapnic mixture. Concentrations were verified using calibrated gas analyzers before each trial and the Douglas bags were periodically 'fluffed' during experimentation to prevent gases from settling.

4.7 Maximal flow-volume curves and inspiratory capacity

Maximum flow-volume curves for each participant were determined by having them perform several forced vital capacity (FVC) maneuvers before and after exercise at varying efforts. Performing the FVC maneuvers after exercise accounts for any exercise-induced bronchodilation and the varying efforts accounts for thoracic gas compression (28). During both the pre and post exercise, the participant was asked to perform a few breaths at maximum effort and at varying efforts (e.g., 90%, 80% and 70%).

The inspiratory capacity was determined at rest and each exercise stage to determine lung volumes and EFL. During the last 30 seconds of each exercise stage the participant was asked to perform an inspiratory capacity maneuver with the prompting phrase "after a normal breath out take a quick and forceful breath all the way in". These were used to determine the lung volume where the tidal volume should be placed within the flow volume loop.

4.8 Data collection

The raw data was recorded at 200 Hz with a 16-channel analog-to-digital data acquisition system (PowerLab/16SP model ML 795; ADInstruments, Colorado Springs, CO). Two separate pneumotachometers (model 3813; Hans Rudolph) were used to measure flow, one for inspired and one for expired. The expired pneumotach was heated to 37°C while the inspired was kept at room temperature. Both pneumotachometers were calibrated independently using room air and a 3-L syringe. End-tidal gases were sampled at the mouth using a sample line connected to calibrated O_2 and CO_2 analyzers (AEI Technologies S-3-A/I and CD-3Am, respectively; Applied Electrochemistry, Bastrop, TX). The collected gas was dried using nafion tubing inside a sealed glass jar filled with Drierite to ensure the gases contain 0% humidity prior to entering the gas analyzers. The end-tidal gases were collected continuously during the first 3 stages while the participant was given the breaths of CO_2. During the last 20 seconds of each of these stages, after the chemosensitivity measures finished, the sample line was connected to the mixing chamber in order to collect mixed expired gases which allowed for the calculation of $\dot{V}O_2$ and $\dot{V}CO_2$ for each stage. After the first three stages were completed, the sample line was connected to the mixing chamber for the remainder of the maximum exercise test. Once the sample line was connected to the mixing chamber, a second sample line was connected at the mouth and to an identical separate CO_2 gas analyzer (AEI Technologies CD-3 Am) for continuous measurement of end-tidal CO_2 throughout the remainder of the test.

Heart rate was assessed with a polar heart rate monitor throughout the entire test. A Nonin pulse oximeter ear clip was used to measure SpO_2 throughout the study, as well as a separate pulse oximeter on the finger as a secondary measurement for SpO_2 to determine hypoxemia at the end of exercise. The highest value of SpO_2 was used.

4.9 Data analyses

We accounted for the gas sampling delay to ensure that both expired flow and the gas concentration are temporally aligned to provide accurate end-tidal values. Delays are accounted for by using the measured values provided during the calibration of the 'Gas Mixer' program and these are input into data collection software as a "shift" in order to move the expired CO_2 measurement back to correspond with the time point of the breath where that measurement occurred.

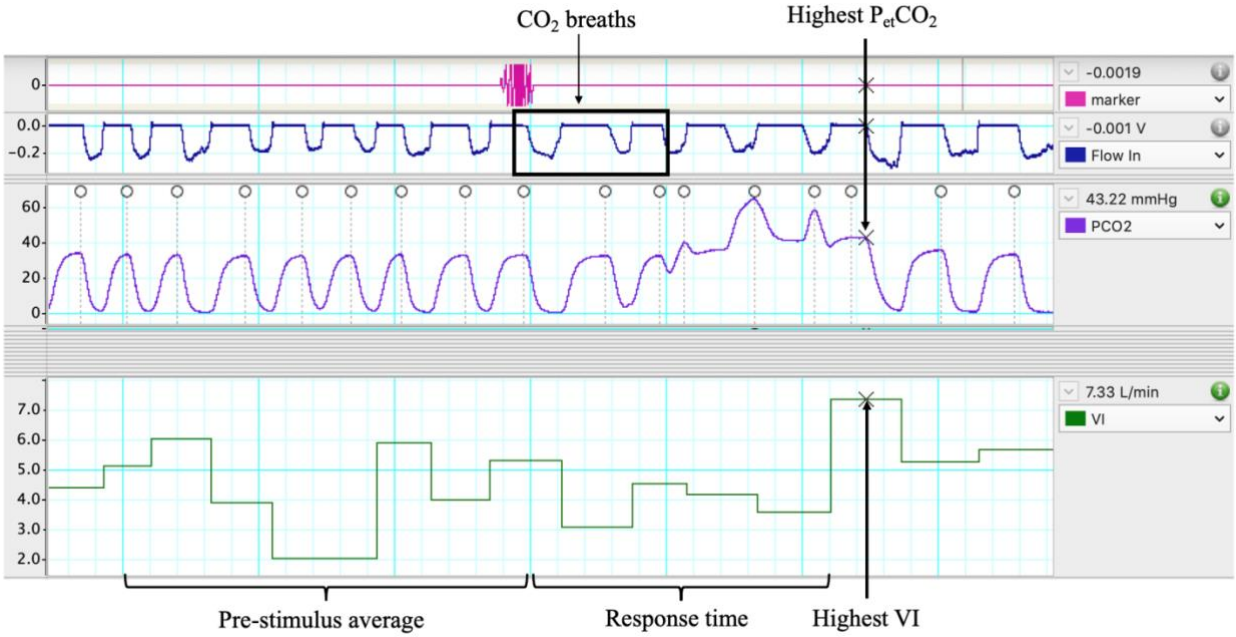

Figure 3: Data acquisitions software (LabChart) image showing one CO_2 response from a female participant during rest. The 15 seconds average used to determine the pre-stimulus is indicated at the bottom of the image. The marker is shown in pink, indicating the start of the two breaths of CO_2, indicated by the box. The response time is also indicated, measured from the start of the first breath to the highest $\dot{V}_I$ breath, shown in green. The peak $P_{ET}CO_2$, shown in purple, is determined visually as the highest pressure at the end of expiration. On the right side of the figure there are values provided for the PCO_2 and the $\dot{V}_I$, these values correspond with the placement of the "X" which are the values that would be used for analysis.

The HCVR was determined by the delta between the peak and the pre stimulus average for inspired ventilation ($\dot{V}_I$, shown in green in figure 3) and $P_{ET}CO_2$ (shown in purple in figure 3). The pre stimulus average was approximately 20 seconds immediately prior to the stimulus breath for both inspiratory ventilation ($\dot{V}_I$) and $P_{ET}CO_2$. The peak response was visually determined for $\dot{V}_I$ as the single highest breath within 30 seconds of the stimulus breaths, as shown in figure 3. The peak $P_{ET}CO_2$ was visually determined as the highest pressure at the end of expiration following the stimulus breaths. This peak value was specifically identified at the end of expiration, aligned by using the "shift", instead of taking the highest $P_{ET}CO_2$ value because the inspired CO_2 value became higher than the expired as the participant breathes the 10% CO_2. Therefore, to overcome this we used the visually identified highest $P_{ET}CO_2$ value at the end of expiration.

The ventilatory response to the hypercapnic gas mixture was calculated as the quotient of the delta $\dot{V}_I$ by the delta $P_{ET}CO_2$. The delta for $\dot{V}_I$ and $P_{ET}CO_2$ was determined as the difference between the peak response for each value and their corresponding pre-stimulus average. The response from all 5 stimulus breaths was averaged to provide the overall response. This was repeated for each stage of hypercapnic sensitivity testing.

Ventilatory capacity ($\dot{V}_{Ecap}$) was determined by first aligning a maximal exercise tidal volume loop within the MEFV based on the EELV. The volume of the tidal breath was divided into equal segments to determine the expiratory duration, the sum of each segment determined the expiratory time (Te) for the tidal breaths. The measured percent of the time spent expiring (%Te) was used with the Te to determine the total expiratory time to determine the maximal Fb. The calculated maximal Fb and the measured V_T product provided the $\dot{V}_{Ecap}$ for each participant.

EFL was determined by placing tidal breaths within the MEFV curves and calculating the percentage of overlap. The tidal breaths are a composite average of 8-10 breaths prior to the IC maneuver during the last few stages of exercise (18). The tidal breaths are placed in the MEFV curves according to the EELV. The EELV was determined by subtracting the inspiratory capacity from the FVC. Following the placement of the tidal breaths within the MEFV, the EFL was calculated by dividing the tidal breath volume overlapped with the MEFV curve by the total tidal breath volume (18). If this percentage was 5% or less the participants are considered not flow limited (18).

The measured flow was numerically integrated to determine volume, allowing for the calculation of tidal volume, and to determine breathing frequency. $\dot{V}_I$ was calculated as the product of the tidal volume and breathing frequency, which was then converted into $\dot{V}_E$ using the Haldane transformation, both are expressed in BTPS. $\dot{V}O_2$ was determined as the difference between the percent inspired and expired oxygen using $\dot{V}_I$ and $\dot{V}_E$. Metabolic volumes ($\dot{V}O_2$ and $\dot{V}CO_2$) are expressed in STPD

$\dot{V}O_{2max}$ was determined by taking a 30 second average during the final stage of their exercise test. This was used to categorize the trained and untrained participants. Max heart rate, end-exercise $P_{ET}CO_2$, and end exercise $\dot{V}_E$ was taken as an average of the last 30 seconds of the final stage completed by the participant.

Participants were grouped into several categories. To determine the effect of fitness, male and female participants were split into the high and low fitness groups independently (*i.e.,* high fitness males and low fitness males) based on the group mean and then all the high participants from each sex and the low participants from each sex were regrouped. Males typically have a higher $\dot{V}O_{2max}$, so this allowed for an equal number of males and females in each group to

prevent one sex from dominating and thus preventing any fitness effect from being contaminated by a sex bias. The hypercapnic response for males and females was also corrected for body surface area (BSA) to minimize differences in the chemoresponse that could be affected by body size (32, 62).

$$BSA=\sqrt{\frac{Height\ x\ Weight}{3600}}$$

Participants were also grouped into flow limited and nonflow limited groups, as well as those that used a high or low percentage of their $\dot{V}_{Ecap}$ to determine the effect of mechanical limitations on hypercapnic chemosensitivity. Sex differences were analyzed by comparing all male participants to all female participants. Finally, participants were divided into high and low chemoresponse at rest and exercise based on the full group mean for rest and exercise. EIAH was determined as a greater than 3% decrease in the SpO_2 from their baseline measurement (9). Time to response was determined as the time from when the first breath of CO_2 is given to when the peak $\dot{V}_I$ response is identified.

4.10 Statistical analyses

Statistical analyses were conducted in SigmaPlot 14.0. Descriptive variable, maximal exercise variables, and pre $P_{ET}CO_2$ and $\dot{V}_I$ values were analyzed with independent samples t-tests to compare males vs females, high vs low fitness, and flow limited vs nonflow limited. A paired t-test was used to compare the delta $P_{ET}CO_2$ and $\dot{V}_I$ at rest to the $P_{ET}CO_2$ and $\dot{V}_I$ during exercise for all participants. Normality of the data was tested with a Shapiro-Wilk test and any data pairs that were not normally distributed were analyzed with the non-parametric Mann-Whitney Rank Sum Test. A Fisher exact test was used to determine if there was a difference in prevalence of EFL between males and females. The coefficient of variation for the chemoresponse, delta

$P_{ET}CO_2$, and delta $\dot{V}_I$ was determined as the quotient of the standard deviation and mean for each participant during each testing stage.

Analysis of the chemosensitivity between high and low fitness was conducted by using a 2 group [high fitness, low fitness] vs 2 stage [rest, combined exercise] split-plot ANOVA. Analysis for the chemosensitivity between flow limited and non-flow limited group as well as males vs females groups were conducted with the same 2x2 ANOVA. To analyze the effect of exercise a one-way repeated measures ANOVA (rest vs average exercise) was used. A Tukey post-hoc test was used to analyze any significant results from the ANOVA. The relationship between aerobic fitness and hypercapnic chemosensitivity was analyzed with a Pearson product moment correlation. Significance for all statistical tests was set at $p<0.05$. All values are reported as a mean and standard deviation.

5.0 RESULTS

A total of 29 participants (n=16 males, n=13 females) completed this study. The average age of all participants was 24±3 years old, with an average BMI of 24.7±3.7kg/m^2, see table 2 for demographics. There was a significant difference in the height, mass, and BMI of the male and female participants, with males being taller, heavier and a greater BMI ($p<0.05$).

Table 2: Participant Demographics.

Characteristics	High Fitness (n=14)	Low Fitness (n=15)	Males (n=16)	Females (n=13)	Flow limited (n=15)	Nonflow limited (n=14)
Age (years)	23±3	25±3	25±3	24±3	25±3	24±3
Height (cm)	160.8±7.8	169.7±8.9	176.4 ± 6	164.3±5.4*	170.9±7.6	170.9±9.3
Mass (kg)	66.5±15.4	74.7±16.5	82.9 ± 13.3	60.9 ± 8.7*	75.1±17.7	71.1±14.2
BMI (kg/m^2)	22.1±3.3	25.7±3.9	26.5 ± 3.3	22.6 ± 3.0*	25.4±4.2	24.1±3.1
Hormonal birth control	4 participants	3 participants	NA	7 participants	4 participants	3 participants

* Indicates significantly different to males, $p<0.05$.

Overall, the pre- hypercapnic chemosensitivity test's values for $P_{ET}CO_2$ and $\dot{V}_I$ were consistent between participants (table 3). For each stage individually, $P_{ET}CO_2$ and $\dot{V}_I$ typically had a SD of 5 mmHg or L/min between groups, showing the variance was relatively equal between groups, see table 3 for more specific values. The delta response for $P_{ET}CO_2$ and $\dot{V}_I$ after 10% CO_2 showed a significant increase from rest to exercise for all participants; rest and average exercise $P_{ET}CO_2$ were 9.9±2.8mmHg and 18.9±3.0mmHg respectively ($p<0.05$), with rest and average exercise $\dot{V}_I$ delta's 6.6±4.1L/min and 17.0±6.7L/min respectively ($p<0.05$). However, the response within participants had a considerable amount of variation in the chemoresponse. This variation is highlighted in figure 4, where 5 CO_2 breaths and responses are shown for one participant cycling at 80W. When each group was compared for the coefficient of variation for

the chemoresponse, the delta $\dot{V}_I$ and the delta $P_{ET}CO_2$ showed significant differences between rest and the exercise stages within a group (e.g Low fitness rest vs low fitness stage 1), but no differences between groups, (table 4). Finally, the time to the peak ventilatory response was determined for each group at rest, stage 1, and stage 2, and a significant decrease was shown between the rest and exercise, (table 5)

Table 3: The average pre-CO_2 stimulus $P_{ET}CO_2$ and $\dot{V}_I$ and delta $P_{ET}CO_2$ and $\dot{V}_I$ for rest, stage 1, and stage 2, as well as the $\dot{V}O_2$ and $\dot{V}CO_2$ for stage 1 and stage 2, for each group.

	High Fitness	Low Fitness	Males	Females	Flow Limited	Nonflow Limited
Rest Pre-$P_{ET}CO_2$ (mmHg)	38.4±10.5	39.1±3.6	40.3±3.0	36.9±3.1*	39.6±3.2	37.9±3.7
Rest Pre-$\dot{V}_I$ (L/min)	11.1±3.8	9.8±2.2	10.9±2.6	9.8±2.2	10.8±2.7	9.9±2.2
Rest Pre-$P_{ET}O_2$ (mmHg)	111.9±4.2	110.9±4.2	109.9±4.6	113.2±2.9*	110.7±3.7	112.1±4.7
Rest Delta $P_{ET}CO_2$ (mmHg)	10.2±2.9	9.6±2.8	9.6±2.6	10.3±3.2	9.6±2.7	10.2±3.0
Rest Delta $\dot{V}_I$ (L/min)	7.9±5.1	5.4±2.4	7.2±5.0	5.8±2.7	6.8±4.2	6.4±4.2
Stage 1 Pre-$P_{ET}CO_2$ (mmHg)	41.9±11.3	42.8±4.5	43.6±2.7	40.8±4.7	43.0±3.2	41.6±4.6
Stage 1 Pre-$\dot{V}_I$ (L/min)	30.8±9.9	28.5±4.9	32.0±6.1	26.6±3.4*	29.3±6.8	29.9±4.3
Stage 1 Pre-$P_{ET}O_2$ (mmHg)	109.7±4.1	111.9±6.6	108.7±5.5	113.5±4.5*	109.9±5.0	111.9±6.1
Stage 1 Delta $P_{ET}CO_2$ (mmHg)	17.6±3.9	17.4±2.1	16.4±2.8	18.9±2.9*	16.4±3.1	18.6±2.6
Stage 1 Delta $\dot{V}_I$ (L/min)	16.8±8.1	16.4±5.8	17.5±7.4	15.5±6.3	15.7±5.7	17.6±8.1
Stage 1 $\dot{V}O_2$ (L/min)	1.23±0.28	1.24±0.32	1.40±0.27	1.02±0.18*	1.23±0.30	1.25±0.30
Stage 1 $\dot{V}CO_2$ (L/min)	1.32±0.23	1.35±0.26	1.48±0.20	1.16±0.15*	1.32±0.24	1.36±0.24
Stage 2 Pre-$P_{ET}CO_2$ (mmHg)	41.8±11.3	40.9±5.6	43.2±2.6	39.2±5.5	42.1±3.4	40.5±5.5
Stage 2 Pre-$\dot{V}_I$ (L/min)	42.1±12.4	44.6±6.0	45.2±4.9	41.2±6.9	42.8±6.2	44.0±6.2
Stage 2 Pre-$P_{ET}O_2$ (mmHg)	114.9±4.2	118.1±7.0	113.4±4.3	120.5±5.3*	115.4±5.3	117.8±6.5
Stage 2 Delta $P_{ET}CO_2$ (mmHg)	20.6±3.6	20.1±3.4	19.1±2.9	21.8±3.5*	19.4±3.4	21.3±3.3
Stage 2 Delta $\dot{V}_I$ (L/min)	17.6±7.9	17.2±7.1	18.5±7.6	16.1±7.2	16.9±6.9	17.9±8.1
Stage 2 $\dot{V}O_2$ (L/min)	1.64±0.27	1.63±0.3	1.82±0.21	1.41±0.17*	1.64±0.28	1.63±0.30
Stage 2 $\dot{V}CO_2$ (L/min)	1.64±0.24	1.68±0.23	1.81±0.18	1.47±0.12*	1.63±0.30	1.66±0.23

Abbreviations: $P_{ET}CO_2$: end-tidal CO_2, $\dot{V}_I$: Inspiratory ventilation, $\dot{V}O_2$: oxygen uptake, $\dot{V}CO_2$: Carbon dioxide production. $P_{ET}O_2$: end-tidal O_2. * Indicates significantly different from the group counterpart, $p<0.05$.

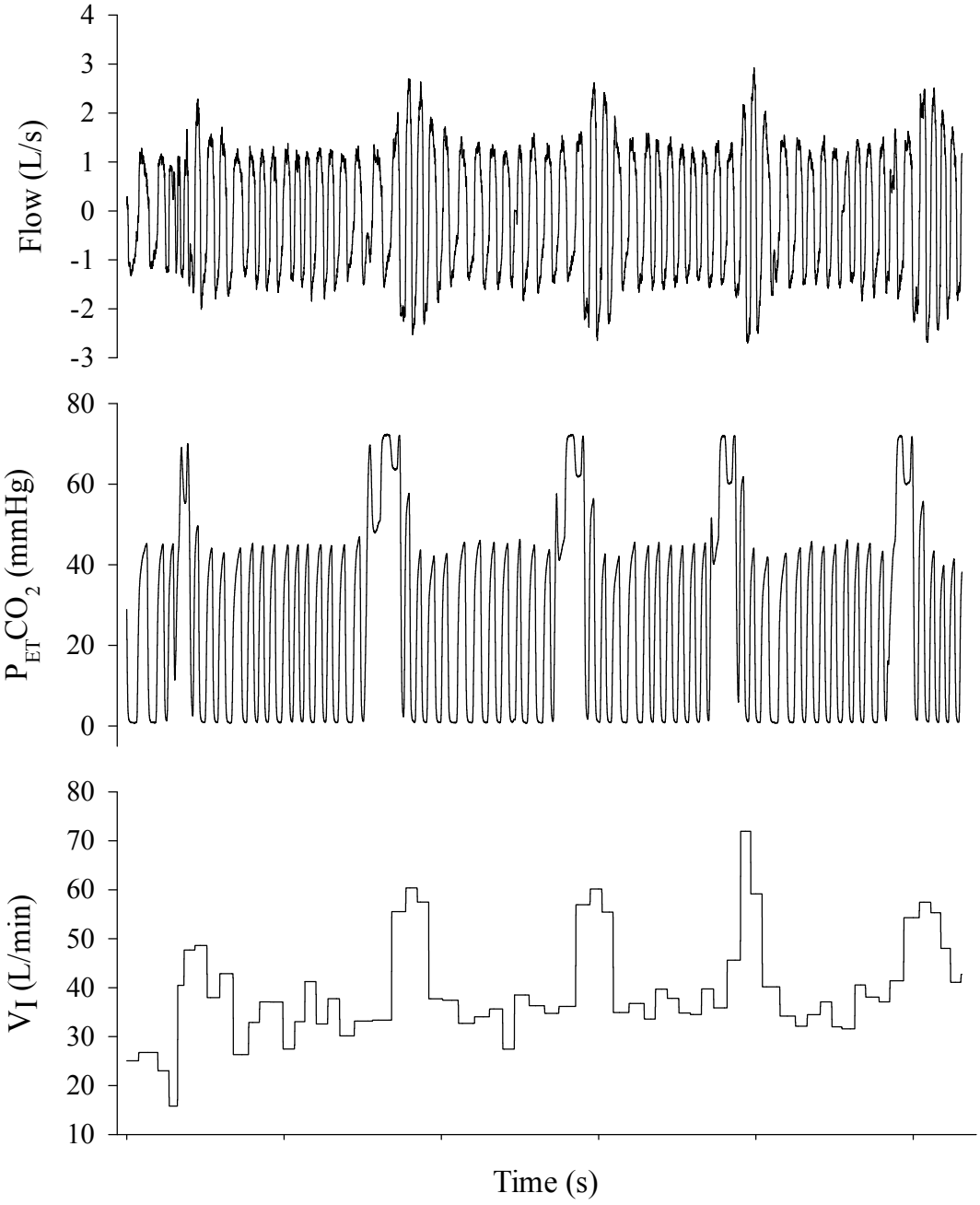

Figure 4: A raw tracing showing flow, $P_{ET}CO_2$, and $\dot{V}_I$ for one male participant cycling at 80W. The tracing shows all 5 sets of 2 breaths of CO_2 that the participant was given and the ventilatory response for each stimulus, highlighting the variability of the hypercapnic chemoresponse within an individual.

Table 4: The coefficient of variation for Δ $P_{ET}CO_2$, Δ $\dot{V}_I$, and the chemoresponse for each group at each stage. Also included is the coefficient of variation for all participants together.

Coefficient of Variation	High Fitness	Low Fitness	Males	Females	Flow Limited	Nonflow Limited	Full group
Rest Δ $P_{ET}CO_2$	15.9±4.9†	12.9±7.4	12.6±5.0†	16.4±7.6†	12.2±4.5	16.6±7.9*†	14.4±6.3†
Rest Δ $\dot{V}_I$	48.5±20.3*†	40.6±19.4†	45.4±20.8*†	43.3±18.8*†	45.5±23.5*†	43.4±15.9*†	44.5±19.2*†
Rest chemoresponse	54.2±22.2*†	48.1±24.5*†	49.5±23.8*†	52.9±23.4*†	48.5±27.2†	53.8±20.3*†	51.0±23.3*†
Stage 1 Δ $P_{ET}CO_2$	10.8±7.1	11.9±5.4	11.1±5.1‡	11.7±7.3	12.3±6.1	10.5±6.5	11.4±6.3‡
Stage 1 Δ $\dot{V}_I$	27.3±15.2	29.7±12.4	30.8±15.6	25.7±10.9	29.5±13.2	27.5±14.9	28.5±13.6
Stage 1 chemoresponse	30.0±17.1	35.9±13.2	33.9±15.0	32.1±15.3	35.8±11.6	30.0±18.2	33.1±14.8
Stage 2 Δ $P_{ET}CO_2$	7.9±4.5	8.8±3.7	7.6±4.2	9.3±3.9	9.3±4.3	7.4±4.2	8.3±4.1
Stage 2 Δ $\dot{V}_I$	29.3±14.5	29.4±14.3	26.8±10.7	32.5±17.6	29.7±13.1	29.0±16.1	29.4±14.4
Stage 2 chemoresponse	31.0±14.5	31.8±15.6	28.9±12.2	34.6±17.8	32.3±13.1	30.5±17.4	31.4±15.0

Abbreviations: $\dot{V}_I$: Inspiratory ventilation, $P_{ET}CO_2$: End-tidal CO_2. * Indicates a significant difference between rest and stage 1 within a group (e.g. resting Δ $\dot{V}_I$ and Stage 1 Δ $\dot{V}_I$ for the high fitness group) $p<0.05$. † Indicates a significant difference between rest and stage 2 within a group, $p<0.05$. ‡ Indicates a significant difference between stage 1 and stage 2 within a group $p<0.05$.

Table 5: Time to peak $\dot{V}_I$ from the onset of the CO_2 stimulus.

	High Fitness	Low Fitness	Males	Females	Flow Limited	Non-Flow Limited	Full Group
Rest Time to Peak $\dot{V}_I$ (s)	16.5±3.8*†	17.9±3.2*†	17.2±3.1*†	17.2±4.1*†	16.4±4.0*†	18.1±2.8*†	17.2±3.5*†
Stage 1 Time to Peak $\dot{V}_I$ (s)	9.1±2.3	9.2±3.1	9.9±3.1	8.2±1.7	9.1±2.3	9.2±3.2	9.2±2.7
Stage 2 Time to Peak $\dot{V}_I$ (s)	8.5±2.6	8.3±3.2	9.1±3.2	7.6±2.2	8.6±3.1	8.2±2.7	8.4±2.9

Abbreviations: $\dot{V}_I$: Inspiratory ventilation. * Indicates a significant difference from rest to stage 1 $p<0.05$, † indicates a significant different from rest to stage 2, $p<0.05$.

5.1 High and low fitness

The maximal exercise results are summarized in table 6. While the groups' high fitness and low fitness based on $\dot{V}O_2max$ were statistically different in terms of fitness, there were no

difference in RER, max heart rate, and metabolic equivalents (table 6), suggesting both groups reached a $\dot{V}O_{2max}$. The pre-CO_2 stimulus $P_{ET}CO_2$ and $\dot{V}_I$ at each stage were not different between the fitness groups ($p>0.05$), (table 3). When comparing the hypercapnic chemosensitivity for the high and low fitness groups, there were no significant differences in the response between the two groups at any stage ($p>0.05$) (figure 5). However, the was a main effect for intensity whereby the average response during exercise was significantly greater than during rest (means 0.76±0.53 vs. 0.94±0.42 L/mmHg*min for rest and exercise respectively, ($p=0.026$; figure 6).

Within the low fitness group there was also a significant difference between the chemoresponse of the participants at rest and the average exercise response (rest mean 0.63±0.39 L/mmHg*min, average exercise mean 0.92±0.32 L/mmHg*min, $p=0.013$). There was no relationship between fitness and the average exercise chemosensitivity ($R=0.159$, $p=0.409$) (figure 7). There was no relationship between fitness and the resting chemosensitivity ($R=0.250$, $p=0.109$) (figure 8). Finally, the chemoresponse of the high and low fitness groups were compared at the percentage of their $\dot{V}O_{2max}$ the exercise chemosensitivity trials, see figure 9. At an iso- relative $\dot{V}O_2$, stage 1 for the low fitness group and stage 2 for the high fitness group, there was no significant difference between the chemoresponse ($p>0.05$).

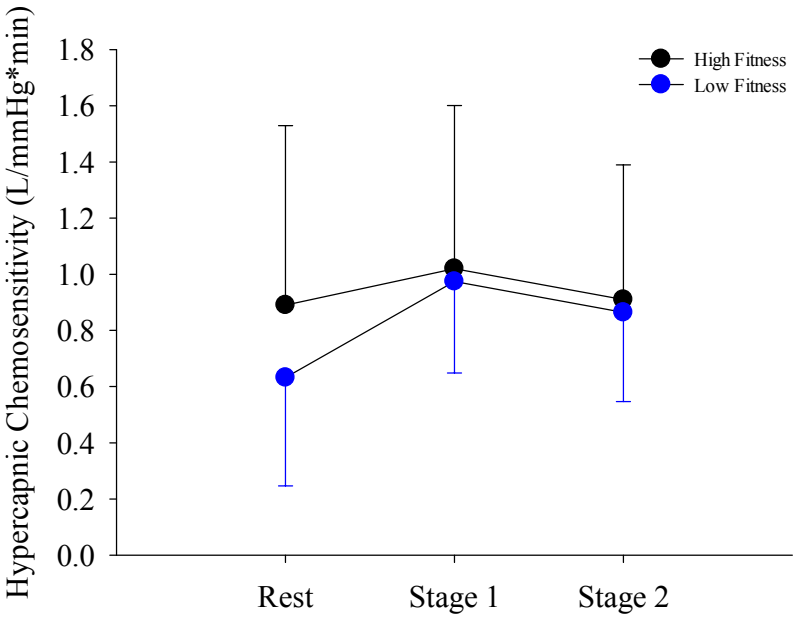

Figure 5: A comparison of the hypercapnic chemoresponse of the high fitness group to the low fitness group at each stage. There is no significant different between the two groups at any stage, p>0.05.

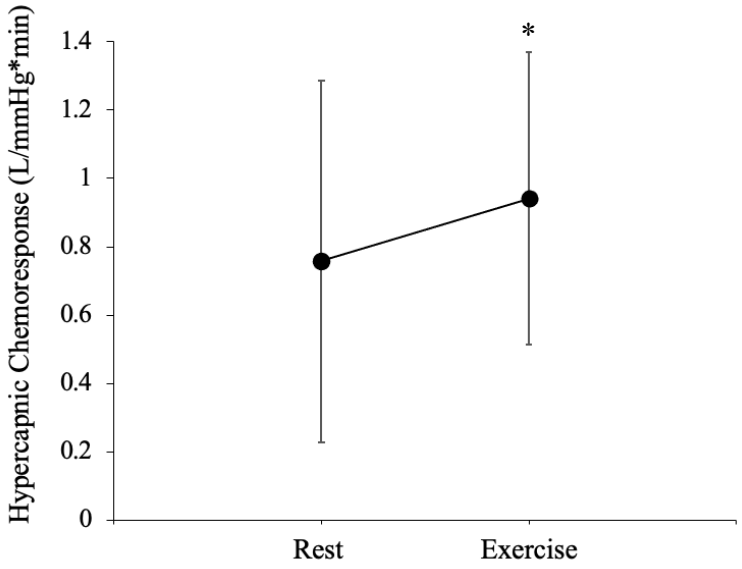

Figure 6: A comparison between the hypercapnic chemoresponse of all participants at rest and their average exercise response (stage 1 and 2). There is a significant increase in the chemoresponse during exercise compared to rest, p<0.05.

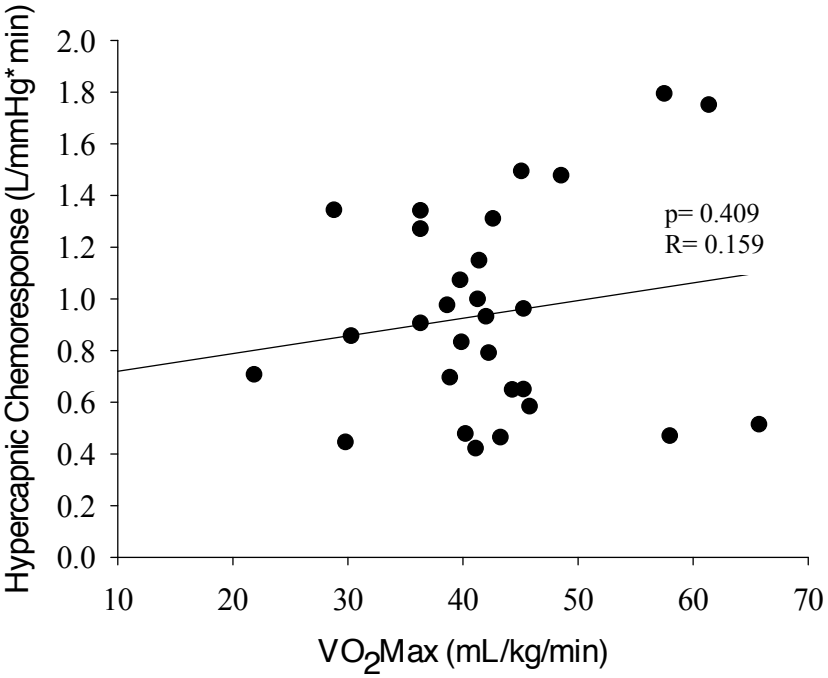

Figure 7: A regression showing the relationship between the hypercapnic chemosensitivity and $\dot{V}O_{2max}$ for the exercise response, average of stage 1 and 2, for each participant. There is no significant relationship as indicated by the R and p values.

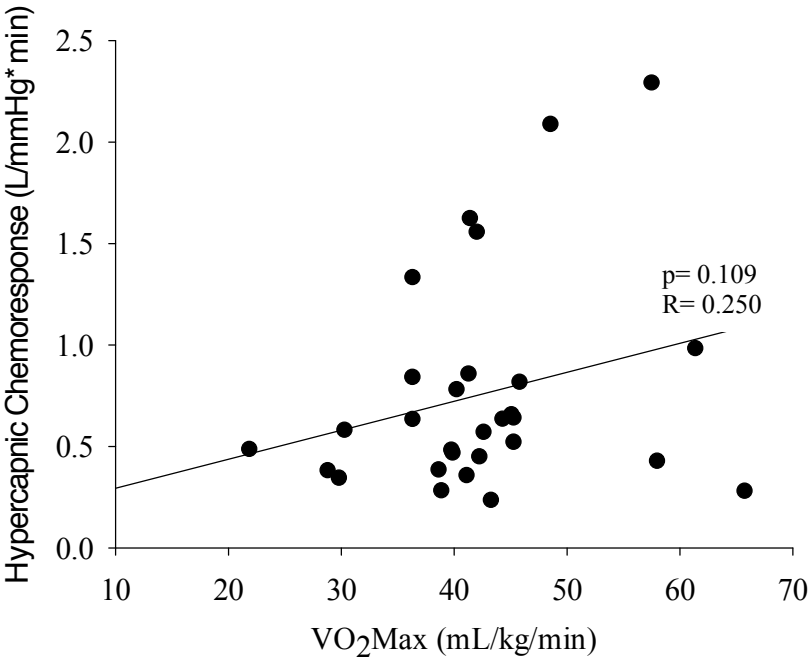

Figure 8: A regression showing the relationship between the hypercapnic chemosensitivity and $\dot{V}O_{2max}$ for the rest response, for each participant. There is no significant relationship as indicated by the R and p values.

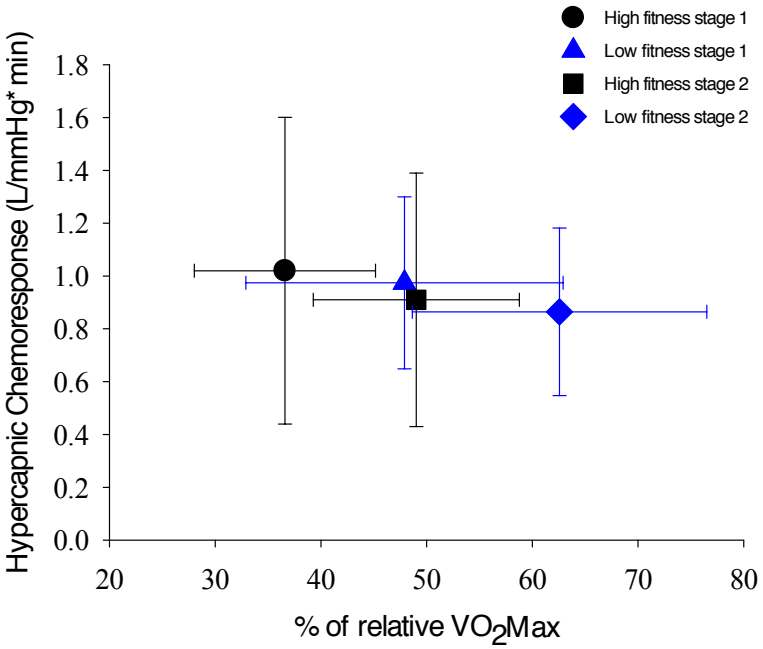

Figure 9: The hypercapnic chemoresponse for the high and low fitness groups shown at the average percentage of $\dot{V}O_{2max}$ during stage 1 exercise and stage 2 exercise. There is no significant difference between the hypercapnic response. p>0.05.

Table 6: Maximal exercise results for each group

Parameter	High fitness	Low fitness	Males	Females	Flow limited	Non flow limited
Participants n	14	15	16	13	15	14
Work rate (W)	264±75	204±61*	278±61	177±42*	244±74	222±73
HR (BPM)	187±8	189±9	190±6	185±10	188±9	187±8
$\dot{V}O_2$ max (ml/kg/min)	49±8	36±6*	46±10	38±7	45±11	41±8
$\dot{V}O_2$ (L/min)	3.5±1.0	2.7±0.8*	3.8±0.8	2.3±0.5*	3.2±1.0	3.0±0.9
$\dot{V}CO_2$ (L/min)	4.0±1.2	3.2±0.8*	4.3±0.9	2.7±0.5*	3.8±1.1	3.4±1.0
V_T (L/min)	2.5±0.7	2.1±0.4	2.8±0.4	1.8±0.3*	2.5±0.6	2.2±0.6
Fb (breaths/min)	51.1±5.4	50.0±8.7	50.4±7.0	50.6±7.5	49.4±7.3	50.9±7.6
$P_{ET}CO_2$ (mmHg)	30.3±5.2	25.6±4.7*	29.0±5.4	27.0±5.5	28.3±4.6	27.8±6.6
$\dot{V}_E$ (L/min)	145±44	120±28	157±33	102±17*	138±41	126±34
RER	1.14±0.06	1.17±0.08	1.15±0.05	1.17±0.08	1.17±0.08	1.14±0.04
$\dot{V}_E/\dot{V}CO_2$	36±4	38±4	36±2	38±5	37±3	37±4
$\dot{V}_E/\dot{V}O_2$	41±4	45±6	41±4	44±6	43±5	43±5
SpO_2 (%)	98.2±1.6	98.2±2.1	98.5±1.3	97.9±2.4	98.2±2.3	98.4±1.2

Abbreviations: HR: Heart rate, $\dot{V}O_{2max}$: Maximal oxygen uptake, $\dot{V}O_2$: Oxygen uptake, $\dot{V}CO_2$: Carbon dioxide production, V_T: Tidal Volume, Fb: Breathing frequency, $P_{ET}CO_2$: End-tidal CO_2, $\dot{V}_E$: Ventilation, RER: Respiratory exchange ratio, SpO_2: Oxygen saturation. * Indicates significantly different from their group counterpart (high fitness and low fitness, male and female, flow limited and nonflow limited), $p<0.05$.

5.2 Male and female

Males had a significantly greater $\dot{V}O_{2max}$, 46±10 vs. 38±7 ml/kg/min ($p<0.05$), and peak work rate, 279±61 vs 177±42 W, ($p<0.05$) compared to females. Prior to the hypercapnic stimulus at rest there was a significant difference in the $P_{ET}CO_2$ between males and females, as well as the $\dot{V}_I$ at stage 1 was significantly lower in females, (table 3). Despite these differences, the hypercapnic chemoresponse was not significantly different between the sexes at each stage ($p>0.05$; figure 10 and 11). The males and females were also compared at an iso-$\dot{V}CO_2$, at 80W, the males had a higher chemoresponse than females (means 1.10±0.5 vs 0.75±0.3 L/mmHg∗min respectively, $p=0.03$; figure 12). However, when the hypercapnic response for males and females was corrected for BSA, there was no difference observed in the response between the sexes at an iso-$\dot{V}CO_2$ (figure 13).

We also examined the percentage of $\dot{V}O_{2max}$ that the participants were using at each exercise stage. As shown in figure 14, the females were using a higher percentage of their $\dot{V}O_{2max}$ compared to the males at the same stage, but it was only significantly different for stage 2 (females 63±14%, males 50±10%, $p=0.005$). When compared at a similar %$\dot{V}O_{2max}$, stage 1 for females and stage 2 for males, there was no difference in the chemoresponse ($p>0.05$; figure 14). Finally, the average percent increase in the Fb and V_T for the ventilatory response at rest, stage 1, and stage 2 were analyzed for males and females (table 7). There was no significant difference in the response between the sexes there was a significant difference between the percent of Fb and V_T used within each sex ($p<0.05$), except for males at rest and females at stage 2 ($p>0.05$).

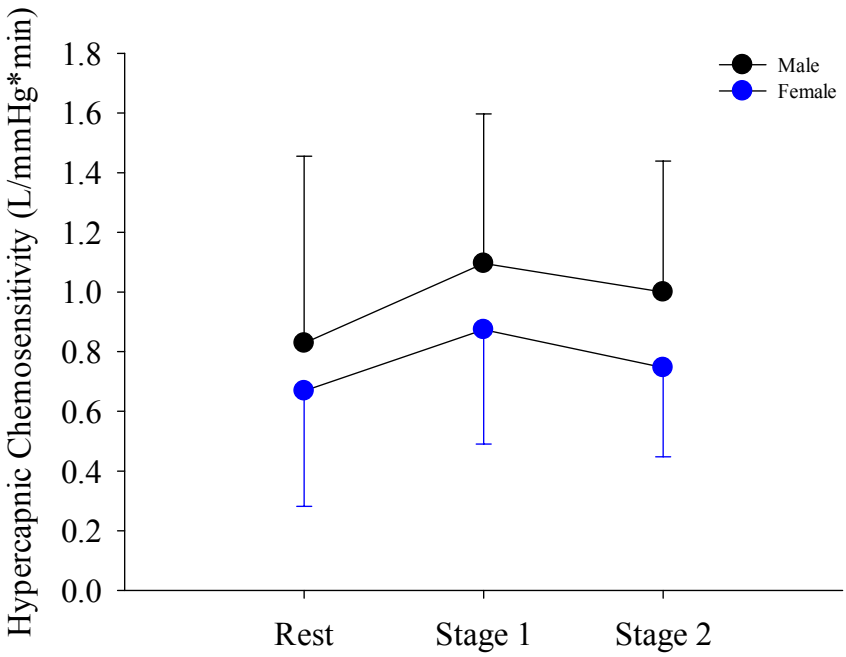

Figure 10: A comparison of the hypercapnic chemoresponse of the males and females at each stage. There is no significant different between the two groups at any stage, p>0.05.

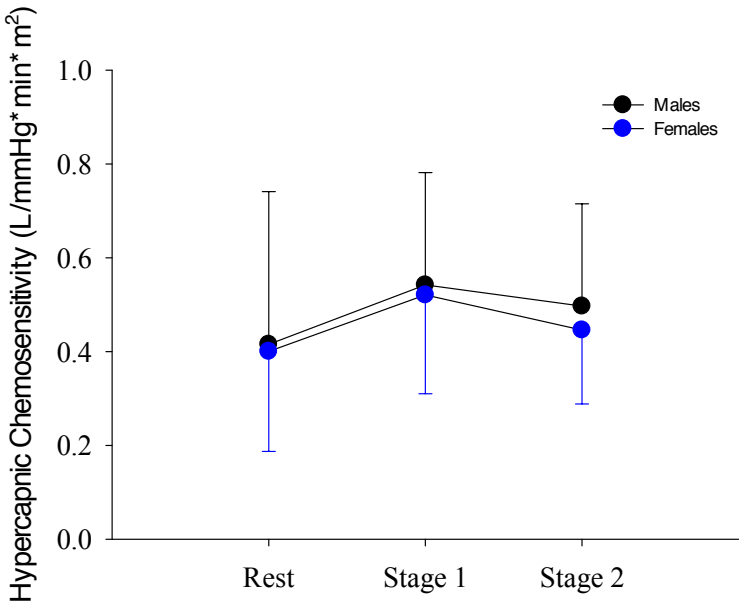

Figure 11: A comparison of the hypercapnic chemoresponse of the males and females at each stage when corrected for BSA. There is no significant different between the two groups at any stage, p>0.05.

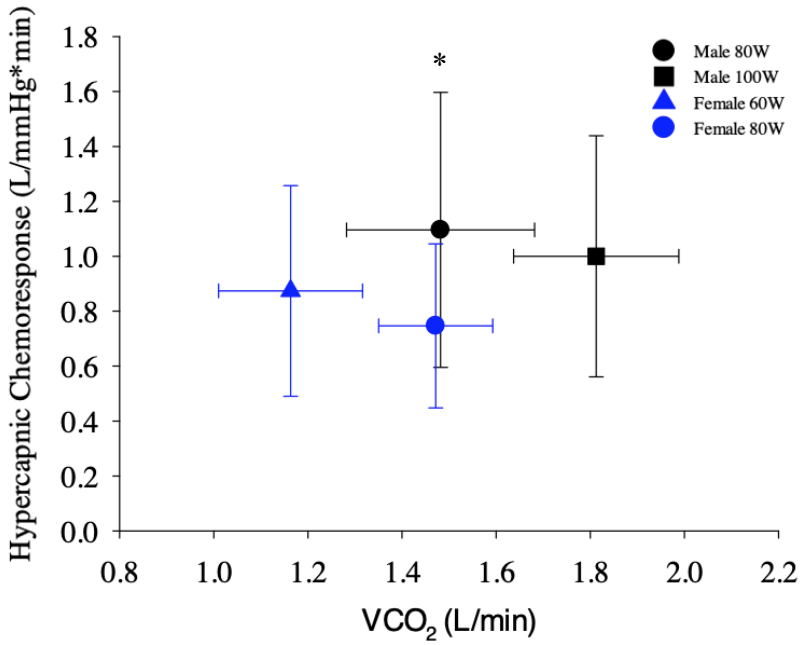

Figure 12: The hypercapnic chemoresponse of males and females at the $\dot{V}CO_2$ for each exercise work rate. Male and female hypercapnic chemoresponse was compared at an iso-$\dot{V}CO_2$, approximately 1.5L/min, and males had a significantly greater response. * Indicates significant difference, p=0.03.

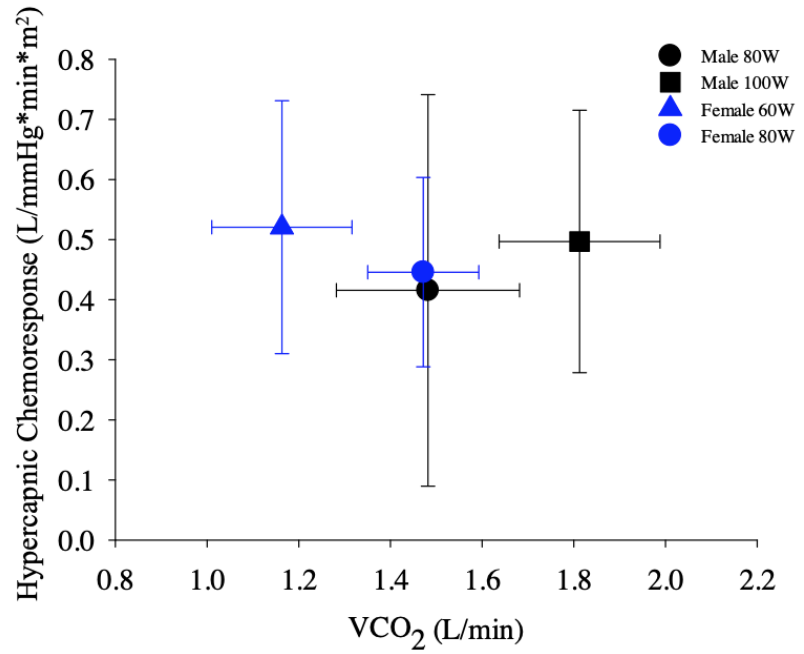

Figure 13: The hypercapnic chemoresponse of males and females at the $\dot{V}CO_2$ for each exercise work rate. Male and female hypercapnic chemoresponse was corrected for BSA and compared at an iso-$\dot{V}CO_2$, approximately 1.5L/min, there was no significant difference, p>0.05.

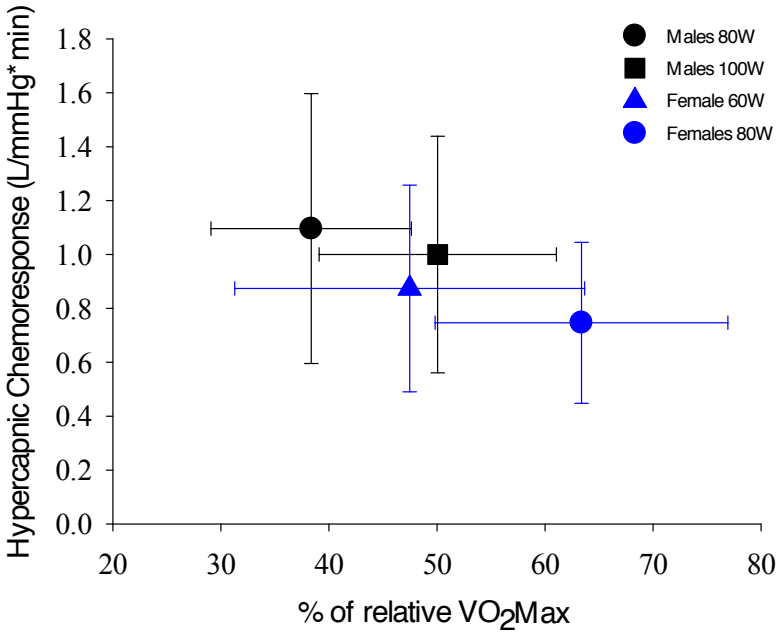

Figure 14: The hypercapnic chemoresponse for the male and female groups shown at the average percentage of $\dot{V}O_{2max}$ during stage 1 exercise and stage 2 exercise. There is no significant difference between the hypercapnic response, $p>0.05$. The female participants were using a significantly higher percentage of their $\dot{V}O_{2max}$ overall, $p<0.05$.

Table 7: The % increase in breathing frequency and tidal volume for each chemosensitivity stage for male and female participants.

	Fb % increase rest	V_T % increase Rest	Fb % increase Stage 1	V_T % increase Stage 1	Fb % increase Stage 2	V_T % increase Stage 2
Males	14±19	25±61	6±10	34±36*	5±11	27±19*
Females	6±14	48±24*	-2±15	42±22*	5±16	22±37

Abbreviations: Fb: Breathing frequency, V_T: tidal volume. * Indicates significant difference between Fb and Vt within female or male participants, $p<0.05$.

5.3 Mechanical ventilatory limitations

Out of the 29 total participants, 15 were flow limited, made up by 50% of the males and 54% of the females developed expiratory flow limitation. The expiratory flow limitation ranged from 17-65%. The prevalence of expiratory flow limitation was not different between the sexes

(p>0.05). Figure 15 provides an example of a participant that developed EFL during progressive exercise.

The chemoresponse of the flow limited and the nonflow limited groups are shown in figure 16. The pre-stimulus $P_{ET}CO_2$ and $\dot{V}_I$ are shown in table 3 and were not significantly different between the two groups. Overall, there was no significant difference between the mean hypercapnic chemoresponse for each group at rest or during exercise (p>0.05). Those that were flow limited also used a higher percentage of their $\dot{V}_{Ecap}$ compared to the nonflow limited group, 78±13% vs 62±7% respectively (p<0.05). Finally, participants were split into two groups, high and low, based on the percent they used of their $\dot{V}_{Ecap}$ and when their chemoresponse was analyzed there was no significant difference at rest between the high and low group (0.75±0.52 L/mmHg*min, 0.76±0.55 L/mmHg*min respectively, p>0.05) or during exercise (high 1.0±0.45 L/mmHg*min, low 0.89± 0.36 L/mmHg*min, p>0.05).

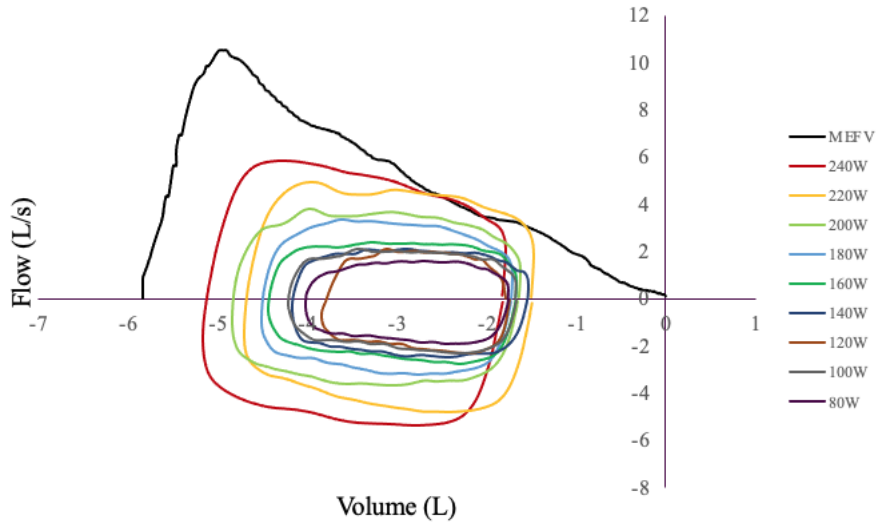

Figure 15: The MEFV and tidal volume loops for one participant during the progressive maximal exercise test. During 220W and 240W the participant developed EFL.

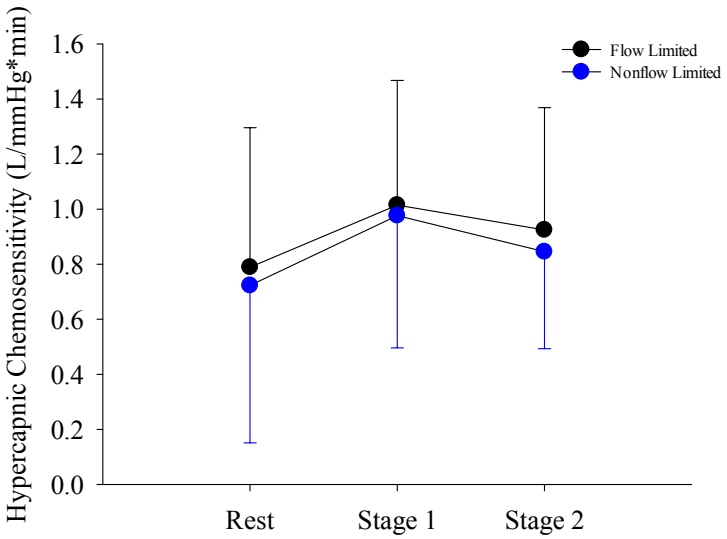

Figure 16: The hypercapnic chemoresponse for the flow limited and the nonflow limited groups at each stage. There was no significant difference between the groups at any stage, p>0.05.

5.4 Chemosensitivity

Finally, the participants grouped by high and low hypercapnic chemoresponse for rest and combined exercise response. We compared the end exercise $\dot{V}_E/\dot{V}CO_2$, $\dot{V}_E/\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_{Ecap}$, and $\dot{V}O_2$ to determine other potential differences between the participants with a high vs low hypercapnic chemoresponse. Overall, there was no significant difference between the maximal exercise values of the high and low chemoresponse groups during exercise ($p>0.05$; table 8).

Table 8: Maximal exercise values for participants when divided into high and low chemoresponse at rest and during exercise (average response of stage 1 and two).

Chemoresponse Group	$\dot{V}_E / \dot{V}O_2$	$\dot{V}_E / \dot{V}CO_2$	$\dot{V}O_2$ (L/min)	% $\dot{V}O_{2max}$	$\dot{V}CO_2$ (L/min)	% $\dot{V}_{Ecap}$
High Rest	44±5	38±3	3.2±1.0	102±18	3.7±1.1	75±15
Low Rest	42±6	37±5	3.0±1.0	94±20	3.4±1.1	66±10
High Exercise	44±4	39±3	3.4±0.9	94±15	4.0±1.1	74±15
Low Exercise	42±6	37±4	2.8±0.9	101±22	3.2±1.0	67±11

The end exercise values for participants divided into high and low chemoresponse groups at rest and exercise (stage 1 + stage 2). There were no significant differences between groups, $p>0.05$.

5.5 EIAH

Out of the 29 participants that completed this study, 3 participants, 2 females and 1 male, were confirmed to develop EIAH, defined as a >3% drop in SpO_2. The male participant had a 4% decrease in SpO_2, and the females had a 4% and 7% decrease in SpO_2. The female participants had a $\dot{V}O_{2max}$ of 36.4 and 46.5 ml/kg/min, and the male participant had a $\dot{V}O_{2max}$ 57.9 ml/kg/min respectively. One female and the male participant were flow limited while the other female participant was not flow limited. At rest, one female participant had a high chemo response, whereas the other participants had a low chemoresponse. During exercise all three participants had a low chemoresponse, average 0.61±0.25 L/mmHg*min vs the full group average of 0.94±0.4 L/mmHg*min.

6.0 DISCUSSION

6.1 Main findings

The purpose of this study was to determine if there was an effect of fitness and acute exercise on hypercapnic chemosensitivity. I also sought to determine if there was a difference in the chemosensitivity of those with or without mechanical limitations during maximal exercise. The main results of this study are two-fold. First, there is no significant difference in the hypercapnic peripheral chemoresponse at each test stage between any of the groups. We interpret this to mean that the peripheral chemosensitivity is not altered through regular aerobic training. Second, being that there is a significant difference in the chemoresponse at rest compared to during acute exercise. We interpret this to mean that acute exercise increases the hypercapnic peripheral chemoreceptors regardless of sex or fitness. Overall, this research suggests that the chemosensitivity response at rest cannot be extrapolated to the response during exercise as previously done by many studies, and that while the effect of acute exercise is present, more research is needed to elucidate the mechanisms of action.

6.2 Technical Considerations

To ensure consistent data collection, the $P_{ET}CO_2$ and $\dot{V}_I$ were monitored between each set of stimulus breaths during each CO_2 trial to ensure the participants were back to their baseline values (table 3). By ensuring the participants were back to their pre-stimulus values we prevented the response from being impacted by any residual response from the previous CO_2 breaths. Participants also all underwent 5 trials at each stage because of the variability for chemosensitivity testing. The average of 5 trials allowed us to have a true average chemosensitivity value for a more accurate analysis of the chemosensitivity between groups.

Participants were also blinded to when they were given the breaths of CO_2 to ensure a true response to the hypercapnic stimulus.

6.3 Central vs Peripheral chemoreceptors

Previous studies examining hypercapnic chemosensitivity primarily involved prolonged exposure to CO_2 through steady state or rebreathing methods of testing. These methods stimulate both the central and peripheral chemoreceptors and thus the response is a combination of both. Some have tried to quantify the peripheral and central chemoresponse from the same test by also incorporating a hyperoxic test to "turn off" the peripheral chemoreceptors to determine the central-only response (37). Since this research however, work in dogs where the carotid bodies were surgically isolated showed that the activity of the peripheral chemoreceptors impacts the activity of the central receptors (67). When the carotid body was hypercapnic, the steady-state central hypercapnic activity was increased in comparison to when the carotid body was hypocapnic (67). The result from Smith et al. 2015 suggests that due to the connection between the receptors, it is unlikely that there is a way to turn off the peripheral chemoreceptors and isolate the central receptors.

For this study we chose to target only the peripheral chemoreceptors because they are likely responsible for the fine tuning of control of breathing during exercise (24). Smith et al. 2015 showed that increased central activity occurred after several minutes of carotid body stimulus, due to the time required for the PCO_2 to be translated into a change in H+, and because our protocol only provided a transient stimulus, there was not enough time or concentration for the central chemoreceptors to be involved. The transient stimulus does provide a large enough

change in PCO_2 to stimulate the peripheral receptors however because they are highly perfused. Thus, we are confident that we only targeted the peripheral receptors.

6.4 Fitness effect

Previous work examining the hypercapnic chemoresponse of athletic vs nonathletic populations has been conflicting; however, it has been suggested that those that are more athletic will have a lower chemoresponse due to decreased chemoreceptor activity (8) and reduced ventilatory output (52). Research has also suggested that the opioid peptide B-endorphin released during exercise increases the carotid body activity, which adds to the increase in ventilation seen during exercise and changes in production of this endorphin will alter the chemoresponse response (40). A study has shown that training, 2 months of high intensity exercise, causes a reduced production of this endorphin which could be a cause for a reduced ventilatory response to CO_2 in athletes compared to a nonathletic population (9).

The results of this current study looking at the relationship between the hypercapnic chemoresponse and aerobic fitness found no significant results (figures 5, 7, 8). These results conflict with previous work that has demonstrated a difference between trained and untrained participants (8, 51, 52). We were unable to take any measurements for the B-endorphin, so we are unable to confirm any differences in our participants on this topic. One possible explanation for this discrepancy in results could be the fitness of the participants. The previous study's athletic group was very cohesive with the fitness level being in the range of 50-60ml/kg/min (8) whereas our high fitness group had a wider range (40-66ml/kg/min) that ultimately could have led to no significant difference. Because several of our high fitness participants had a $\dot{V}O_{2max}$ below 50 ml/kg/min it is possible that they may have not had the ventilatory adaptations that

come from training or increased fitness that would lead to a decreased CO_2 responsiveness that previous work suggested (8, 51). Though there were not enough participants with a $\dot{V}O_{2max}$ above 50ml/kg/min for a comparison to the low fitness group, those with the highest fitness (55+ ml/kg/min, figure 7) during exercise had both the highest and the lowest hypercapnic chemoresponse, highlighting the variability between participants. Similarly, there was no significant relationship between the chemoresponse at rest and fitness (figure 8).

Our results are consistent with the results of Mahler et al. 1982 that compared the central chemoresponse of athletes and control participants during a rebreathing test at rest and found that there were no differences between the two groups (43). Mahler et al. 1982 suggested that a lack of difference may simply be due to a wide spectrum of ventilatory control present between individuals. Martin et al. 1978 also found similar central hypercapnic chemoresponses between endurance trained athletes and nonendurance trained athletes during a rebreathing test at rest (47), suggesting that endurance is not a contributing factor for differences in chemoresponsiveness. Though our results agree with the previously mentioned studies, all their measurements were made at rest which could have impacted their findings compared to other studies that did find a difference. By comparing trained and untrained participants during exercise a more well-rounded comparison can be made to determine if there is truly no difference or if the difference in the chemoresponse is only present under specific conditions.

6.5 Acute exercise

In comparison to the effect of fitness on the hypercapnic chemoresponse, research on the results of acute exercise on the chemoresponse has a greater agreement. Several papers have demonstrated that with the onset of acute exercise there is an increase in the ventilatory response

to CO_2 (35, 59, 76, 81). Several mechanisms have been suggested for this increased response, such as increased sensitivity of the chemoreceptors, increased sympathetic nerve activity, impulses sent from active muscles, increased concentrations of metabolites, pH oscillation changes, etc. (71). Consistent with several other studies, the results of this study showed an increased average hypercapnia response during exercise when compared to rest (figure 6). Unfortunately, in this study we were unable to collect data regarding sympathetic nerve activity, metabolites, or pH, so we are unable to confirm whether they played a role in the increased response in these results. However, based on previous research MSNA is unlikely to be directly influencing the hypercapnic chemoresponse because research has shown that MSNA increases linearly at exercise intensities above 40% of $\dot{V}O_{2max}$ (39), which should directly relate to a linear increase in chemoresponse; however that is not shown in these results (figures 5, 10, 11, 16). Also consistent with other research was that these results showed no further increase in the hypercapnic chemoresponse with additional increases in exercise (figures 5, 10, 11, 16). A few studies have shown the opposite result with a decrease in the ventilatory response to CO_2 during exercise (10). However, they suggested that this trend was not entirely unexpected and was not actually a decrease in chemosensitivity but instead reflected the expected decrease in PCO_2 due to the increased transit time and the increase in ventilation. The results of our study highlight the importance of taking these measurements during exercise compared to extrapolating the data from rest, because of the differences in chemosensitivity that have been shown here. These results are also important because they demonstrate the tight control of breathing during exercise because the hypercapnic ventilatory response remained similar at different work rates.

6.6 Males vs Females

Research comparing sex differences for the hypercapnic chemosensitivity is limited, primarily because historically animal studies or males made up the majority of the research participants. We hypothesized that there could be a difference between male and female participants because of inconclusive results from previous studies, as well as because it has been suggested that estrogen and progesterone have an influence on ventilation which could translate into a different hypercapnic chemoresponse (63). Animal research has found conflicting results including those that looked at the response after gonad removal, causing a reduction in testosterone and estrogen, where some saw differences between the hypercapnic response of male and female rats (45, 46) but some research found no difference between male and female cats (73). Some research in humans and animals has suggested that increased testosterone may also increase the sensitivity to CO_2 (2, 74), however there is an equal amount of research suggesting that there may not be an effect (48, 79).

The results of the present study comparing the response of males and females found no difference in the hypercapnic ventilatory response between sexes at each specific stage (figure 10). When the males and females were compared at an iso-$\dot{V}CO_2$ the males had a higher chemoresponse compared to females (figure 12), however when scaled for BSA this difference was absent (figure 13). Previous research has shown a difference between the response of males and females at similar stages, conflicting with our results, however, different techniques were used which may account for the differences seen (42, 56, 78). Research in sheep has shown that $\dot{V}CO_2$ plays an important role in ventilation. When $\dot{V}CO_2$ was removed completely the $\dot{V}_E$ was reduced to zero, and as CO_2 was added back into the blood the ventilation increased proportionally (60), however it is still unknown what is sensing this change in $\dot{V}CO_2$. Our

research shows that the potentially higher chemoresponse in males at an iso-$\dot{V}CO_2$ is likely related to a mechanism outside of the influence of $\dot{V}CO_2$ on ventilation, however due to the limited research around the sexes and chemosensitivity there is no clear explanation as to why this response has occurred.

6.7 Mechanical Limitations

Initially we thought that chemosensitivity could be linked to mechanical limitations or a lack of mechanical limitations because CO_2 stimulates an increase in ventilation; those that have a greater sensitivity to hypercapnia would be more likely to have a higher ventilation overall. When two healthy individuals of a similar size and age are compared, their MEFV is likely to be very similar, providing them with similar ventilatory mechanics. However, if one of those individuals has a greater ventilatory sensitivity and thus a higher ventilation, they have a greater potential to become flow limited during exercise. The results of this study showed that when divided into the flow limited and nonflow limited groups there was no difference in the hypercapnic chemoresponse at each stage (figure 16). By showing no significant difference between the groups that were and were not flow limited, these results suggests that hypercapnic chemosensitivity does not play a large role in the development in EFL. EFL is more likely related to other factors such as airway and lung size than chemosensitivity (18).

There was also no difference between the metabolic equivalents at maximal exercise for those that had a high or low hypercapnic chemoresponse at rest or exercise (table 8). Mercier et al. 1992 suggested the pattern of ventilation could be different between those with a high and low chemosensitivity, specifically whether the change in frequency or V_T was the cause of the ventilatory response (50). Overall, it was found that those with a higher V_T prior to the CO_2

stimulus had a greater ventilatory response than those that had a greater breathing frequency prior to the CO_2 stimulus (50). Another study found that the recovery ventilation after intermittent hypoxic stimulus was similar between males and females it was accomplished with different breathing patterns where females tended to use a greater breathing frequency and males a greater tidal volume (75). In comparison, our study found no difference between the frequency and V_T but saw that both male and female participants primarily had a greater increase in their V_T compared to their Fb in response to the hypercapnic stimulus. This is an expected increase because of the mechanical efficiency of ventilation.

6.8 Exercise Induced Arterial Hypoxemia (EIAH)

Mechanical constraints have been shown to play a role in the development of EIAH as well. Dominelli et al. 2013 demonstrated that though EIAH can develop in those that are flow limited because of being unable to increase their ventilation to maintain the required PaO_2 (16). Though we did not find a large sample of those with EIAH in this study to examine the difference in hypercapnic chemosensitivity, we did see 2/3 EIAH participants demonstrate flow limitations. The flow limited EIAH participants we had were also not part of our high chemoresponse group, which supports the idea that hypercapnic chemosensitivity is not a driving factor in the development of EFL; however, further research is needed to confirm.

6.9 Future directions

The EIAH phenomenon where individuals desaturate during exercise is one direction for future chemosensitivity research. It is thought that EIAH could be related to the CO_2 sensitivity because a reduced sensitivity would lead to a reduced ventilatory response which could

ultimately cause the reduction in SpO_2 (27). A $\dot{V}_E$ would result in a lower $\dot{V}_A$ and a reduced compensatory hyperpnea which will lead to a lower PaO2 causing the desaturation

A previous study found that those with greatest desaturation also had the lowest response to CO_2 during exercise and there was a significant relationship between a higher sensitivity and a higher end exercise SpO_2 (22). In the current study 3 participants, 2 females and 1 male, were identified to develop EIAH, defined as a greater than 3% decrease in SpO_2 from rest. At rest one of these three participants had a high chemo response, whereas during exercise all three had a low chemoresponse. Within this group though, only one participant was not flow limited so while they did have a lower chemoresponse compared to other participants, we are unable to make any kind of inferences based on this single data point. This does suggest though that there may be some potential variations within those that develop EIAH and their hypercapnic chemoresponse.

6.10 Limitations

There are some limitations that warrant discussion. The tubing for each participant between the facemask and the bag was also quite long; while it was consistent between each participant it did create a significant amount of space. The space would have impacted the amount of inhaled CO_2 because depending on their tidal volume participants may have needed more breaths to clear the room air before they received CO_2, this was not accounted for, and each participant only got 2 breaths total once the three-way valve for CO_2 was opened. There are also several other stimuli that can impact ventilation, including body temperature, pH, K+, H+, all of which were not measured within this study. By testing individuals at a submaximal work load these changes should have primarily been avoided, however we cannot say for certain because the intensity of this workload would have changed between individuals. We also compared the

data gathered at the submaximal intensities to the response at maximal exercise because of the other stimuli that would have impacted the results at maximal exercise. While the data suggests that the chemoresponse would not continue to change as exercise intensity increases, more data is needed to confirm this finding. The protocol for this study did not include any testing of O_2 sensitivity, so while we kept the participants iso-oxic to prevent any changes to the hypercapnic chemoresponse due to hyperoxic and hypoxic conditions, we did not look at any changes to the oxygen sensitivity that might have been occurring. There was also the potential for an interactive effect of the peripheral and central chemoreceptors as previously mentioned, however this is less likely to have taken place because studies that have demonstrated this showed the effect happening when the peripheral chemoreceptors were hyper- or hypocapnic for prolonged periods of time, not with transient breaths. Unfortunately, for the purposes of the current study we were unable to assess any genetic components or relationship in any potential findings. Lastly, due to the various lockdowns and time constraints on testing during the COVID-19 pandemic, some females were not able to be tested during the low hormonal phase of their menstrual cycle. However, this only impacted 2 participants, so there is unlikely to be a significant impact.

7.0 CONCLUSION

The hypercapnic chemosensitivity of the peripheral chemoreceptors has been shown to have a large amount of conflict about how their sensitivity changes with exercise, fitness, between sexes, etc. This study sought to determine the difference of the peripheral chemoresponse to a transient hypercapnic test at rest and during exercise in individuals of various fitness. Overall, we found that there was no significant difference in the hypercapnic chemoresponse at rest or during exercise between males and females, those with or without flow limitations, or between different fitness levels. An effect of acute exercise was seen and suggests that the sensitivity of the peripheral chemoreceptors increases with the onset of exercise, enabling the fine tuning of the ventilatory response. In conclusion, these findings demonstrate the importance of collecting these measurements at both rest and during exercise and demonstrates the fine-tuning control that the peripheral receptors have over ventilation during exercise.

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