What happens to metabolic rate when breath-holding in humans?

by

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Abstract

Objective:

Despite suggestions of a human dive response, a few studies > 50 years ago concurred that humans do not reduce metabolic rate when breath-holding. However, these studies had limited participant numbers, did not account fully account for oxygen consumed from the blood, or consider additional oxygen supplied to the blood from contraction of the spleen. Nor did they consider that the loss of respiratory muscle contraction alone may result in a reduction in metabolic rate. Here we are address these limitations.

Methods:

In 20 subjects we measured metabolic rate at rest, over the course of breath-holds, and in the period following breath-holds from air and 60% oxygen using Douglas bags. In our investigation we have also 1) measured SpO_2 and included extra oxygen consumed from the blood, 2) accounted for additional oxygen supplied to the blood from contraction of the spleen, and 3) measured the oxygen requirement for breathing via mechanical ventilation (n = 10).

Results:

Metabolic rate at rest was 227 ± 39 millilitres.oxygen · $minute^{-1}$ STPD (n = 20). The metabolic cost of breathing was 60 ± 40 SD millilitres oxygen · $minute^{-1}$, STPD (n =10). Over breath-holds from air and after accounting for arterial desaturation and spleen contraction, metabolic rate slightly fell to 152 ± 48 SD millilitres oxygen · $minute^{-1}$, STPD ($p < 0.001$ *vs* SpO_2*, n = 20*) ($p < 0.05$ *vs* mechanical ventilation*, n = 10*). When breath-holding from oxygen, when arterial desaturation does not occur but still accounting for contraction of the spleen, reductions in metabolic rate were potentiated and it fell to 96 ± 26 SD millilitres

oxygen · minute^{-1}, STPD ($p < 0.001$ *vs rest, n = 20*) ($p < 0.001$ *vs breath-hold from air, n = 20*) ($p < 0.01$ *vs mechanical ventilation, n = 10)*.

Conclusions:

For the first time we show that metabolic rate falls when breath-holding in humans, even after accounting for oxygen consumed from the blood and additional oxygen consumed from the blood by spleen contraction. When breath-holds were prolonged with oxygen, reductions in metabolic rate were accentuated. Such reductions are greater than measured reductions associated with the absence of respiratory muscle contraction. Whilst suggestions of the dive response resurface, detailed literature examination highlights humans do not exhibit a response of a magnitude comparable to that of diving mammals.

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rises immediately following breakpoint and does so by a greater extent following breath-holds from oxygen. Over 5 minutes following, metabolic rate is not different from resting values after breath-holds from air but remains elevated following breath-holds from oxygen.

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List of definitions and abbreviations

~	Approximate number
Δ	Change
AIDA	International association for the development of apnoea
ANOVA	Analysis of variance
Apnoea	Temporary cessation of breathing and term often used in lieu of breath-hold
Bradycardia	Slow resting heart rate typically below 60 beats minute^{-1}
Breakpoint	The resumption of breathing at the end of breath-holding
Breath-holding	The sustained contraction of the expiratory respiratory muscles against the passive recoil of the lungs that usually occurs at rest
BTPS	Body temperature, ambient pressure, saturated with water vapour
^{o}C	Degrees Celsius
CaO_2	Blood oxygen concentration
Central respiratory rhythm	Higher brainstem generated pattern responsible for producing rhythmic synaptic drive for motoneurons controlling the respiratory muscles
cm	Centimetres
CO	Cardiac output; the amount of blood pumped by the heart per unit of time
Constant weight freediving	Diver descends to and ascends from a depth with/without the use of fins

Dive response	Reflex physiological response to conserve oxygen seen in diving mammals. Characterised by large reductions in heart rate and cardiac output and complete cessation of blood flow to non-essential organs
Douglas bag	Large airtight bag introduced by Oxford physiologist Claude Gordon Douglas in 1911 for collecting expired gas samples
Dynamic freediving	Diver travels in a horizontal direction to cover the greatest distance possible with/without fins
ECG	Echocardiogram; recording of the hearts electrical activity
Eucapnia	Normal arterial carbon dioxide levels; $P_aCO_2 = 40$ mmHg
Eupnoea	Normal, unlaboured breathing at rest
F_eCO_2	Fraction expired carbon dioxide
F_eN_2	Fraction expired nitrogen
F_eO_2	Fraction expired oxygen
F_iCO_2	Fraction inspired carbon dioxide
F_iN_2	Fraction inspired nitrogen
F_iO_2	Fraction inspired oxygen
Free immersion freediving	Diver uses a rope to pull on the descent and ascent
HCT	Haematocrit
Hypercapnia	High arterial carbon dioxide levels; $P_aCO_2 > 40$ mmHg
Hyperventilation	Breathing in exceeds metabolic requirements resulting in hypocapnia
Hypocapnia	Low arterial carbon dioxide levels; $P_aCO_2 < 40$ mmHg
Mechanical ventilation	Passive ventilation achieved where a gas mixture is pushed into the lungs

Metabolic cost of breathing	The oxygen requirement necessary to meet the requirements of the respiratory musculature to maintain ventilation
Metabolic rate	The amount of energy expended per unit of time; the rate at which oxygen consumed by the body and amount of carbon dioxide produced by the body
mM	Millimolar; unit of measurement of a molecule within a solution. 1 mM = 1 mole litre^{-1}
mmHg	Millimetres of mercury; units of pressure.
Mueller manoeuvre	A manoeuvre performed by attempting to inhaled against a closed airway
n =	Number
P_{50} *value*	Oxygen tension of haemoglobin where 50% is saturated
P_ACO_2	Partial pressure of carbon dioxide in the alveolae
P_aCO_2	Partial pressure of carbon dioxide dissolved in the blood
P_AO_2	Partial pressure of oxygen in the alveolae
P_aO_2	Partial pressure of oxygen dissolved in the blood
$P_{ET}CO_2$	Partial pressure of end tidal carbon dioxide
$P_{ET}O_2$	Partial pressure of end tidal oxygen
PiO_2	Partial pressure of inspired oxygen
$PiCO_2$	Partial pressure of inspired carbon dioxide
Respiratory sinus arrhythmia	Naturally occurring heart rate variation from the breathing cycle; heart rate increases during inspiration and decreases during expiration
SD	Standard deviation
SE	Standard error of the mean

S_pO_2	Indirect arterial oxygen saturation measure detailing the amount of oxygen bound to haemoglobin in arterial blood measured by pulse oximetry
Static dry breath-holding	Individuals remains still and holds breath for as long as possible on land without any immersion
Static freediving	Diver holds their breath for as long as possible with their nose and mouth immersed whilst floating or standing on the bottom of a pool and is based on the duration of the breath-hold rather than the distance travelled
STPD	Standard temperature and pressure, dry
Valsalva manoeuvre	A manoeuvre performed by attempting to forcefully exhale against a closed airway
Variable weight freediving	Diver descends to a depth with the help of a ballast weight and ascends using own power
V_B	Blood volume.
VCO_2	Rate of carbon dioxide production
V_e	Minute ventilation; volume of gas inhaled/exhaled from a person's lungs per minute.
VO_2	Rate of oxygen consumption

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1. General introduction

Breath-holding related activities, for both recreational and commercial purposes, have been performed for centuries (Bain et al., 2018). The Ama have been diving for shellfish in Japan and Korea for ~ 2000 years and in recent years, breath-holding and breath-hold diving have developed into highly competitive sports. In 1992, the International Association for the Development of Apnoea (AIDA) was established to organise and develop rules for competitions. Breath-holders now compete in regulated events relating to either the deepest or furthest distance travelled underwater (with subcategories including constant weight, variable weight, free immersion, no limits, and dynamic apnoea) or the maximal breath-hold duration (static apnoea). As this study is primarily concerned with breath-holding without immersion or facial immersion, all breath-holds reported here will relate to dry, static breath-holds unless otherwise stated.

Humans normally hold their breath for ~ 1 minute and do so without losing consciousness ((Schneider, 1930; Engel et al., 1946; Flume et al., 1994; Flume et al., 1996; Cherouveim et al., 2013) and reviewed by Parkes (2006)). Although, competitive breath-holders and free divers can hold for longer, ~ 4 minutes, and may also lose consciousness (Lindholm and Lundgren, 2006; Lemaître et al., 2008; Pingitore et al., 2008; Heusser et al., 2010; Schagatay and Lodin-Sundström, 2014).

Diving mammals, in particular seals, possess a reflex response that allows for a reduction in metabolic rate. This happens due to a complete cessation of blood flow to skeletal muscle and a reduction in viscera blood flow which limits peripheral oxygen consumption (Scholander, 1940; Zapol et al., 1979). In addition to this, a reduction in cardiac output, resultant from a large fall in heart rate reduces the oxygen requirements of myocardium as well as preventing an intolerable rise in blood pressure (Irving, 1939;

Scholander, 1940; Kooyman and Campbell, 1972; Zapol et al., 1979; Butler and Jones, 1997). These physiological adaptations, in part, explain why diving mammals are capable of spending prolonged periods underwater. Some diving mammals can spend up to ~ 1 hour when forcibly submerged, and up to ~ 40 minutes whilst actively foraging (Irving, 1939; Scholander, 1940; Kooyman et al., 1980; Castellini and Somero, 1981; Davis et al., 1999; Watwood et al., 2006).

There has been persistent suggestion that humans have a similar response during breath-holding without facial immersion. which conserves oxygen consumption and prolong breath-holding (Scholander, 1962a; Irving, 1963; Heistad et al., 1968; Gooden, 1994; Schagatay and Andersson, 1998). Despite this suggestion, there is no evidence of complete shutdown of organ blood flow, cardiac output does not fall below resting values of ~ 6 litre · $minute^{-1}$ (Hong et al., 1971; Pingitore et al., 2008; Heusser et al., 2010), heart rate does not fall below ~ 50 beats · $minute^{-1}$, and stroke volume does not fall below resting values of 70 millilitres (Scholander et al., 1962; Hong et al., 1971; Lin, 1982; Heusser et al., 2010). Further to this, the few but incomplete studies, carried out > 50 years ago that have assessed metabolic rate averaged over breath-holds in humans suggest that it does not fall (Stevens et al., 1946; Klocke and Rahn, 1959; Hong et al., 1971).

Until now, there has been little direct measurement of metabolic rate before, during and following breath-holding in a plausible number of participants. Therefore, the question of 'What happens to metabolic rate when breath-holding?' remains to be definitively established. To comprehensively answer this, it is necessary to consider the underlying physiology of breath-holding.

1.1 Literature review

1.1.1 How long can humans hold their breath?

Breath-holding is the sustained contraction of the expiratory respiratory muscles against the passive recoil of the lungs that usually occurs at rest (Parkes, 2006). In humans, breath-holding is notoriously variable and depends on several different factors. But, when breath-holding from air humans can typically only hold their breath for a maximum of ~ 1 minute. The largest cross-sectional study examining breath-hold durations was conducted by Edward Schneider (Schneider, 1930), who measured maximal breath-hold time in 318 American aviators in France between 1918-1919 (during World War I). Mean breath-hold duration was 1.1 minutes. Others have also found mean breath-hold duration to be ~ 1 minute. For example, 10 healthy volunteers held their breath for 1.4 minutes (Harty et al., 1996), while another experiment found 10 different volunteers were able to hold their breath for 1.2 minutes (Flume et al., 1996).

Schneider noted the vast disparity in breath-hold duration, with the observed breath-hold durations ranging from 29 to 128 seconds. He concluded that the variability between individuals' ability to breath-hold was because the discomfort associated with breath-holding was not equally tolerated (Schneider, 1930). This meant that it was only those who were able to withstand a degree of this discomfort and were suitably motivated could reach the upper limits of this range. This disparity can also be identified by comparing different studies' mean breath-hold durations when participants breath-hold under the same conditions. For instance, Lin et al. (1974) found breath-holds to last on ~ double that of as Schneider's conditions (2.2 minutes), but only had 5 participants. Likewise, competitive breath-holders can hold their breath for even longer. Heusser et al. (2010) measured mean breath-hold duration of 14 competitive breath-holders to be 3.8 minutes.

Breath-hold duration does not appear to be affected by sex. Cherouveim et al. (2013) found no difference in mean breath-hold duration between 8 females (1.8 minutes) and 8 males (1.7 minutes). Nor is breath-hold duration affected by age. Trembach and Zabolotskikh found no significant correlation between breath-hold duration ($r = 0.13$) and age in 47 participants aged between 25 and 85 (Trembach and Zabolotskikh, 2017).

1.1.1.1 Complications of practice, distraction and lung volume

Breath-hold duration is complicated by practice, as repetition improves performance by ~ 30%. Following a practice breath-hold, 15 breast cancer patients were able to improve breath-hold duration from 0.7 to 1.0 minutes ($n = 15$) (Parkes et al., 2016b). End expiration breath-holds, were prolonged by 37% after six successive trials from 0.6 to 0.8 minutes ($n = 6$) (Bartlett, 1977). As well as two weeks of twice daily breath-hold training, improving maximal breath-hold duration by 31% in 10 healthy untrained volunteers (Engan et al., 2013).

Breath-hold duration is further complicated as distraction from the discomfort of breath-holding also prolongs duration. Performing a Valsava manoeuver, Mueller manoeuvre or squeezing a bulb with one hand improves end-expiration breath-hold duration by 15%, 12% and 19% respectively ($n = 6$) (Bartlett, 1977). This occurs as a result of distraction rather than the physical action of a motor task. Alpher et al. (1986) demonstrated this by performing a similar experiment in which the effects of a bulb squeeze and mental arithmetic on breath-hold duration were examined. Mean breath-hold duration, at functional residual volume, was extended from 0.4 minutes to 0.5 minutes ($n = 20$) in both tasks, indicating that distraction alone is capable of prolonging breath-holding.

The experiments above conducted by Bartlett (1977) and Alpher et al. (1986), had participants breath-hold from end expiration at functional residual lung volume. These breath-holds are relatively short in comparison to breath-holds by participants from studies which

performed breath-hold from total lung capacity discussed above (Schneider, 1930; Norfleet and Bradley, 1987; Harty et al., 1996; Parkes et al., 2014; Trembach and Zabolotskikh, 2017). Flume et al. (1996) confirmed this finding using a paired experimental model, eliminating variance in breath-hold capability between individuals. They found mean breath-hold duration from total lung capacity was 1.2 minutes compared to 0.6 ± 0.1 minutes at functional residual volume.

1.1.1.2 Complications of oxygen and carbon dioxide

The typical ~ 1 minute breath-hold can be influenced by altering the amount of 1) oxygen breathed prior to the breath-hold, 2) carbon dioxide breathed prior to the breath-hold or extent of hyperventilation, and 3) a combined alteration in oxygen and carbon dioxide levels.

1.1.1.2.1 Oxygen

Breath-holds performed following the breathing of increased levels of oxygen, or 'pre-oxygenation' are longer than the average ~ 1 minute room air breath-hold. For example, Klocke and Rahn (1959) recorded a mean breath-hold of 5.7 (n = 7) following a breath of pure oxygen. This is well established and has been confirmed many times (Lin et al., 1974; Cooper et al., 2004; Parkes et al., 2014). Pre-oxygenation typically allows participants to breath-hold for ~ twice as long as breath-holding from air. In 1908, Hill and Flack demonstrated this doubling effect with mean room air breath-holds increasing from 0.7 minutes to 1.4 ± 0.6 minutes in 13 volunteers following 3 breaths of pure oxygen (Hill and Flack, 1908). Similarly, Norfleet and Bradley (1987) also showed mean room air breath-holds were extended from 1.6 to 4.1 ± 0.6 (n = 5) minutes with pre-oxygenation.

Conversely, breath-hold duration is diminished at altitude where the partial pressure of oxygen is lower. In the same experimental series, Hill and Flack (1908) found that breath-

hold duration at sea-level in Berlin (where PaO_2 = 160 mmHg) was halved when breath-holding at altitude in Turin (where PO_2 = 89 mmHg). Ferris et al. (1946) conducted a similar investigation and presented that mean breath-hold durations of 6 participants fell from 2.0 ± 0.5 (± SD) minutes at sea-level to 1.5 ± 0.4 (± SD) minutes when breath-holding at altitude (where PO_2 = 85 mmHg). Likewise, simulating this by lowering the oxygen contribution to a gas mixture has an equivalent effect. Engel et al. (1946) showed in 40 individuals that breathing a gas mixture consisting of 10% oxygen would cause P_aO_2 to fall (< 62 mmHg), and breath-hold duration was ~ halved from room air (1.7 ± 0.5 *vs.* 0.9 ± 0.3 (± SD) minutes).

1.1.1.2.2 Carbon dioxide

Increasing the level of inspired carbon dioxide prior to breath-holding shortens the breath-hold duration. In 1 participant, Godfrey and Campbell (1969) increased P_ICO_2 to 50, 52, 56 and 60 mmHg and breath-hold duration was shortened to 0.35, 0.25, 0.15 and 0.08 minutes respectively. Kelman and Wann (1971) also examined this over a larger range by having participants rebreathe expired gas (from an oxygen gas mixture). As end-tidal PCO_2 ($P_{ET}CO_2$) rose, breath-holds were performed at 30, 40, 50, 60 mmHg and durations decreased linearly. Although 6 participants performed this, data presented was again only for 1 'typical subject' and breath-holds at each $P_{ET}CO_2$ were 3, 2, 1 and 0.5 minutes respectively.

As room air consists of such a small amount of carbon dioxide (~ 0.04%), it is not possible to lower this concentration to examine the effect this has on breath-hold duration. But, it is possible to lower the body's store of carbon dioxide with hyperventilation and examine the effects that this has on breath-holding. Burton et al. (1997) found mean breath-hold duration (from functional residual volume) was increased from 32 seconds (range = 13 - 75 seconds) to 41 seconds (range = 17 – 106) following 6 rapid breaths in 31 healthy controls. Similarly, Hill (1973) demonstrated that room air breath-holds were extended with

hyperventilation alone. Their 5 volunteers performed breath-holds from eupnoea (normal, unlaboured breathing at rest) and following 8, 16 and 24 breaths at vital capacity. Mean breath-hold durations increased from 1.5 minutes in room air to 1.6, 1.7 and 1.9 minutes with each respective increase in breaths at vital capacity.

1.1.1.2.3 Combined alterations in oxygen and carbon dioxide

Pre-oxygenation paired with hypocapnia has an additive effect, which commonly extends breath-holds for periods exceeding ~ 6 minutes. The longest recorded experimental breath-hold was 14 minutes long and performed by Hermann Rahn himself having breathed pure oxygen for 3 breaths and voluntarily hyperventilated (Klocke and Rahn, 1959). In the same paper, they showed in 7 participants that breath-hold duration was increased from 5.7 to 10.5 minutes. Norfleet and Bradley (1987) showed likewise results. They had participant's breath oxygen, and hyperventilate following a metronome at a rate of 30 breaths · minute^{-1} and depth of 2 litres. Mean breath-hold duration as 6.3 minutes. When the experiment was repeated with participants breathing an oxygen mixture with elevated carbon dioxide (at 4.37%), the prescribed level of hyperventilation prevented hypocapnia from occurring. This then shortened pre-oxygenated eucapnic breath-hold duration to 4.1 minutes. These studies establish that the combined effect of pre-oxygenation and hypocapnia on breath-hold duration is greater when they are combined, than when administered alone. However, they do not establish the extent and consistency of the $PaCO_2$ fall prior to breath-holding in each subject. Cooper et al. (2003) did this and found that breath-holding with mechanical ventilation reducing $P_{ET}CO_2$ consistently to 20 mmHg increased mean pre-oxygenated breath-hold duration from 4.0 to 6.6 minutes. This, therefore, corroborates the findings of previous experiments.

1.1.2 Do humans have a diving response?

There has long been the suggestion that humans have a rudimentary diving response which enables them to hold their breath for longer by conserving oxygen (Scholander et al., 1962; Irving, 1963; Heistad et al., 1968; Gooden, 1994; Schagatay and Andersson, 1998; Foster and Sheel, 2005). The dive response is a reflexive set of physiological responses present in diving mammals which results in a lowering of metabolic rate (Irving, 1939; Scholander, 1940; Zapol et al., 1979). This, alongside large oxygen depots, explains why diving mammals can spend such prolonged periods actively swimming underwater (Kooyman et al., 1980; Castellini and Somero, 1981; Davis et al., 1999; Watwood et al., 2006). This response is characterised by a complete cessation of blood flow to non-essential organs and a reduction cardiac output resultant from a large drop in heart rate unaccompanied by increases in stroke volume (Irving, 1939; Scholander, 1940; Kooyman and Campbell, 1972; Zapol et al., 1979; Butler and Jones, 1997).

The following sections review evidence of the dive response in humans elicited by breath-holding alone, but accentuated with facial immersion and immersion (Andersson et al., 2000; Espersen et al., 2002; Foster and Sheel, 2005; Lemaître et al., 2008). As this study is concerned with breath-holding without immersion or facial immersion, this review will focus on studies concerning breath-holding alone, unless otherwise stated.

1.2.2.1 No evidence of organ blood flow shut down

In his seminal paper, Scholander (1940) cut into an exposed seals fin during a forced submersion and it did not bleed. During the dive, muscle lactate rose to 44 mM but arterial lactate had not risen above resting values of 4 mM. Once breathing was resumed and blood flow restored, lactate trapped in the muscle as washed out and arterial values were elevated.

Thereby indicating peripheral blood flow to skeletal muscle during diving had been entirely cut off (Scholander, 1940).

To date, there is no experimental evidence directly examining organ blood flow in humans during breath-holding. One early investigation, using plethysmography to measure finger and forearm blood flow during breath-holding does exist but does not indicate that widespread vasoconstriction and organ blood flow shut down is present. Heistad et al. (1968) only measured a slight (but significant) reduction in finger blood flow (21 *vs* 14 millilitres · minute^{-1}) but no change in forearm blood flow following a 30 second breath-hold in 18 volunteers. Similar has been found by others (Andersson and Schagatay, 1998).

Additionally, following breath-holding humans do not appear to produce large elevations in arterial lactate concentrations that would be expected with profound vasoconstriction. Hong et al. (1971) measured only a small rise in lactate concentration 20 seconds (of 0.8 mM) after a 2 minute room air breath-hold in 9 volunteers. Similarly, measurements in elite breath-holders do not indicate elevations in anaerobic metabolism within skeletal muscle. As only modest elevations in circulating lactate have been measured (~ 0.3 mM litre^{-1}) (Bain et al., 2016; Bain et al., 2017). Even when breath-holds are performed with physical exercise, are comparatively small. Andersson et al. (2004) measured an 11% rise in plasma lactate concentration in 15 trained breath-holders following a 40 second breath-hold when cycling at 80 Watts (resting plasma lactate was 1.1 ± 0.1 mM litre^{-1}, no absolute post breath-hold value reported).

1.2.2.2 No fall in cardiac output or heart rate

Cardiac output falls in Weddell seals (*Leptonychotes weddellii)* from 40 to 6 litre · minute^{-1} during forced submersion which is characterised by a fall in heart rate without any associated increases in stroke volume (Zapol et al., 1979). Scholander (1940) observed a fall

in seals heart rate from 100 to 10 beats · minute^{-1} within one cardiac cycle of forced submersion. In addition to preventing intolerable rises in central blood pressure, a reduction in cardiac output will result in a reduction in the myocardial oxygen requirements.

However, humans' cardiac output does not appear to fall below resting values of ~ 6 litre · minute^{-1}. Hong et al., (1971) assessed cardiac output during breath-holds of 2 – 4 minutes in duration in 9 volunteers. They did so by injecting a green dye into the right atrium of the heart 1.5 minutes into breath-holds and employed the dye-dilation method to determine any changes. Their findings reported no change in cardiac output from rest (5.8 *vs* 6.1 litre · minute^{-1}), a slight increase in stroke volume (72 *vs* 78 millilitres) and heart rate did not fall more than 4 beats · minute^{-1} from resting values (85 *vs* 81 beats · minute^{-1}). In contrast, in trained breath-holders, reductions in cardiac output during breath-holding have been reported. Heusser et al., (2010) reported a reduction in cardiac output by 2.4 litres · minute^{-1} during a minute breath-hold in 14 trained volunteers through a reduction in heart rate (77 to 66 beats · minute^{-1}) as no indication of stroke volume was given. However, resting cardiac output of these breath-holders was measured to be 8.4 litres · minute^{-1} and therefore cardiac output only fell to 6.2 litres · minute^{-1}. Other experiments without immersion in trained breath-holders have indicated that cardiac output does not fall below 6 litres · minute^{-1} (Pingitore et al., 2008).

Bradycardia, perhaps as it is so easily measurable, has commonly been used to indicate the presence of the dive response (Foster and Sheel, 2005; Alboni et al., 2011; Schagatay, 2014). It often reported that humans can decrease their heart rate when breath-holding in air. Scholander (1962a) himself reported heart rate fell following measurements in Australian divers, but did not employ any numerical or statistical analysis and did not show heart rates falling below 50 beats · minute^{-1}. In a review of heart rate changes during breath-

holding in humans it was reported that mean heart rate does not fall below 55 beats · minute^{-1} (Lin, 1982). Even in breath-holds, without facial immersion, elite breath-holders do not show a reduction in heart rate more than 5 beats · minute^{-1} (Perini et al., 2008; Pingitore et al., 2008; Bain et al., 2015a; Willie et al., 2015; Bain et al., 2016).

However, many of these studies commonly misinterpret heart rate data during breath-holding. This misinterpretation occurs because these studies compare breath-holding heart rates with heart rate immediately prior to breath-holding as opposed to a true resting baseline (Parkes, 2012; Bain et al., 2018). The implication of this is that large increases in lung volumes, from maximally inflating the lungs, results in an acceleration in heart rate (respiratory sinus arrhythmia) and exaggerating a decline in heart rate (Manzotti, 1958; Angelone et al., 1964; Cooper et al., 2004). Lemaître et al. (2008) reported a 50% reduction in heart rate over a 3.8 minute breath-hold from the immediate start of breath-holding and the breakpoint (with no absolute values being presented) in 11 trained breath-holders. Furthermore, 15% reduction in heart rate, from 90 beats · minute^{-1} immediately prior to breath-holding to 81 beats · minute^{-1} at breakpoint, was reported by Hong et al. (1971). But, re-examination of Hong et al's., (1971) data to compare changes in heart rate using true resting values, was 85 beats · minute^{-1}, shows a fall of only 4 beats · minute^{-1} opposed to 9 beats · minute^{-1} when using the elevated heart rate value immediately prior breath-holing. Further studies show that heart rate does not fall with breath-holding. Figure 2 in Parkes et al. (2014) heart rate analysis also emphasises that resting heart rate (72 ± 2 (mean ± SE) beats · minute^{-1}) is elevated immediately prior to breath-holding (~ 85 beats · minute^{-1}), and does not fall below resting values during breath-holding in air, from pre-oxygenation and from pre-oxygenation with hypocapnia.

1.2 Previous experiments measuring metabolic rate over breath-holding

Resting metabolic rate in humans is typically ~ 250 millilitres oxygen • $minute^{-1}$ STPD (Aub and Du, 1917; Harris and Benedict, 1918; Ferraro et al., 1992; Haugen et al., 2003). Manipulating resting metabolic rate has predictable effects on breath-hold duration. Exercising submaximally and elevating metabolic rate, reduces breath-hold capability in humans. Ward et al. (2001) found that cycling shortened breath-hold duration in 9 volunteers. Mean breath-hold duration when cycling at 10 Watts was reduced from 59 seconds to 11 seconds when cycling at 180 Watts. Although, humans have a limited capacity to depress metabolic requirements, breath-hold duration can be extended with fasting, which lowers resting rates of oxygen consumption. The resting rate of oxygen consumption is lower following a 12 hour fast (217 ± 0.5 (± SE) millilitres oxygen · $minute^{-1}$) compared to a 4 hour fast (230 ± 0.5 (± SE) minutes oxygen $minute^{-1}$) (Haugen et al., 2003). Mean breath-hold duration was increased from 2.2 to 2.4 ± 0.6 minutes in (Lindholm et al., 2007) in 10 volunteers following a 18 hour fast. Similarly, fasting for 13 hours improved 13 competitive breath-holders mean breath-hold duration from 3.9 to 4.7 minutes (Schagatay and Lodin-Sundström, 2014).

There has been little direct measurement of metabolic rate before, over and following breath-holding in a plausible number of participants. This is quite possibly due to the impracticality of making such measurements (Parkes, 2012). Metabolic rate is typically measured at rest and during exercise via indirect calorimetry, which requires the measurement of oxygen consumption and carbon dioxide excretion with expired gas samples being collected with the 'gold standard' Douglas bag (Carter and Jeukendrup, 2002). Several assumptions are made when implementing this method including that steady state oxygen dynamics are present (Haugen et al., 2007). This is the case at rest and with exercise as

oxygen desaturation does not occur (Dempsey and Wagner, 1999). However, during breath-holding this is not the case as arterial desaturation occurs. Over a 2 minute breath-hold from air, partial pressure of arterial oxygen falls from a resting value of ~ 100 mmHg to 54 mmHg (Lin et al., 1974). Hong *et al*., (1971) measured a reduction in arterial oxygen content 3.5% over a 2 minute breath-hold. Additionally, this fall is greater, the longer the breath-hold. Pingitore et al. (2008) measured arterial saturation (SaO_2) to fall from ~ 99 to 84% in trained breath-holders over breath-holds of 3.7 minutes (n = 10). Any oxygen consumed from the blood store is not accounted for when using this method. Therefore, measurement of arterial oxygen content or prevention of this fall with pre-oxygenation is necessary.

The three studies (Stevens *et al.,* 1946; Klocke and Rahn 1959; Hong *et al*. 1971). that have attempted to address what happens to metabolic rate during breath-holding in humans concluded that metabolic rate does not fall. However, the data quality and clarity of these studies is difficult to follow. Rates of metabolic rate at rest and during breath-holding are summarised in *Table 1*.

Table 1 – Summary of limited data from previous studies assessing metabolic rate prior to and during breath-holding

	Resting metabolic rate	Breath-hold metabolic rate
	(millilitres oxygen minute^{-1} STPD)	
Stevens *et al*., (1946) (n = 3)	291	250 – 500
Klocke and Rahn (1959) (n = 6)	-	300
Hong *et al*., (1971) (n = 2)	-	212

Stevens et al. (1946) indirectly assessed metabolic rate during breath-holding by assessing changes in buoyancy in 6 participants who performed breath-holds from total lung capacity and using room air during underwater weighing to assess pulmonary gas exchange. Participants' gradually gained weight (their buoyancy decreased) at a constant rate of 250 – 500 grams minute^{-1} for as long as they held their breath. The authors attributed weight gain to be oxygen taken from the lungs into the blood stream and not being equally replaced by metabolically produced carbon dioxide. This subsequently was directly examined by Hong et al. (1971) where they performed alveolar gas sampling over 4 minute breath-holds (dry static breath-hold form air at total lung capacity) and found that 698 millilitres of oxygen was extracted from the lung whereas only 160 millilitres of carbon dioxide was replaced. The rate of weight gain matched the rate of oxygen consumption at rest, measured to be 291 millilitres oxygen · minute^{-1} STPD using the Benedict-Roth metabolism apparatus following inhalation of air. But only in 3 participants. As oxygen consumed from the blood during these breath-holds was not accounted for, they used pre-oxygenated breath-holds (which prevents a fall in arterial oxygen saturation (Lin et al., 1974; Parkes, 2012; Parkes et al., 2016a; Parkes et al.,

2016b). With these breath-holds they found that the reduction in buoyancy (weight gain) was still present at a constant rate but exceeded the rate in which weight was gained when breath-holding from air. This suggests that even when accounting for oxygen consumed from the intrinsic blood store, the rate of oxygen consumption when breath-holding does not fall below resting values.

Klocke and Rahn (1959) measured the change in lung volume and alveolar gas composition with spirometry in 6 participants in breath-holds from total lung capacity, pre-breathing an oxygen gas mixture and voluntarily hyperventilating. They concluded that the measurable large changes in lung volume when breath-holding occurred as a result of oxygen taken up by the blood not being entirely replaced by the carbon dioxide produced: the total amount of carbon dioxide in the lungs at the start of the breath-hold was within a ~ 3 millimetres at the end of the breath-hold when accounting for the initial values and changes in lung volume in all 6 participants. Therefore, all changes in lung volume are attributed to oxygen uptake (as in Stevens *et al*., 1946) and this corresponded to a rate of oxygen consumption of 300 millilitres oxygen · minute^{-1} STPD. This value corresponds with Stevens *et al*. (1946) indicating that the rate of oxygen consumption does not fall below resting with a larger number of participants.

In 1971, Hong *et al.* attempted to directly measure the actual rate of oxygen consumption combining changes in lung volume over breath-holding and performing arterial blood sampling over a 4 minute breath-hold. Participant's mean initial lung volume was 5800 millilitres BTPS and over the course of the breath-hold this was reduced by 657 millilitres (STPD). Using Stevens *et al.* (1946) and Klocke and Rahn (1959) calculations this would have equated to a fall in resting oxygen consumption to 164 millilitres oxygen · minute^{-1} STPD. However, arterial sampling revealed that an extra 193 millilitres oxygen · minute^{-1}

STPD had been utilised from the blood, and therefore, total oxygen consumption over the 4 minute breath-hold was 212 millilitres oxygen · minute^{-1} STPD. These values fall under that of Stevens *et al.* (1946) and Klocke and Rahn (1959) (~ 300 millilitres oxygen · minute^{-1} STPD) and slightly below classic values of resting metabolic rate (~ 250 millilitres oxygen · minute^{-1} STPD) (Aub & DuBois, 1917; Harris and Benedict, 1918; Ferraro *et al*., 1992; Haugen et al., 2003) suggesting that metabolic rate may fall. Unfortunately, Hong et al., (1971) did not provide a resting measurement of metabolic rate to compare this to directly and only made measurements in 2 participants which is not a substantial number to definitively conclude what happens to metabolic rate when breath-holding (Parkes, 2006; McLoughlin and Drummond, 2017).

1.3 Present study

Despite these previous studies concluding metabolic rate during breath-holding does not fall below resting values they do not all assess this in a reasonable number of subjects or account for 1) arterial desaturation that occurs during breath-holding, 2) contraction of the spleen supplying additional oxygen to the circulation, and 3) anticipated fall in metabolic rate from the withdrawal of the metabolic cost associated with sustaining respiratory muscles. This study aims to overcome the short-comings of previous experiments to directly assess the rate at which oxygen is consumed over a breath-hold in a credible number of participants (n = 20).

1.3.1 Metabolic cost of breathing

It may be expected that metabolic rate may fall slightly when breath-holding. This is because contraction of the respiratory muscles are no longer at work and their associated metabolic cost is removed. To date there has been no discussion of the removal of the

metabolic cost of breathing whilst breath-holding influencing metabolic rate when breath-holding.

The metabolic cost of breathing has been previously quantified in exercise as ~ 10% of the maximum rate of oxygen consumption (Aaron et al., 1992) and accounts for 14 – 16% of cardiac output (Harms et al., 1998). Similar values, up to 15% of metabolic rate also during maximal exercise have been reported elsewhere (Bevegaard and Shepherd, 1966; Ishii et al., 2018). Values for the metabolic cost of breathing at rest remain unquantified.

1.3.2 Oxygen consumed from the blood

To limit the extent of arterial desaturation, breath-holds will be terminated at 90% of each participant's maximal breath-hold time. Any measured reduction in arterial saturation will be incorporated into calculations determining metabolic rate. The early termination of breath-holds also ensure that participants exhaled at breakpoint to allow for collection of expired gases. After very long breath-holds, it is possible to breathe in at breakpoint (Hill and Flack, 1908). To fully prevent any arterial desaturation from occurring, breath-holds will also be performed using pre-oxygenation.

1.3.3 Additional oxygen supplied from contraction of the spleen

Extra oxygen is supplied to the blood during breath-holding as a result of spleen contraction, which acts as a dynamic oxygen store containing red blood cells. Diving mammals have large spleens with Weddell seals (*Leptonychotes weddelli*) storing 20 litres of blood and ~ 75% of their total haemoglobin content in their spleen (Qvist et al., 1986). This is then injected into the circulation during dives. Qvist et al. (1986) measured that following a 12 minute dive, haematocrit had risen by 60% in Weddell seals (*Leptonychotes weddelli*). Others have made similar measurements (Hurford et al., 1996) and the spleen has since been described as a 'scuba tank' providing oxygen during dives (Zapol, 1987).

Humans have a comparably small spleens, (~ 10 times smaller than seals), containing only 200 – 250 millilitres of blood which accounts for ~ 8% of the total red blood cell content (Koga, 1979). Spleen volume is significantly correlated (r = 0.57) to breath-hold diving ability amongst elite divers (Schagatay, 2012) implying the importance of the spleen in humans.

There is substantial evidence of contraction of the spleen during breath-hold diving in humans. Hurford et al. (1990) ultrasonically measured spleen size before and after 6 metre dives in 5 Korean Ama divers and found a 20% reduction in spleen size (from 206 to 160 millilitres), a 10% increase in haemoglobin concentration and 6% increase in haematocrit. Schagatay et al. (2001) performed 5 maximal breath-hold with facial immersion in healthy volunteers and volunteers who had undergone a splenectomy. They measured a 6% increase in haematocrit and 3% increase in haemoglobin concentration in healthy participants. There was no change in these variables in participants who had a splenectomy and they could only breath-hold for half as long as the healthy participants. The magnitude of spleen contraction increases over the course of breath-holding. Palada et al. (2008) showed a reduction from a resting spleen volume of 283 millilitres in 7 trained divers during maximal breath-holds of 4.3 minutes to 227 millilitres at 2.7 minutes (described as the easy-going phase of breath-holding) and even further to 152 millilitres towards the end of breath-holding (described as the struggle phase). Subsequently, an increase in haemoglobin and haematocrit concentration results in enhanced oxygen transport when breath-holding.

Albeit a muted effect, breath-holding alone also results in contraction of the spleen. Espersen et al. (2002) determined contraction of the spleen in participants with and without breath-hold diving experience during breath-holding from air and breath-holding and facial immersion using erythrocyte radiolabelling technique and scintigraphic measuring. Responses

were greater with facial immersion but were similar regardless of training status. Following 2 minutes of breath-holding alone, the spleens relative content of red blood cells was significantly reduced by ~ 13% and the relative splenic area was ~ 12% smaller. With facial immersion, this was reduced by ~ 25% and ~19% respectively.

From the above studies, the general consensus is that haematocrit rises ~ 5% from contraction of the spleen during breath-hold diving which equates to 80 millilitres of additional blood volume being supplied to the blood (Fitz-Clarke, 2018).

1.4 Aims

The aims of this study are to measure and assess in 20 participants:

- ➢ Metabolic rate over breath-holds from air whilst accounting for oxygen consumed from the lungs, blood and spleen.
- ➢ Metabolic rate over breath-holds from oxygen accounting for oxygen consumed from the lungs and spleen, but where breath-hold duration is longer and arterial desaturation is prevented.
- ➢ The metabolic cost of breathing at rest and determine whether this accounts for any reductions in metabolic rate when breath-holding.

2. Methods

This experiment was approved by Walsall Local Research Ethics Committee (reference: 05/Q2708/53 RRK 3310) and the University of Birmingham (reference: ERN_16-0767). It conformed to both the Declaration of Helsinki and the Data Protection Act (1998). Informed, written consent was obtained from all participants prior to commencement of their involvement. Each participant completed a health check form, which self-reported height and weight measurements and admitted as participants to the Wellcome Trust Clinical Research Facility, Heritage Building, Queen Elizabeth Hospital Birmingham, UK. All experiments procedures following were conducted within this facility.

2.1 Participants

Twenty healthy participants (12 male and 8 female) aged 24 ± 5 (mean ± SD) years volunteered for and completed all experimental procedures detailed below. All were normotensive, non-smokers and of a healthy weight. Descriptive participant characteristics can be seen in Table 2.

Table 2 – Participant information.

Height	175 ± 6 cm
Weight	74 ± 10 kg
BMI	24 ± 6 kg.m^2
Resting blood pressure	
Systolic	111 ± 10 mmHg
Diastolic	56 ± 6 mmHg
Mean	74 ± 5 mmHg

Data presented as mean ± SD

All participants were recruited from The University of Birmingham. Exclusion criteria was as follows 1) history of respiratory, metabolic or cardiovascular disease/illness including asthma, coronary artery disease, hypertension, diabetes, renal failure, 2) latex allergy, 3) taking prescribed medication other than the contraceptive pill, 4) cold/flu like symptoms in the past 7 days.

Half of our participants (n = 10) had previous experience performing breath-holds (> 10 occasions) from pre-oxygenation and hypocapnia ($P_{ET}CO_2$ of 20 mmHg) as outlined in (Cooper et al., 2004; Parkes et al., 2014; Parkes et al., 2016a; Parkes et al., 2016b). The remaining 10 participants had no previous experience of breath-holding and were not familiar with the laboratory or experimental procedures outside of their involvement of this study.

2.2 Familiarisation and safety limits

Prior to the 3 experimental trials, participants were familiarised with the laboratory set up and measurements below. During this training session participants were 1) instructed on how to hold their breath, 2) accustomed with mechanical ventilation, and 3) given information regarding their safety whilst breath-holding.

Throughout all parts of this study, participants lay, at rest in a semi-recumbent position and listened to music, as shown in Figure 1. The following continuous measurements were made: 1) beat by beat blood pressure analysis using a finger plethysmograph (Finapres® 2300, Ohmeda, Englewood, CA) set at heart level; 2) heart rate analysis via a 3-lead chest electrocardiogram (lead configuration I); 3) arterial oxygen saturation (S_pO_2) using a finger pulse oximeter (Ohmeda, Engelwood, CA); 4) end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) was measured using inline capnography (Hewlett Packard 78536A; Hewlett Packard, Palo Alto).

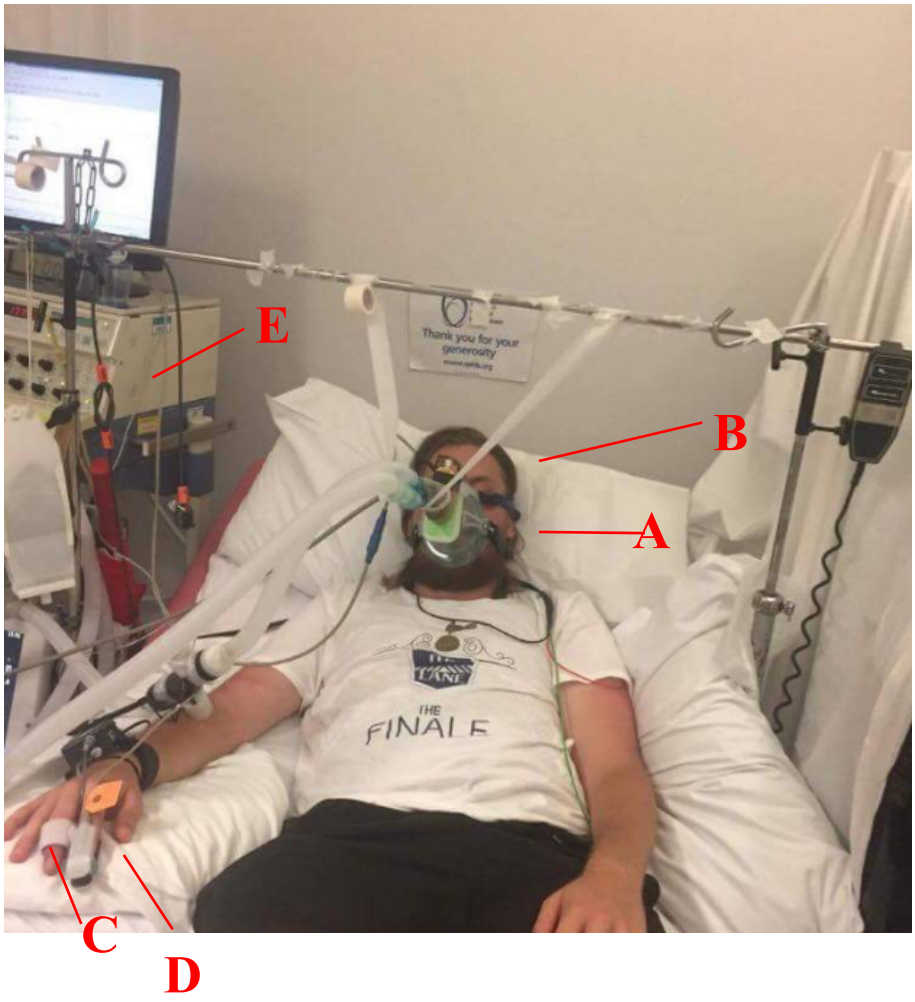

Figure 1 – Participant at rest during experiments breathing through disposable facemask and filter (A) with in line capnograph and pressure transducer (B). Blood pressure measured continuously through Finapres (C) and oxygen saturation levels via an oximeter (D). Mechanical ventilator is also visible (E) and Douglas bags located on the expiratory side of this (not visible).

2.2.1 Breath-hold familiarisation

To ensure all participants held their breath in a similar fashion, and were confident in doing so, they were given instruction by the investigator on this. In their own time, they were asked to take the largest breath they could, maximally inflating their lungs and pushing their diaphragm down and outwards. They were then instructed to fully exhale, repeat previous maximal inhalation and hold their breath whilst remaining as still and as relaxed possible. Participants were also reminded to close off their mouth and nasal palate internally. Any exhalation or inspiration during the breath-hold could be easily identified by changes in pressure detected by a pressure transducer and by eye.

As practice is known to influence breath-hold duration, participants were given the opportunity to complete two practice maximal breath-holds following this initial instruction. They then, when instructed, performed a maximal breath-hold from air and with pre-oxygenation without hyperventilation. We measured $P_{ET}CO_2$ throughout training and experiments, which did not fall below resting values prior to breath-holds on any occasion, confirming that no hyperventilation occurred.

2.2.2 Mechanical ventilation familiarisation

Participants who were unaccustomed to mechanical ventilation (n = 10) were familiarised with this procedure. At first, each participant held the face mask over their mouth and nose whilst the mechanical ventilator (Dräger Evita 2 ventilator, Däger, Luebeck, Germany) in the positive intermittent pressure setting, where air is pushed towards the participant, at their measured rate and depth. This allowed them to become accustomed with the sensation of ventilation whilst remaining in full control because this allowing them to quickly remove the facemask if necessary. Participants were then coached to breathe in when

the ventilator pushed air at their mouth. They were then encouraged to stop breathing in with the ventilator, to allow the ventilator to take over, and be passively ventilated.

Once participants were happy with this, the facemask was secured with a strap around the back of the head. Participants then underwent 5 minutes of mechanical ventilation in order to practice this.

2.2.3 Safety limits

Participants were reminded breath-holds were safe to perform in this way and informed of the conservative safety limits in place. As breath-holding from pure oxygen increases the risk of atelectasis (Klocke and Rahn, 1959; Agostoni, 1963), all breath-hold with oxygen were made using a gas mixture consisting of 60% oxygen. All breath-holds were carried out according to Parkes et al. (2014) safety guidelines where breath-holds were terminated if systolic blood pressure rose above 180 mmHg and/or SpO_2 fell below 94%.

2.3 Experimental protocol

For clarity the experimental visits will also be referred to as days 1 to 3. Figure 2 outlines the protocols for the following visits and indicates the durations over which a measurement of metabolic rate was made. Resting metabolic rate was measured over 5 minutes of eupnoea at the start of each day.

2.3.1 Day 1

Within this 'control trial' in which no breath-holding occurred, we wished to confirm metabolic rate could be consistently measured over a single breath using indirect calorimetry. This was necessary as our measurement of metabolic rate over breath-holding had to be made from one single breath.

We wanted to confirm that we could measure resting metabolic rate from one normal single breath. Therefore, metabolic rate was measured over two separate single breaths.

As breath-holding occurred following a maximum breath in and breath out, we also wished to confirm that this manoeuvre did not result in metabolic rate readings falling below resting values. Therefore, we also measured metabolic rate over two separate maximal breaths.

2.3.2 Day 2

On this visit, we measured participant's metabolic rate when breath-holding from air. Participants were asked to break the breath-hold at 90% of their maximum duration (the terminated breakpoint) measured during familiarisation. This was done to 1) prevent substantial arterial desaturation occurring and 2) ensure that participants were able to exhale their lung contents before taking a breath

2.3.3 Day 3

On this visit, we measured participant's metabolic rate when breath-holding from oxygen. Again, participants were asked to break the breath-hold at 90% of their maximum duration (the terminated breakpoint) measured during familiarisation. Here this was done primarily to ensure that participants were able to exhale their lung contents before taking a breath, as no arterial desaturation occurs in these breath-holds.

During this visit we also measured the metabolic cost of breathing by measuring metabolic rate. This was done by mechanical ventilating participants with positive pressure at their normal rate and depth which measured during the initial resting 5 minutes of metabolic rate measurement in air.

To confirm breathing oxygen did not alter metabolic rate, we made an additional measurement of metabolic rate during 5 minutes of eupnoea whilst participants breathed oxygen.

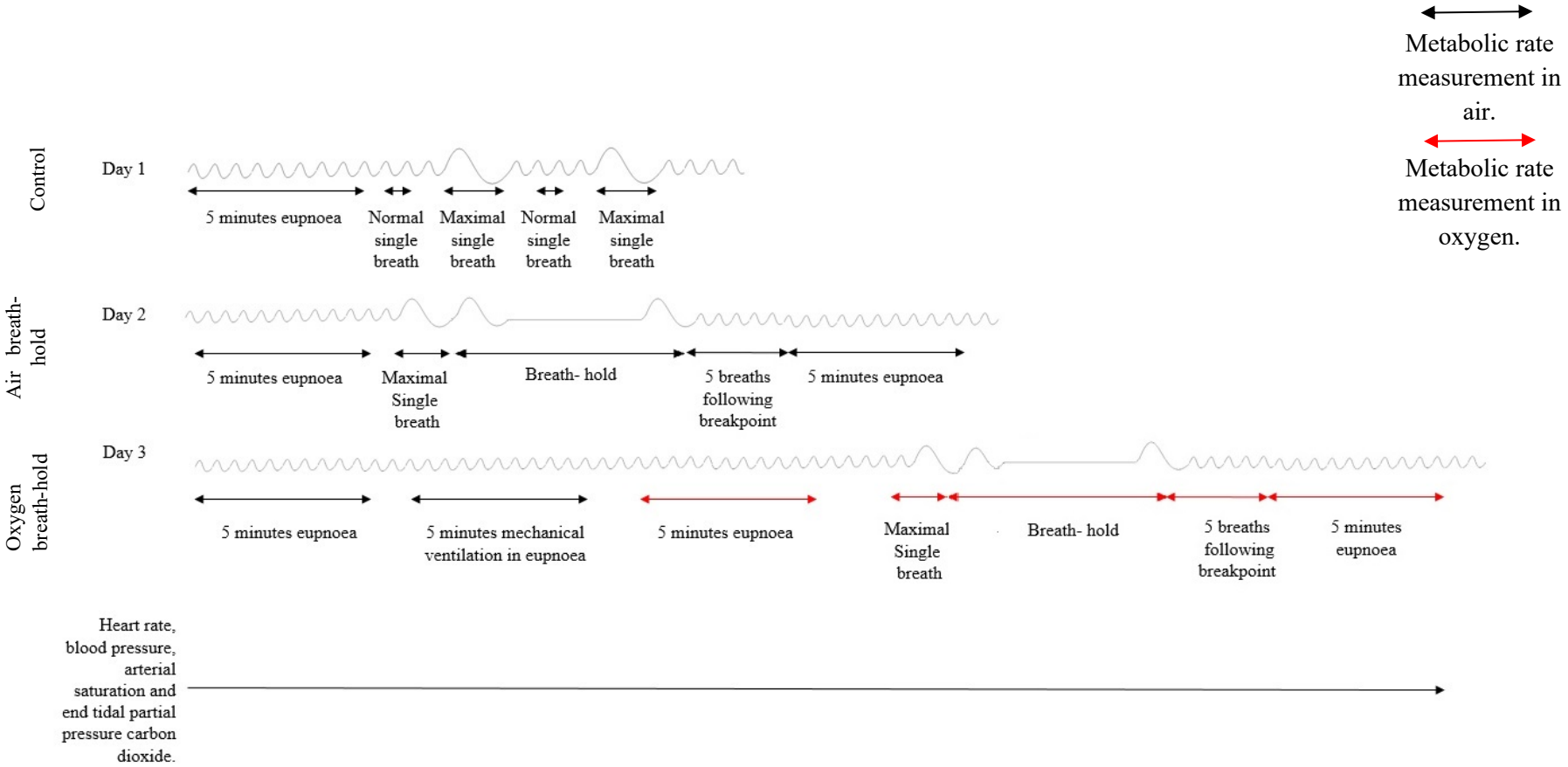

Figure 2 – Experimental protocol on each visit. Trace is representative of airflow. ↔ indicates period over which metabolic rate measurement is taken via collection of expired air into a Douglas bag. When in red ↔ denotes when participants are breathing oxygen. Continuous measures of heart rate, blood pressure, arterial saturation and end tidal partial pressure of carbon dioxide are also taken.

2.4 Measurement of metabolic rate

To assess the rate of oxygen consumption, expired gas samples were collected using Douglas bags (Cranlea, Birmingham, UK). To ensure that the entire respiratory cycle was accounted for, sampling was made from end-expiration to end-expiration. Samples were then analysed for oxygen and carbon dioxide gas composition (Dräger vamos anaesthetic gas analyser, Datex-Ohmeda, General Electric Company Healthcare, Worldwide) which was calibrated with gas samples of (21.0%, 15.1% and 0.0% oxygen). Expiratory gas volume was measured using a flowmeter (Branta, United Kingdom) positioned on the expiratory side of the respiratory circuitry. This was validated with linear calibration over 0.02 – 3 litres (Hans Rudolph Inc, Shawnee, KS) prior to each experiment and to ensure accuracy, recoveries over the same volume range were performed.

The numerical analyses used to calculate the respective volumes of oxygen consumed and carbon dioxide produced were as follows:

$$VO_2 = (ViXFiO_2) - (VeXFeO_2)$$

$$VCO_2 = (ViXFiCO_2) - (VeXFeCO_2)$$

For measurements from air it was assumed that inspired air contained 20.9% oxygen, 79.1% nitrogen, and 0.04% carbon dioxide. When participants breathed 60% oxygen, we confirmed FiO_2 = 60% by briefly attaching the gas analyser to the inspiratory circuit. Therefore, the inspired gas during the oxygen trial was 60% oxygen, 40% nitrogen, and 0.04% carbon dioxide.

In lieu of measuring the inspired gas volume by means of a flowmeter placed on the inspiratory side of the circuitry or the participant inspiring air from a Douglas bag filled with a known gas volume, the Haldane Transformation:

$$V_t = V_E X \frac{F_{EN_2}}{F_{IN_2}}$$

$$V_{O_2} = V_E X \frac{F_{EN_2}}{F_{I_{N_2}}} X F_{IO_2} - V_E X F_{EO_2}$$

was used. This is based on the assumption that N_2 content remains constant between inspired and expired samples as it is neither consumed nor produced by the body. The barometric pressure and room temperature during each experiment were taken in order to convert values into STPD.

When calculating the rate of oxygen consumption when participants were given 60% oxygen to breathe we had to use VCO_2 and extrapolate from each participant's ratio of VO_2:VCO_2 production when breathing at eupnoea in air because this level of hyperoxia is outside the normal functioning range of the gas analyser. To confirm that this extrapolation was accurate we measured separately the VO_2 and VCO_2 in air at rest and from breath-holding. We then confirmed that the measured VO_2 produced for the breath-hold in air was not different from what it was calculated using the extrapolation from VCO_2 during the breath-hold.

2.5 Measurement of the metabolic cost of breathing

We measured the decrease in metabolic rate when participants were passively ventilated via positive pressure mechanical ventilation (Dräger Evita 2 ventilator, Dräger, Luebeck, Germany) over a 5 minute period. Participants were ventilated at their measured eupnoeic depth and frequency at their eupnoeic level of $P_{ET}CO2$. This was calculated by averaging the tidal volume and breaths · minute^{-1} for each individual during an initial 5 minute period in which participants were at rest.

2.6 Allowance for measured arterial desaturation

Measurement of metabolic rate using indirect calorimetry does not account for oxygen consumed from the blood store. We attempted to prevent any reductions in arterial saturation by forcing participants to resume breathing at 90% of their maximum breath-hold (as well as to ensure they were able to fully expire). In addition, we measured arterial oxygen saturation throughout all experiments and any reductions in arterial saturation following breakpoint were measured. The finite lag present using indirect pulse oximetry (Parkes et al., 2014) meant that arterial desaturation at breakpoint was measured to be the lowest trough within the immediate 20 seconds following the resumption of breathing.

We calculated the additional oxygen provided by reductions in arterial saturation. This was done by calculating the reduction in SpO_2 over a breath-hold, establishing the content of oxygen this accounted for per decilitre of blood using the oxygen content equation:

$$CaO_2 = (SaO_2 x Hb x 1.34) + o.oo4(PaO_2)$$

Assuming normal haemoglobin concentration (15 grams decilitre^{-1} of blood) and PaO_2 being established from SaO_2 values using the oxyhaemoglobin curve (assuming a pH of 7.4 and body temperature of 37^{o}C). No to measures establish if participants had normal haematology were carried out. We then established total blood volume for each participant based on their sex, height and weight (Nadler et al., 1962):

$$Males\ total\ blood\ volume\ (millilitres) = (0.006012\ x\ height\ (centimeters)^3) + (14.6\ x\ weight\ (kilograms)) + 604$$

$$Females\ total\ blood\ volume\ (millilitres) = (0.005835\ x\ height\ (centimeters)^3) + \big(15\ x\ weight\ (kilograms)\big) + 183$$

The amount of additional oxygen consumed for each participant's total body blood volume was then added to each individuals measured rate of oxygen consumption from the expired gas samples.

We confirmed this adjustment was unnecessary for breath-holds with pre-oxygenation because we established that SpO_2 does not fall.

2.7 Allowance for contraction of the spleen

As the spleen contracts during breath-holding in humans, resulting in available oxygen being added into the circulation, it is also necessary to make reasonable adjustments to the measured rate of oxygen consumption. Typical values for human breath-hold diving indicate a ~ 5% increase in blood haematocrit (HCT) (Hurford et al., 1990; Schagatay et al., 2001; Espersen et al., 2002; Schagatay et al., 2012) resulting from a 200 millilitre reduction in splenic volume (Fitz-Clarke, 2018) (assuming a blood volume (V_B) = 5 litres, spleen contents = 100% red blood cells at 1.0 haematocrit and normal typical circulating haematocrit volume of 0.45) raises circulating haematocrit to 0.47:

$$\Delta Hct = [V_B(Hct1) + \Delta V_B(1.0)]/(V_B + \Delta V_B)$$

$$= [5.0(0.45) + 0.2(1.0)]/(5.0 + 0.2)$$

$$= 0.47$$

Our use of the Nadler Equation to estimate participant's blood volume was substituted instead of using 5 litre value. But as our participants estimated blood volume yielded a mean of 4.9 ± 0.6 litres the same result was found.

Fitz-Clarke (2018) used this to calculate that 80 millilitres of oxygen of additional oxygen was added to the blood volume (assuming blood oxygen concentration (CaO_2) consisted of the following: red blood cells are 100% saturated, haemoglobin concentration is 140 grams $litre^{-1}$ and each gram of fully saturated haemoglobin holds 1.34 millilitres oxygen):

$$\Delta O2 = CaO_2 \Delta V_B / Hct$$

$$= 1.34(140)(0.2)/0.47$$

$$= 80mL$$

Therefore, 80 millilitres oxygen was added to the total oxygen consumed over a breath-hold and metabolic rate during breath-holding was then expressed as millilitres oxygen per minute of breath-holding.

2.8 Data analysis

Data were sampled at a rate of 2 kHz and analysed using a CED1401 data acquisition system (Spike2, Cambridge Electronics Design, Cambridge, UK). We identified each heartbeat, blood pressure peaks and troughs in order to plot lines of instantaneous heart rate (in beats per minute) and blood pressure. To assess changes that occur in heart rate and blood pressure that occur during breath-holding, data were normalised (0-100%) over each breath-hold during experimental trials. This allowed us to correctly align data in all participants at the same time points. Data were sampled 5 seconds prior to the onset of breath-holding (-5%), which is defined as beginning of the maximum inhalation prior to the breath-hold (0%), and the first inhalation at breakpoint (100%). For the air breath-holds, data were sampled at 240 time points. To maintain resolution during the prolonged breath-holds from oxygen, data were sampled at 960 time points. Data were then exported to Excel (Microsoft, Redmond, WA), where for each participant we calculated mean heart rate, blood pressure, end tidal partial pressure of carbon dioxide and arterial saturation for the period of each Douglas bag sample. Once rates of oxygen consumption had been calculated these were also inputted into the spreadsheet for each participant over each time period.

2.9 Statistical analysis

Statistical analyses were conducted using PASW® statistics v. 22.0 (SPSS Inc., Chicago IL). We used a paired experimental design in which each participant was compared against their own experiment for each condition. For each participant, their mean values were compared and, where appropriate, the following tests were used 1) repeated-measures analysis of variance (ANOVA) using within subject contrasts, 2) paired-samples t-tests, and 3) two-tailed correlation analysis. There was no difference between males and females and all data were combined unless otherwise specified, with the exception of the metabolic cost of breathing where n = 10.

Significance was set at $p < 0.05$. Data presented as means for n = 20 participants (unless otherwise stated) and ± standard deviation of the mean.

3 Results

3.1 At rest

Resting minute ventilation, $P_{ET}CO_2$, arterial saturation, heart rate and blood pressure measured over 5 minutes was consistent between experimental days (days 1-3) and when participants breathed oxygen (day 3). Therefore, these data were combined and the average resting values were as follows: minute ventilation = 9.8 ± 1.2 breaths · minute^{-1} BTPS (days 1-3 in air and oxygen), $P_{ET}CO_2$ = 37 ± 2 mmHg, heart rate = 64 ± 8 beats · minute^{-1}, systolic blood pressure 111 ± 12 mmHg, mean arterial blood pressure = 75 ± 8 mmHg, and diastolic blood pressure = 57 ± 8 mmHg.

Resting arterial saturation measured over 5 minutes was consistent between experimental days when breathing air (97 ± 1%) (days 1-3), but, was significantly higher when breathing oxygen at rest (99± 1%) (day 3) ($p < 0.001$).

3.1.1 Metabolic rate

Figure 3 shows measurement of resting metabolic rate measured over 5 minutes was consistent between experimental days (days 1-3) and when participants breathed oxygen (day 1). This data were combined and the average resting metabolic rate measured over 5 minutes was 227 ± 39 millilitres oxygen · minute^{-1} STPD (days 1-3 in air and oxygen).

Figure 4 shows we were able to consistently measure resting metabolic rate from two separate single, normal sized breath from air. Metabolic rate was not different between each separate attempt (227 ± 59 *vs* 247 ± 71 millilitres oxygen · minute^{-1} STPD) nor was the average resting metabolic rate for the two single breaths (237 ± 71 millilitres oxygen · minute^{-1} STPD) different from measurements made over 5 minutes of eupnoea (days 1-3 in air and oxygen).

Also shown in Figure 4 is that performing maximal single breaths apparently increased metabolic rate to 467 ± 134 millilitres oxygen · minute^{-1} STPD (days 1-3 in air and

oxygen) both above average resting metabolic rate measured over 5 minutes of eupnoea (days 1-3 in air and oxygen) ($p < 0.001$) and averaged metabolic rate from a single, normal sized breath (day 1 in air, $p < 0.001$). Metabolic rate data were combined for all maximal single breaths as there was no difference across experimental days (days 1-2) and was not affected by breathing oxygen (day 3).

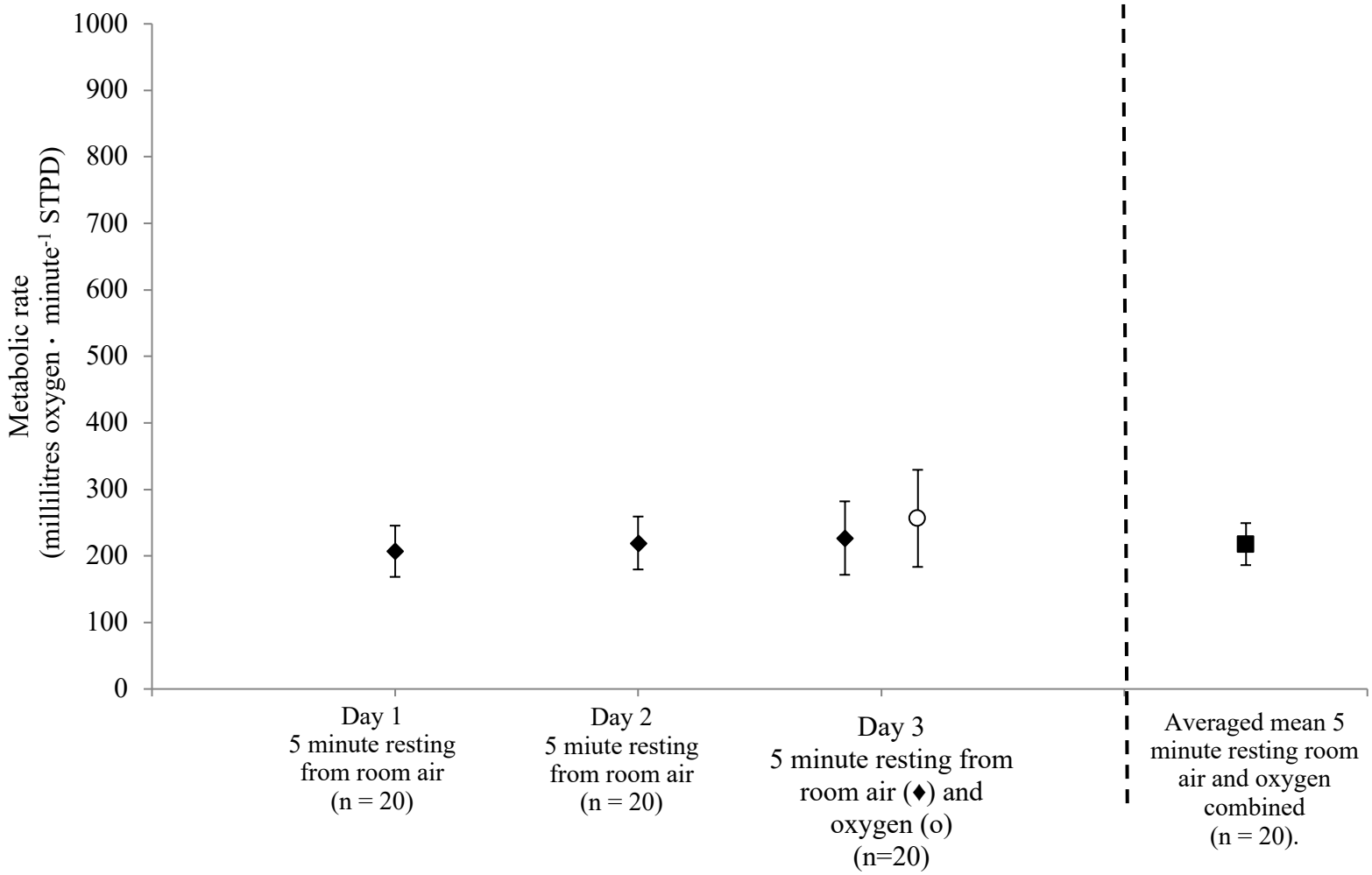

Figure 3 – Resting metabolic rate measured over 5 minutes of eupnoea did not differ on experimental days ($p > 0.05$, *ns*) nor when participants breathed oxygen ($p > 0.05$, *ns*). Room air represented by ♦, oxygen by o, and combined average from air and oxygen represented by ■.

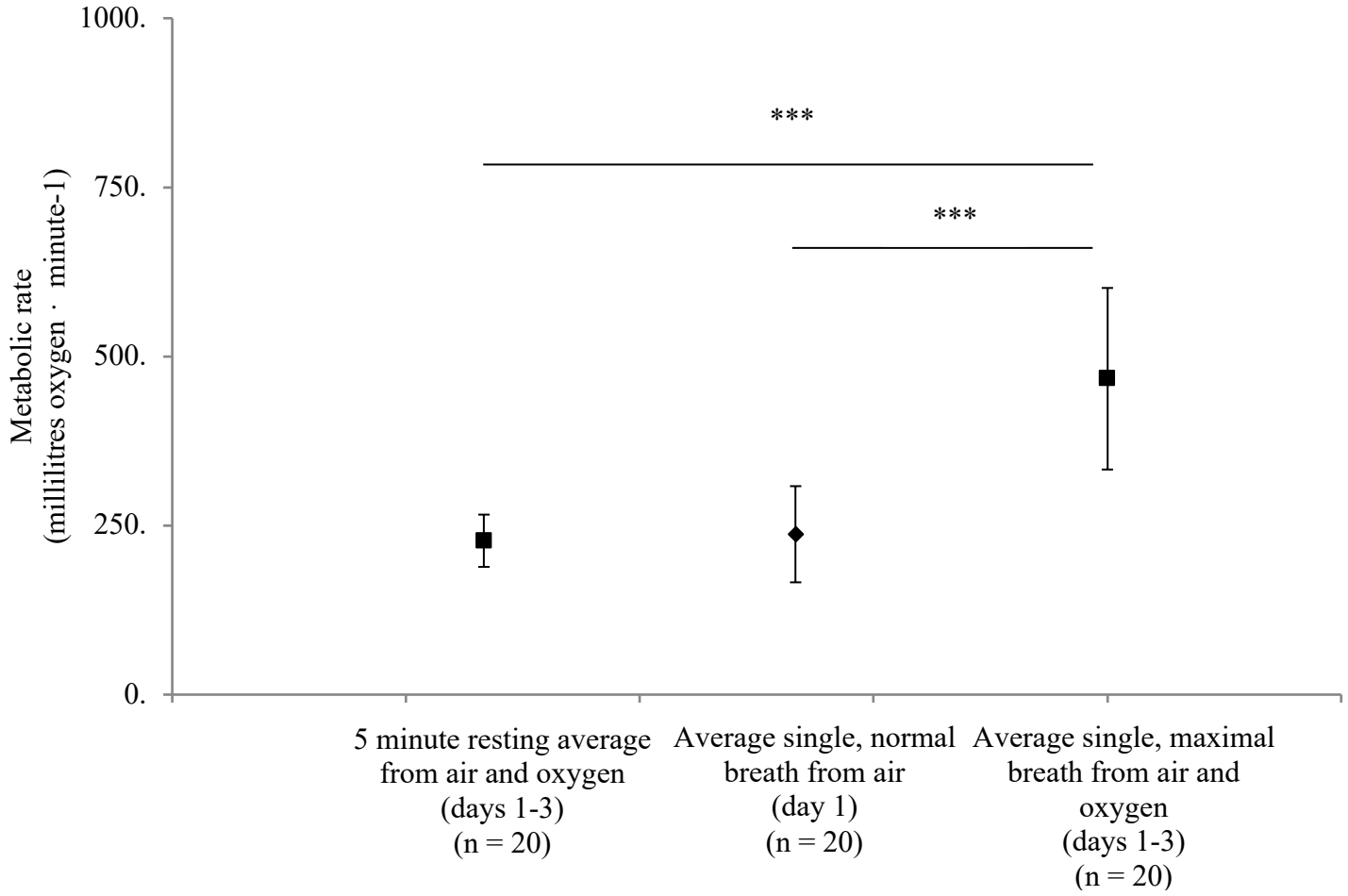

Figure 4 – Average resting rate of oxygen consumption measured over 5 minute in air and oxygen (days 1-3) was not different from the average of one normal single breath in air (day 1). But, was elevated when performing a single maximal breath from the resting rate measured over 5 minutes ($p < 0.001$) and a normal single breath ($p < 0.001$). Room air represented by ♦ and combined average from air and oxygen represented by ■. *** denotes $p < 0.001$.

3.1.2 Metabolic cost of breathing

During mechanical ventilation, participants ventilated at the same rate and frequency as in eupnoea. Minute ventilation was 8.8 ± 2.7 litre · minute^{-1} BTPS and this was not different from average resting minute ventilation measured over 5 minutes in air and oxygen (days 1-3).

Mean resting metabolic rate in the 10 experienced subjects fell by 60 ± 40 millilitres oxygen · minute^{-1} STPD ($p < 0.001$) from their average resting metabolic rate from air and oxygen (days 1-3) (225 ± 41 millilitres oxygen · minute^{-1} STPD) when they were mechanically ventilated (165 ± 72 millilitres oxygen · minute^{-1} STPD) ($p < 0.01$) (*Figure 6*).

Measurements were made in the 10 inexperienced participants, but they were actively breathing whilst being mechanically ventilated. The Spike2 polygraph (*Figure 5*) highlights this as there is no uniformity between breaths applied by the ventilator. Therefore, we measured no change in averaged mean resting metabolic rate in these 10 participants (*Figure 6*).

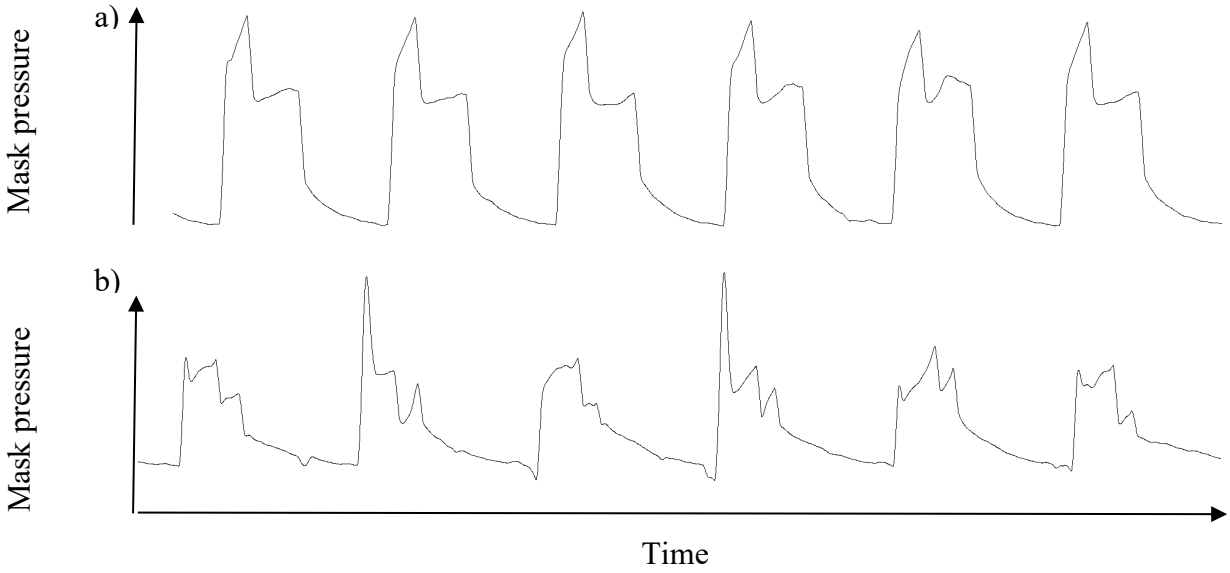

Figure 5 – Spike2 polygraph with from 2 different participants. 5a) is an example participant who is being passively ventilated (n = 1) and 5b) is an example of a participant who is actively breathing in with the ventilator (n = 1).

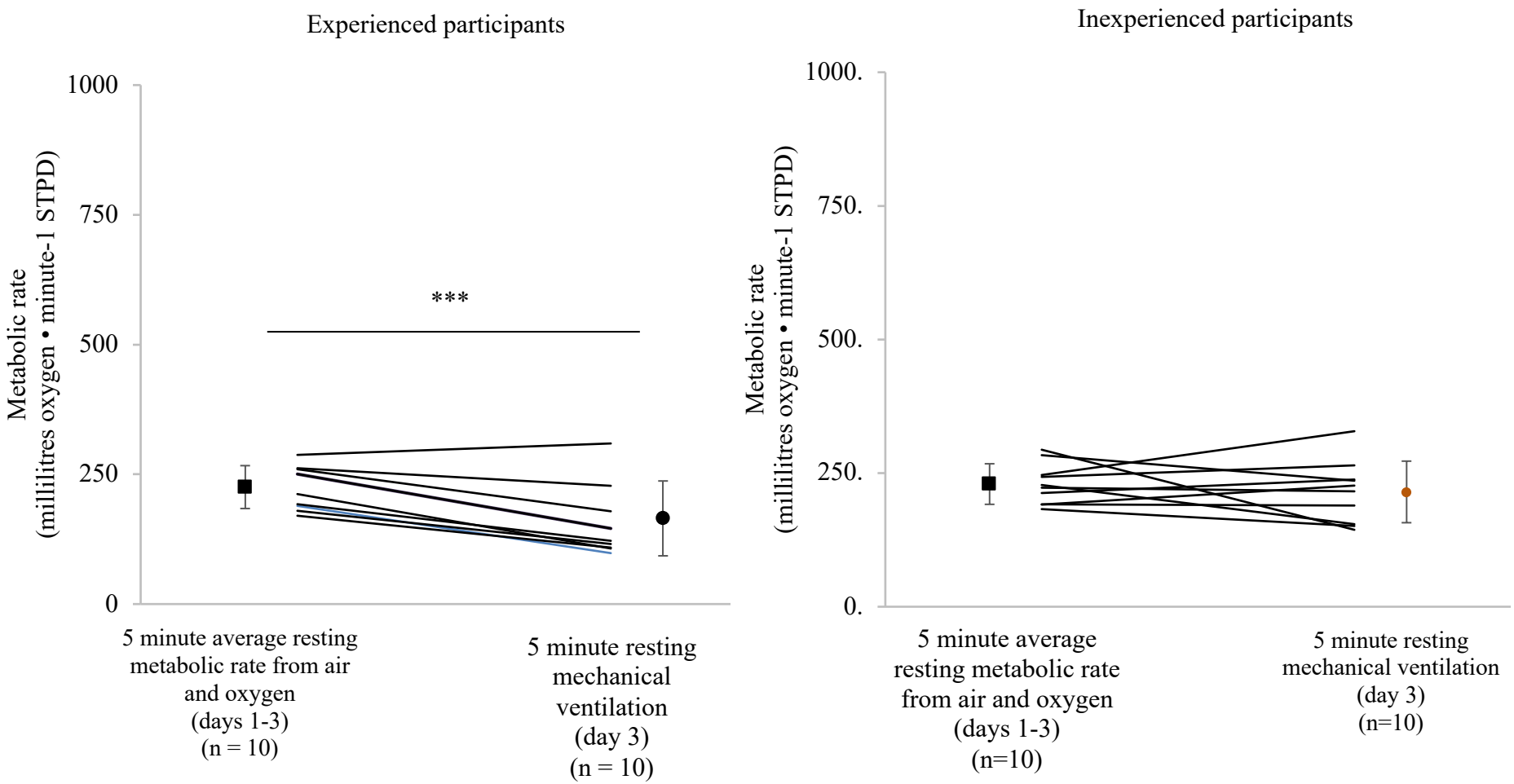

Figure 6 – Resting metabolic rate measured over 5 minutes in air and oxygen (days 1 – 3) falls when experienced participants are mechanically ventilated ($p < 0.001$) ($n = 10$), but not when inexperienced participants are mechanically ventilated ($n = 10$). Each line represents $n = 1$. Room air represented by ♦ and combined average from air and oxygen represented by ■. *** denotes $p < 0.001$.

3.2 Breath-holding

During experimental breath holds, the imposed 90% maximum breath-hold duration in air was 1.3 ± 0.4 minutes (93 ± 5% of maximum) and significantly longer at 2.2 ± 0.7 minutes (88 ± 4% of maximum) in oxygen ($p < 0.001$).

$P_{ET}CO_2$ was significantly greater than average resting values from air and oxygen (days 1 - 3) at the breakpoint when breath-holding from both air (43 ± 3 mmHg) ($p < 0.01$) and from oxygen (49 ± 3 mmHg) ($p < 0.01$).

At the breakpoint of the maximal breath-holds, heart rate did not change from average resting values (days 1 – 3) when breath-holding from air nor from when breath-holding from oxygen. *Figure 7* shows that mean continuous heart rate throughout breath-hold from air and oxygen does not fall below resting values.

Figure 8 depicts blood pressure changes with breath-holding from air and oxygen. When breath-holding from air, systolic blood pressure progressively rose by 21 ± 11 mmHg ($p < 0.001$), mean arterial blood pressure rose by 18 ± 13 mmHg ($p < 0.001$), and diastolic rose to 17 ± 13 mmHg ($p < 0.001$). When breath-holding from oxygen, systolic blood pressure progressively from average resting values (days 1-3) by 20 ± 19 mmHg ($p < 0.001$), average blood pressure rose by 13 ± 9 mmHg ($p < 0.001$) and diastolic rose by 15 ± 12 mmHg ($p < 0.001$). There was no significant difference between the rise at breakpoint in the experimental trials between air and oxygen.

At breakpoint following breath-holds from air, SpO_2 fell from average resting SpO_2 in air only (days 1 – 3) values by 2 ± 4% ($p < 0.01$). SpO_2 did not change from resting values during breath-holds from oxygen (SpO_2 at breakpoint was 99 ± 1%).

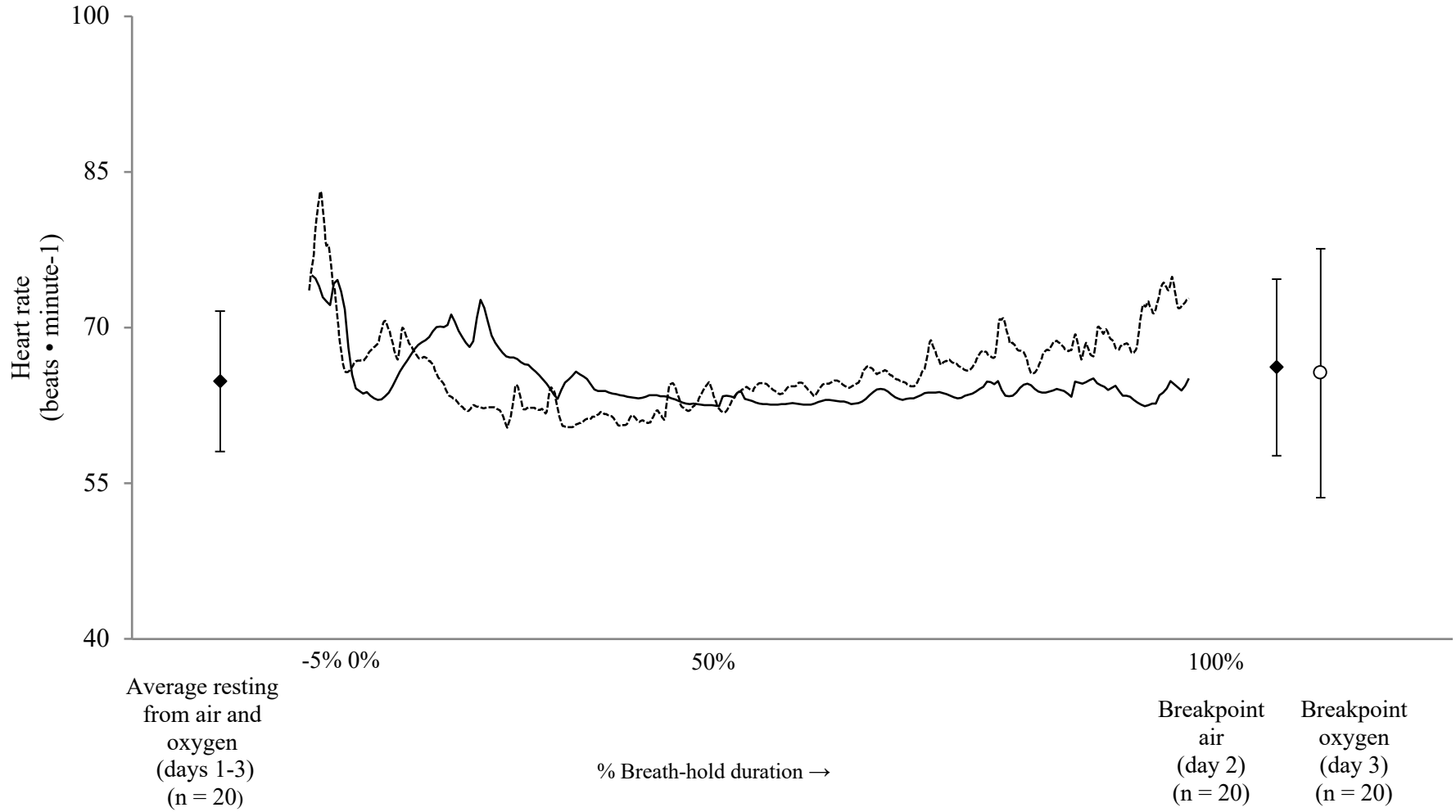

Figure 7 – Mean heart rate does not change from resting values during 90% maximal breath-holding from air (solid line) and oxygen (broken line). Average resting heart rate from air and oxygen (days 1-3) was not different at breakpoint (last 10 seconds of breath-holding, when performing 90% terminated breath-holds from air (day 2) and from oxygen (day 3). Room air represented by ♦, oxygen by o, and combined average from air and oxygen represented by ■.

Figu).

Average resting blood pressure in air and oxygen (days 1-3) and averaged over the last 10 seconds prior to breakpoint when performing 90% terminated breath-holds from air (day 2) ($p < 0.001$) and from oxygen (day 3) ($p < 0.001$). Room air represented by ♦, oxygen by o, and combined average from air and oxygen represented by ■. *** denotes $p < 0.001$

3.2.1 Metabolic rate over breath-holds from air

After adjusting for arterial desaturation and contraction of the spleen, metabolic rate when breath-holding in air fell in all 20 participants by a mean of 75 ± 49 millilitres oxygen · minute^{-1} from average metabolic rate at rest in air and oxygen (days 1-3) ($p < 0.001$) (*Figure 9*).

The greatest reduction in metabolic rate was not indicative of the longest breath-hold from air, as the associated fall in metabolic rate was not correlated with breath-hold duration ($r(18) = 0.36$).

In the 10 experienced participants, when breath-holding from air metabolic rate fell by a further 9 ± 36 millilitres oxygen · minute^{-1} STPD ($p < 0.05$) than metabolic rate when they were being mechanically ventilated. There was no significant difference in the reduction in metabolic rate from average resting metabolic rate from air and oxygen (days 1-3) between the experienced (reduced by 77 ± 35 millilitres oxygen · minute^{-1} STPD) ($n = 10$) and inexperienced ($n = 10$) participants (reduced by 75 ± 42 millilitres oxygen · minute^{-1} STPD) ($p < 0.05$, *ns*).

3.2.2 Metabolic rate over breath-holds from oxygen

Arterial desaturation did not occur during breath-holds from oxygen, but after accounting for oxygen from contraction of the spleen, we measured metabolic rate to be 96 ± 26 millilitres oxygen · minute^{-1} STPD. This is significantly lower than average metabolic rate at rest in air and oxygen (days 1-3) ($p < 0.001$) (*Figure 9*) and metabolic rate when breath-holding from air ($p < 0.001$) (*Figure 8*).

The greatest reduction in metabolic rate was not indicative of the longest breath-hold from oxygen, as the associated fall in metabolic rate was not correlated with breath-hold duration ($r(18) = 0.41$).

In the 10 experienced participants, when breath-holding from air metabolic rate fell by a further 63 ± 38 millilitres oxygen · minute^{-1} STPD ($p < 0.01$) than metabolic rate when they

were being mechanically ventilated ($p < 0.05$). There was no significant difference in the reduction from average resting metabolic rate from air and oxygen (days 1-3) between the experienced (reduced by 119 ± 49 millilitres oxygen · $minute^{-1}$ STPD) (n = 10) and inexperienced (n = 10) participants (reduced by 131 ± 43 millilitres oxygen · $minute^{-1}$ STPD).

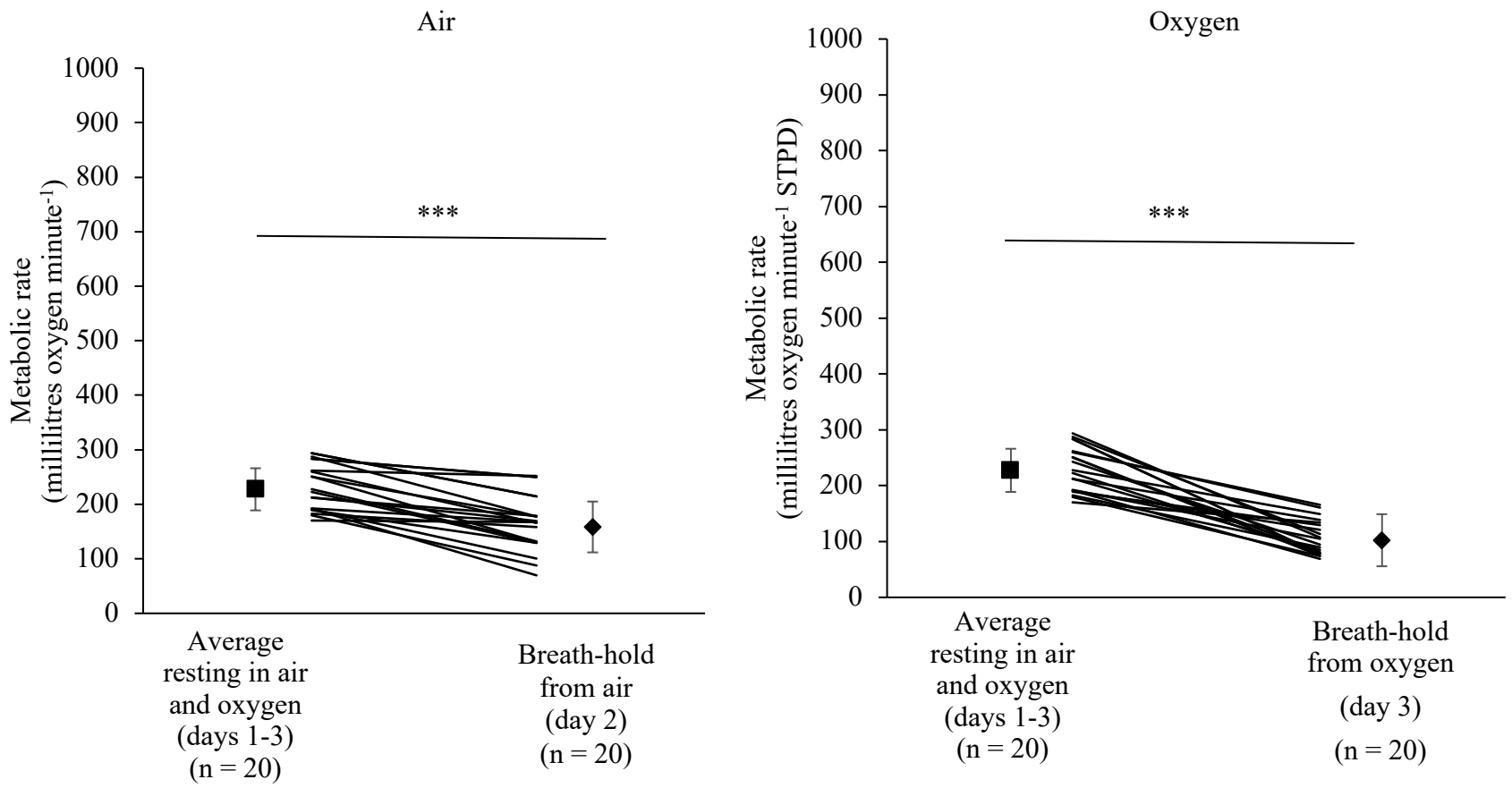

Figure 9 – Metabolic rate fell from average resting values from air and oxygen (days 1 – 3) in all 20 participants when breath-holding from air ($p < 0.001$) and breath-holding with pre-oxygenation ($p < 0.001$). Each line represents (n = 1). Room air represented by ♦ and combined average from air and oxygen represented by ■. *** denotes $p < 0.001$.

3.3 Following breath-holding

3.3.1 Immediately

Immediately, (in the next 5 breaths), average resting minute ventilation in air and oxygen (days 1-3) following the breakpoint was elevated when breath-holding in air to 17 ± 6 litre · minute^{-1} BTPS ($p < 0.001$) and significantly more when breath-holding from oxygen to 22 ± 7 litre · minute^{-1} BTPS ($p < 0.001$ *vs* average resting) ($p < 0.001$ *vs* breath-holding from air).

$P_{ET}CO_2$ following breath-holds from air (36 ± 2 mmHg) and oxygen (36 ± 2 mmHg) were not different from average resting values from air and oxygen (days 1-3) nor each other.

Arterial saturation was not different from average resting values from air only (days 1-3) when breath-holding from air 96 ± 2 mmHg, nor was it different from resting values in oxygen (day 3) (99 ± 1 mmHg).

Average resting heart rate in air and oxygen (days 1-3) following the breakpoint was elevated when breath-holding from air (67 ± 8 beats · minute^{-1}) ($p < 0.01$) and further in oxygen (72 ± 9 beats · minute^{-1}) ($p < 0.01$).

Blood pressure measurements were not different from average resting values in air and oxygen (days 1 – 3) when breath-holding from air (systolic = 108 ± 29 mmHg; mean arterial = 78 ± 10 mmHg; diastolic = 60 ± 9 mmHg) and from oxygen (systolic = 117 ± 16 mmHg; mean arterial = 77 ± 11 mmHg; diastolic = 60 ± 12 mmHg).

3.3.1.1 Metabolic rate immediately following breakpoint

Following breath-hold from air, metabolic rate overshoots average resting metabolic rate in air and oxygen (days 1 – 3) by 199 ± 132 millilitres oxygen · minute^{-1} STPD ($p < 0.001$). This overshoot when breath-holding from oxygen was significantly greater than average resting metabolic rate ($p < 0.001$) and greater than when breath-holding from air ($p < 0.001$) (*Figure 10*).

3.3.2 Recovery

In the following 5 minute period following breath-holding from air, minute ventilation (10 ± 2 litre · minute^{-1} BTPS) fell to average resting minute ventilation in air and oxygen (days 1-3). But, remained slightly elevated when breath-holding from oxygen (13 ± 3 litre · minute^{-1} BTPS) ($p < 0.01$).

Average resting heart rate in air and oxygen (days 1-3) was not different from when breath-holding from air (63 ± 9 beats · minute^{-1}) and from oxygen (64 ± 8 beats · minute^{-1}).

3.3.2.1 Metabolic rate during recovery

In the following 5 minutes, metabolic rate was not different from the average resting values from air and oxygen (days 1-3) when breath-holding from air. But, remained elevated above averaged resting metabolic rate from air and oxygen (days 1-3) ($p < 0.001$) (*Figure 10*).

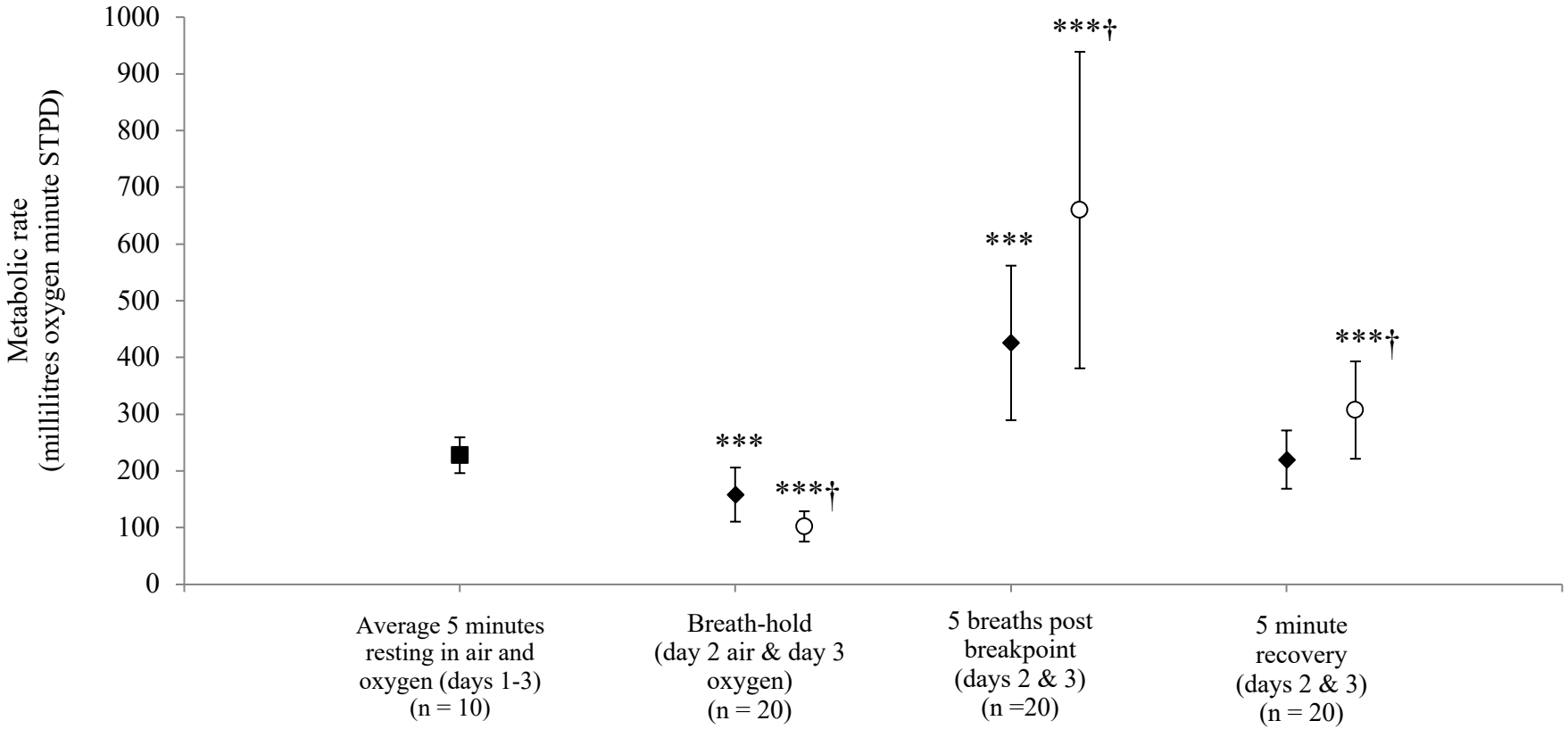

Figure 10 – Average resting rate of oxygen consumption from air and oxygen (days 1-3) falls when breath-holding from air (day 2) ($p < 0.001$) and oxygen (day 3) ($p < 0.001$) and the fall in metabolic rate is greater in the oxygen breath-hold ($p < 0.001$). It is then elevated, above resting values in the 5 breaths in both air ($p < 0.001$) and oxygen ($p < 0.001$) breath-holds and this rise was significantly greater when breath-holding from oxygen ($p < 0.001$) (†). In the 5 minutes following this, metabolic rate only remained elevated following breath-holding from oxygen ($p < 0.001$). Room air represented by ♦, oxygen by o, and combined average from air and oxygen represented by ■. *** denotes $p < 0.001$ *vs* average resting value from air and oxygen (days 1-3). † denotes $p < 0.001$ *vs* when breath-holding from air.

4. Discussion

This study's key aim was to determine, in a credible number of participants, what happens to metabolic rate over breath-holding after estimating the total oxygen consumed by the body. This includes oxygen consumed from the lungs measured via Douglas bags, as well as previously overlooked 1) oxygen consumed from the blood which was calculated by measuring SpO_2 (in air breath-holds) or prevented altogether (in oxygen breath-holds), and 2) additional estimated oxygen supplied to the blood from contraction of the spleen. Our results show that metabolic rate falls when breath-holding and such reductions are accentuated when breath-holds are prolonged with pre-oxygenation.

This study also aimed to examine if any reductions in metabolic rate could be explained by the removal of the metabolic cost of breathing during breath-holding. We quantified this in 10 participants by measuring metabolic rate during passive mechanical ventilation. As expected, metabolic rate fell when these participants were passively ventilated. However, such reductions could not fully explain the extent of the reduction in metabolic rate when breath-holding.

4.1 Metabolic rate at rest

We were consistently able to measure resting metabolic rate on different experimental days within the expected range of 200 – 250 millimetres oxygen $minute^{-1}$ (Aub and Du, 1917; Harris and Benedict, 1918; Ferraro et al., 1992; Haugen et al., 2003). This occurred despite not controlling for factors known to influence resting metabolic rate, such as caffeine, energy intake, prior exercise, menstrual cycle, sleep (Sandiford and Wheeler, 1924; Horst et al., 1936; Solomon et al., 1982; Molé, 1990; Arciero et al., 1993; Speakman and Selman, 2003; Spaeth et al., 2015).

Provision of 60% oxygen did not alter resting metabolic rate. Welch and Pedersen (1981) demonstrated similar findings at rest and during submaximal exercise when providing participants with 60% oxygen. Mean resting metabolic rate from air was 560 ± 20 (± SD)

millilitres oxygen · minute^{-1}, which was not altered with provision of oxygen (580 ± 50 (± SD) millilitres oxygen · minute^{-1}). At rest, their values were somewhat elevated, likely as they were placed on a cycle ergometer, had performed previous exercise, and measurements were taken immediately prior to the onset of a graded exercise test.

Additionally, we confirmed that measuring resting metabolic rate from one single normal sized breath (day 1) is comparable to measuring resting metabolic rate over a 5 minute period. Therefore, measurement of metabolic rate from one single breath when breath-holding is considered valid. As it is possible to breath-hold for longer durations when the lungs are maximally inflated (Flume et al., 1996), all breath-holds were made following a maximal inhalation. Although we expected that this would slightly elevate metabolic rate simply because of the greater effort required, the duration of this breath (8 ± 2 seconds) is much shorter than the time taken for oxygen to travel through the circulatory system (lung to lung circulation time is 55 seconds in healthy individuals) (Morris et al., 2009). Therefore, we anticipate that this apparent rise may be a result of increased recruitment and distention of pulmonary capillaries, resultant from large lung volume, as opposed to a true net increase in oxygen uptake by all tissues.

4.2 The metabolic cost of breathing

We expected a slight decrease in metabolic rate when breath-holding because of the net loss of respiratory muscle activity. We measured the metabolic cost of breathing to be 29 ± 8% of resting metabolic rate in 10 experienced participants. Previous research estimates the metabolic cost of breathing to account for a much lower percentage (~ 10%) of metabolic rate (Bevegaard and Shepherd, 1966; Harms et al., 1998; Ishii et al., 2018). However, these measurements were made when participants were maximally exercising, and when metabolic rate is elevated, rather than at rest.

We excluded data from the 10 inexperienced participants because we were unable to successfully mechanically ventilate them. These participants actively breathed with the

ventilator, preventing the examination of metabolic rate without the associated metabolic cost of breathing. Subsequently they showed no measurable reduction in metabolic rate during mechanical ventilation.

4.3 Metabolic rate when breath-holding

This is the first study to demonstrate that metabolic rate falls during breath-holding. Metabolic rate fell below resting values when breath-holding in all 20 participants and on all occasions it was measured. This reduction was present even after accounting for oxygen consumed from the blood and additional oxygen supplied to the blood by contraction of the spleen. Extension of breath-holds by providing oxygen resulted in a greater reduction in metabolic rate when breath-holding.

In the 10 experienced participants we have shown that when breath-holding from air, metabolic rate fell slightly more (an additional 9 ± 36 millilitres oxygen · minute^{-1} STPD) than during mechanical ventilation when the metabolic cost of breathing was removed. Such reduction was accentuated when these 10 participants held their breath with oxygen, and metabolic rate fell further (an additional 119 ± 49 millilitres oxygen · minute^{-1} STPD) than during mechanical ventilation when the metabolic cost of breathing was removed.

These findings contradict experiments carried out ~ 50 years ago which concluded that there was no net decrease in metabolic rate when breath-holding (Stevens et al., 1946; Klocke and Rahn, 1959; Hong et al., 1971). However, these experiments had inadequate resting measurements to compare to metabolic rate over breath-holding and insufficient participant numbers. Here we have made careful measurement of resting metabolic rate, both over 5 minutes and single breaths, to compare breath-hold values to and have done so in 20 individuals. These experiments also did not sufficiently account for oxygen consumed from the blood during breath-holding, nor did they consider additional oxygen supplied to the blood via contraction of the spleen (Hurford et al., 1990; Schagatay et al., 2001; Espersen et al., 2002; Palada et al., 2008).

4.3.1 Accounting for arterial desaturation

Indirect calorimetry assumes arterial oxygen remains stable (Dempsey and Wagner, 1999) and that all metabolised oxygen is from the lungs. In lieu of direct, invasive arterial blood sampling and direct measurement of blood gas composition during breath-holding, we used non-invasive finger pulse oximetry to determine any changes in arterial saturation levels. As pulse oximetry has an inherent time delay (Andersson and Schagatay, 1998; Parkes et al., 2014), we measured the peak trough in arterial desaturation seen following break point. This was a conservative measure chosen to ensure we did not underestimate the amount of oxygen additionally consumed from the blood. We detected a small amount of arterial desaturation (2 ± 4%) comparable to levels of desaturation seen in previous studies of similar breath-hold durations. Hong et al. (1971) measured a 3.5% reduction during fixed 2 minute breath-holds. As arterial saturation levels are a function of breath-hold duration (Hong et al., 1971; Sasse et al., 1996), larger reductions in arterial saturation (15%) by Pingitore et al. (2008) is expected because mean breath-hold duration was 3.7 ± 0.15 minutes.

As calculating oxygen content for corresponding oxygen saturation values are provided per litre of blood, it was necessary to estimate blood volume. This was done using Nadler's equation (Nadler et al., 1962) which requires participant's height and weight to be factored into calculations. For this we used self-reported values from which there is bound to be a small margin of error (Gorber et al., 2007).

Albeit indirectly, we have accounted for arterial desaturation during breath-holds in air for 20 participants. We also performed prolonged breath-holds in oxygen, omitting this complication, but also measuring and confirming that arterial desaturation did not fall. This is an improvement on previous studies which assess metabolic rate and either made no attempt to account for this (Stevens et al., 1946), performed breath-holds with pre-oxygenation but did not confirm arterial desaturation did not occur (Klocke and Rahn, 1959) or only did so in 2 participants (Hong et al., 1971).

4.3.2 Accounting for contraction of the spleen

To account for additional oxygen supplied to the circulation from contraction of the spleen, we added 80 millilitres oxygen STPD to measurements of metabolic rate over breath-holds from air and oxygen using Fitz-Clarke's (2018) calculation. This is based on measurements of a ~ 5% increase in haematocrit during breath-holds with immersion or facial immersion (Hurford *et al.*, 1990; Schagatay *et al.*, 2001; Palada *et al.*, 2008). Therefore, the supposition that the spleen contracts to the same extent from breath-holding alone was made. Espersen *et al.* (2002) demonstrated that this is not the case by examining changes in spleen size in breath-holds and breath-holds combined with facial immersion in cold (10^{o}C) water. The reduction in splenic area measured via ultrasound following 30 seconds of breath-holding was 13% compared to 27% with facial immersion. Similarly, the reduction in spleen erythrocyte content was reduced by 15% compared with 29% with facial immersion. Therefore, it is possible that the 80 millilitres of oxygen added to measured rates of oxygen consumption might be an overestimation of this value.

Hypoxia is a stimulus for spleen contraction (Stewart et al., 2003) and elevating arterial desaturation by performing repeated breath-holds results in a progressive reduction in spleen volume (Schagatay et al., 2001). Therefore, preventing arterial desaturation during breath-holds with pre-oxygenation will likely impair spleen contraction. Richardson et al. (2009) demonstrated this after providing oxygen prior to breath-holding, which resulted in an offset of reductions in arterial saturation (by 16.8%), blunted elevations in haemoglobin (by 2.4%), and reduced the elevation in haematocrit (by 1.7%) when compared to breath-holds of the same length from air. Subsequently, the oxygen added to the circulation from contraction of the spleen during breath-holds from pre-oxygenation in our protocol may slightly exceed the true value.

4.4 Metabolic rate following breakpoint

In the first 5 breaths immediately following the breakpoint of breath-holding with air, metabolic rate increased from resting values by 88 ± 52%. The magnitude of this immediate overshoot was greater (by 185 ± 98%) when the reductions in metabolic rate when breath-holding were accentuated by prolonging breath-holds with oxygen. From such breath-holds, this elevation above resting values was also prolonged, as over a 5 minute period metabolic rate remained slightly elevated. The manipulation of reductions in metabolic rate when breath-holding, and subsequently measured elevations following breath-holding, in a predictable fashion indicate such reductions are genuine. Metabolic rate in the period following breath-holding has not before been examined in humans. Scholander (1940) described the subsequent rise in metabolic rate following forced submersions as being a consequent compensatory effect to repay oxygen debt. It is possible that such elevations occur following breath-holds in humans to also be a compensatory effect for reductions in metabolic rate.

4.5 Potential mechanisms for the observed reduction in metabolic rate when breath-holding in humans

The simplest explanation for the observed reduction in metabolic rate robustly observed here when breath-holding reaffirms, and potentially strengthens, the notion of a dive response in humans (Scholander *et al.*, 1962; Irving, 1963; Heistad *et al.*, 1968; Gooden, 1994; Andersson and Schagatay, 1998). Thereby, it is necessary to assess the presence of underlying physiological reflexive responses that underpin this response in diving mammals.

The principal oxygen conserving mechanism of diving mammals is their ability to redistribute cardiac output, completely shutting down muscle blood flow and driving a rise in lactate concentration (Scholander, 1940; Zapol *et al*., 1979) and reviewed in section *4.7.2.2 Reduced metabolic rate*.

The assessment of such peripheral vasoconstriction has been previously assessed in humans during breath-holding alone (1.2.2.1 No evidence of organ blood flow shut down). Heistad *et al*. (1968) observed a slight reduction in skin blood flow using plethysmography in finger blood flow (21 *vs* 14 millilitres · minute^{-1}) but no change in forearm blood flow following a 30 second breath-hold in 18 volunteers. Whilst similar has been found by others (Andersson and Schagatay, 1998), the indirect assessment of skin blood flow of just one finger does not provide robust evidence to indicate the presence of substantial organ blood flow reduction.

Assessment of changes in lactate concentration in humans indicate that profound vasoconstriction of skeletal musculature does not occur. Hong *et al*., (1971) assessed alterations in blood lactate 20 seconds following a 2 minute breath-hold from room air in 9 volunteers and found a slight rise (of 0.8 mM). Further to this, elite breath-holders who can withstand breathing for more than 6 minutes show a modest rise in circulating lactate of ~ 0.3 mM litre^{-1} (Bain *et al.*, 2016; Bain *et al.*, 2017). Furthermore, even when breath-holds are combined with physical exercise (cycling at 80 Watts) Andersson *et al.* (2004) measured an 11% rise in plasma lactate concentration in 15 trained breath-holders following a 40 second breath-hold when cycling at 80 Watts (resting plasma lactate was 1.1 ± 0.1 mM litre^{-1} with no absolute post breath-hold value reported).

It may be argued that the progressive rise in blood pressure observed here, and by others in humans (Hong *et al*., 1971; Parkes *et al*., 2014), could be indicative of some peripheral vasoconstriction occurring with no reduction in cardiac output. This would seem fitting with the no observed changes in heart rate seen here, or by others who have correctly measured heart rate previously without the exaggerating the decline seen due to breathing motions at the start of breath-holding (Hong *et* al,, 1971; Parkes, 2012; Parkes *et al.*, 2014; Bain *et al*., 2018). But without assessment of stroke volume, it cannot be certainly inferred that this is the case. Prior assessment of cardiac output during breath-holding indicates that

there is no associated reduction with breath-holding below resting values of ~ 6 litre · minute^{-1} (1.2.2.2 No fall in cardiac output or heart rate) (Hong et al., 1971; Pingitore *et al*., 2008; Heusser *et al*., 2010). However, if this was indeed the case it may then be expected that the total increase in blood pressure would be exaggerated when breath-holding with oxygen than with air as a greater reduction in metabolic rate is observed. However, this does not occur here.

A direct assessment of organ blood flow during breath-holding in humans would be welcomed to definitively confirm if such, regardless of magnitude, a redistribution of cardiac output does occur. Without this clarification, it is not possible to determine this from indirect measures of skin blood flow, changes in lactate concentration, or increases in blood pressure. It cannot for certain be ruled out. However, if the fundamental components of the dive response, namely a reduction in heart rate and cardiac output, are not observed would it be accurate to use this term in humans even to describe even a rudimentary response.

4.6 Effect of facial immersion

It would be anticipated that the measured decline in metabolic rate from breath-holding alone would be exaggerated if breath-holding was combined with facial immersion in cold water and full immersion but this is further complicated with changes in pressure (Fitz-Clarke, 2018) and the cold shock response (Tipton, 1989). Blunted arterial desaturation during breath-holding with facial immersion supports the notion that metabolic rate is suppressed. Andersson and Schagatay (1998) performed breath-holds and breath-holds with facial immersion (in water of 10 ^{o}C) in 21 participants. Arterial saturation fell significantly more following 2-minute breath-holds alone (- 2.7 %), than following breath-holds with facial immersion (- 1.4 %). Others have reported similar results both at rest and during steady state exercise (Lindholm et al., 1999; Andersson et al., 2002; Stewart et al., 2005; Andersson and Evaggelidis, 2009).

Elements of the dive response appear to be somewhat potentiated when breath-holding with facial immersion. During breath-holding alone, Heistad et al. (1968) measured a reduction in mean finger blood flow (from 21.0 to 12.4 millilitres · minute^{-1} per 100 millilitres), but no reduction in forearm blood flow. Conversely, combining breath-holding with facial immersion in water (20 – 25 ^{o}C) resulted in a reduction in finger blood flow (from 18.2 to 11.7 millilitres · minute^{-1} per 100 millilitres) and a reduction in forearm blood flow (from 3.4 to 2.5 millilitres · minute^{-1} per 100 millilitres). Similarly, Andersson and Schagatay (1998) observed a reduction in skin blood flow of the thumb (38 %) when breath-holding, but a greater decrease (56 %) when breath-holding with facial immersion.

Studies have also established that some mild bradycardia may occur with facial immersion. Heistad et al. (1968) observed that heart rate does not change from mean resting values (69 beats · minute^{-1}) but falls slightly (from 71 to 55 beats · minute^{-1}) when combined with facial immersion (20 – 25^{o}C). Similarly, Andersson and Schagatay (1998) observed greater percentage reductions (no absolute values were reported) in heart rate when breath-holding was combined with facial immersion (10^{o}C). They measured an 8 % reduction in heart rate with breath-holding alone, but a larger 14 % reduction when breath-holding was combined with facial immersion. Others have shown similar reductions (Craig, 1963; Brick, 1966), as well as a small (6%) reduction in cardiac output (Sterba and Lundgren, 1988). More extreme reports of decreases in heart rate exist, with heart rate being reported to fall as low as ~ 20 to 30 beats · minute^{-1} (in 25^{o}C water) in trained divers (n = 2) during simulated dives in a pressure chamber (Ferrigno et al., 1997).

Despite this, the physiological relevance of a dive response even when breath-holding is combined with facial immersion is not likely to be meaningful, as breath-holding duration is not prolonged extensively, if at all. Whilst trained individuals can breath-hold with facial immersion for up to 11.6 minutes (world record static breath-hold without supplemented oxygen set by Stephane Misfud in 2009), they do so whilst hyperventilating. Limited data

comparing breath-holding to breath-holding with facial immersion without hyperventilation exists. However, studies that have examined this show breath-holding is not implicitly prolonged more than the typical ~ 1 minute room air breath-hold in untrained non-hyperventilating participants (Schneider, 1930; Norfleet and Bradley, 1987; Flume et al., 1994; Flume et al., 1996; Parkes et al., 2014; Trembach and Zabolotskikh, 2017). Andersson and Schagatay (1998) reported that breath-hold duration was not prolonged with facial immersion (10^{o}C water) (mean duration of both 2.1 ± 0.1 (± SE) minutes). Similarly, Sterba and Lundgren (1988) reported no difference in breath-hold duration compared with breath-holding with facial immersion (20^{o}C water) (mean duration of both was 1.55 ± 0.1 (± SE) minutes). As with breath-holding alone, clearly establishing if peripheral vasoconstriction occurs is difficult because detailed assessment of organ blood flow does not exist during breath-holding with facial immersion. Additionally, it is likely that even the greatest measured reductions in heart rate down to ~20 – 30 beats · minute^{-1} are not of great relevance. Complete cessation of heart rate would only reduce metabolic rate by ~ 14% (Parkes, 2012), as the human heart only consumes ~ 35 millilitres oxygen · minute^{-1} STPD at rest (Takaoka et al., 1992).

4.7 Diving mammals

As we have found a fall in metabolic rate when breath-holding in humans and suggestions of the dive response re-emerge, we are obliged to discuss evidence concerning diving mammals. The following sections compare and contrast findings in humans with the extreme proficiencies diving mammals to survive underwater without oxygen for extended periods of time.

4.7.1 Greater oxygen stores

In his 1962 Harvey Lecture, Scholander aptly said: *'The simplest way to conceive why these animals are capable for diving for so long is that they have both greater oxygen stores and buffering capacity'*. Relative to size, diving mammals have ~ double the total body

oxygen storage of humans, this is highlighted in *Table 3*. Furthermore, the distribution of oxygen between the lungs, blood and peripheral tissues differs between humans and mammals. Humans store most of their total body oxygen within the lungs, whereas diving mammals favour oxygen storage within the blood and peripheral tissues. *Table 3* also shows that this is indeed the case, and demonstrates the stark contrast between humans and select diving mammals. A number of physiological adaptations explain why diving mammals can store greater amounts of oxygen within the blood and peripheral tissues. The following two sections discuss these.

Table 3 – Size and distribution of the total body oxygen stores of humans and select diving mammals.

			Distribution of oxygen stores		
	Body mass (kilograms)	Total body oxygen store (millilitres oxygen kilogram^{-1})	Lungs (%)	Blood (%)	Tissue (%)
Human	70	38	51	35	14
Californian sea lion (*Zalophus californianus)*	35	39	21	45	34
Northern elephant seal (*Callorhinus ursinus*)	400	97	4	71	25
Harbour seal (*Phoca vitulina)*	24	57	13	54	33
Weddell seal (*Leptonychotes weddellii*)	400	87	5	66	29
Hooded seal (*Cystophora cristata*)	252	90	7	51	42
Sperm whale (*Physerter macrocephalus*)	10000	68	4	38	58

Human total body oxygen store calculated by Irving (1939) and distribution of oxygen stores reported by (Kooyman, 1985). Values for Californian sea lion from Ponganis *et al.* (1997); Northern elephant seal, Weddell seal, and Sperm whale from Kooyman and Ponganis (1998); Hooded seal from Burns *et al.* (2007); Harbour seals from Lenfant *et al.* (1970).

4.7.1.1 Blood stores

Diving mammals have a greater total blood volume relative to their size in comparison to their terrestrial counterparts. This allows them to store more oxygen in the blood. French physiologist Paul Bert was the first to show this in 1870, when using the Weckler method, which involved the complete bleeding of an animal. Bert found that the total blood volume of ducks (*Anas platyrhynchos*) accounted for 8.6% of their body weight, but only accounted for 3.9% of hens (*Gallus gallus domesticus*) total body weight (Bert, 1870). Similarly, *Table 4* highlights that relative to size, diving mammals have substantially greater blood volumes than humans.

Secondary to this, diving mammals' blood has a greater capacity to carry oxygen as they have a higher concentration of haemoglobin. Irving (1939) and Scholander (1940) both noted that diving mammals' blood was '*deeply pigmented'*, suggested this colouration was a result a high haemoglobin concentration. This was associated with superior oxygen carrying capacity. Haemoglobin concentration is largely affected by factors such as age and sex, but human blood typically has a haemoglobin concentration of ~ 15 grams decilitre^{-1} (Lentner, 1984). *Table 4* also shows that diving mammals, particularly pinnipeds, have a much greater concentration of haemoglobin than the typical human values.

Despite the higher concentration of haemoglobin per unit of blood, diving mammals' haemoglobin proteins do not have significantly a greater oxygen binding ability. The P_{50} (7.4) value (the partial pressure of oxygen corresponding to 50% oxygen saturation at a pH of 7.4) ranged from 29.7 to 34.4 mmHg in adult pinnipeds (Lenfant et al., 1970). Whereas in humans, the P_{50} (7.4) value of blood is typically 26 mmHg (at 37^{o}C and pH 7.4) (Horvath et al., 1977).

Table 4 – Total blood volume and haemoglobin concentrations of the blood in humans and select diving mammals.

	Total blood volume (millilitres blood kilogram^{-1})	Haemoglobin concentration (grams decilitre^{-1})
Human	71	15
Californian sea lion (*Zalophus californianus)*	120	18
Northern elephant seal (*Callorhinus ursinus*)	216	25
Harbour seal (*Phoca vitulina)*	132	21
Weddell seal (*Leptonychotes weddellii*)	210	26
Hooded seal (*Cystophora cristata*)	106	23
Sperm whale (*Physerter macrocephalus*)	200	22

Human total blood volume calculation from (Irving, 1939) and haemoglobin concentrations from (Lentner, 1984). Values for Californian sea lion from Ponganis *et al.* (1997); Northern elephant seal from Simpson *et al.* (1970); Harbour seal from Burns *et al.* (2005); Weddell seal from Ponganis *et al.* (1993); Hooded seal from Burns *et al.* (2007); Sperm whale from (Ridgway, 1986).

4.7.1.2 Peripheral tissue stores

Diving mammals are able to store large amounts of oxygen in peripheral muscles as they have between ~ 10 – 30 times more myoglobin than humans (Panneton, 2013). The variation between human values and diving mammals can be seen in *Table 5*. Diving mammals which are capable of spending the longest periods underwater appear to have the greatest levels of myoglobin content (Snyder, 1983; Kooyman and Ponganis, 1998). However, similar to that of haemoglobin, the affinity of myoglobin for oxygen does not appear to be superior to terrestrial mammals (Nichols and Weber, 1989).

Diving mammals also have large spleens which act as a reservoir for oxygenated red blood cells. Weddell seals (*Leptonychotes weddelli*) store 20 litres of blood and ~ 75% of total haemoglobin content in their spleen (Qvist et al., 1986). However, humans have a comparably smaller spleen containing only 200 – 250 millilitres of blood. This, accounts for ~ 8% of the total red blood cell content (Koga, 1979). During periods of stress, the spleen releases its contents into circulation, and elevates oxygen transport. This promotes aerobic metabolism by increasing oxygen availability whilst the mammal is spending prolonged periods underwater (Stewart et al., 2003).. Immediately following a voluntary dive, spleen size was observed to be reduced by 71% in Weddell seals (*Leptonychotes weddelli*), which increased haemoglobin concentration by 18 grams decilitre^{-1} and haematocrit from 44 to 55% (Hurford et al., 1996). Similarly, collection of aortic blood samples by a microprocessor-controlled sampling system showed that Weddell seals (*Leptonychotes weddelli*) increase circulating haemoglobin concentration by 60% during the initial 12 minutes of immersion (Qvist et al., 1986).

Table 5 - Myoglobin content of muscle in humans and select diving mammals

	Myoglobin content (grams kilogram^{-1})
Human	6
Californian sea lion (*Zalophus californianus)*	27
Northern elephant seal (*Callorhinus ursinus*)	65
Harbour seal (*Phoca vitulina)*	55
Weddell seal (*Leptonychotes weddellii*)	54
Hooded seal (*Cystophora cristata*)	96
Sperm whale (*Physerter macrocephalus*)	54

Human value from Andersen (1966). Values for Californian sea lion from Ponganis *et al.* (1997); Northern elephant seal from Bryden (1972); Harbour seals from Lenfant *et al.* (1970); Weddell seals from Ponganis *et al.* (1993); Hooded seal from Burns *et al.* (2007); Sperm whale from Lockyer (1976).

4.7.2 Management of oxygen store

4.7.2.1 Behaviour

Behaviour can aid the conservation of oxygen. In early studies using birds, Bert (1870) highlighted the difference between ducks (*Uria triole*) and hens (*Gallus gallus domesticus*) during forced submersion. Ducks could remain submerged for ~ 15 minutes (before drowning) whilst remaining relaxed whereas hens survived only for a ~ 3 minutes and struggled violently when underwater. Diving mammals, such as Weddell seals (*Leptonychotes weddellii)*, can change their behaviour to conserve oxygen. They alter movement kinetics to reduce oxygen demand by performing periods of prolonged gliding, and during descents exceeding 80 metres this can reduce oxygen requirements by up to a 59.6% *(Williams et al., 2000)*. In depth reviews of this are available from Butler and Jones (1997) and (Kooyman and Ponganis, 1998).

4.7.2.2 Reduced metabolic rate

Elevated oxygen stores and behaviour alone are insufficient to account for the diving capacity of diving mammals. A pioneer of the oxygen conserving dive response was Per Scholander, who conducted experiments on seals in the early 1940s. However, as early as 1870 Bert demonstrated this by replacing half of the duck's (*Uria troile*) blood with saline (and thereby largely reducing oxygen stores), and showing that diving capacity of the animals was not impaired. Similarly, Richet (1899) concluded that ducks (*Uria troile*) must be able to reduce the rate at which they consumed oxygen during dives because their total body oxygen stores could not sustain resting metabolic rate for the ~ 20 minutes they could remain submerged for. Total body oxygen stores of a 1.5 kilogram duck (*Uria troile*) was calculated to be 90 millilitres and resting metabolic rate was measured to be 29.1 millilitres oxygen · minute^{-1}. This oxygen store would be depleted in ~ 3 minutes. Both Irving (1939) and

Scholander (1940) agreed that normal metabolism could not be maintained throughout these dives in the seals they studied. Scholander (1940) calculated the total oxygen store of a 29 kilogram hooded seal (*Crystophra cristata*) to be 1520 millilitres and resting metabolic rate to be ~ 200 – 300 millilitres oxygen · minute^{-1}, which would only permit dives of 5 – 6 minutes in length before oxygen stores were depleted. However, these seals regularly spend upwards of 20 minutes submerged. Establishing metabolic rate over breath-holds in seals is methodologically challenging. However, Scholander (1940) did in fact measure oxygen consumption at rest, throughout and following submersion. *Figure* 5 of his paper shows these measurements, with the graph trend line showing a reduction in oxygen consumption from rest during the dive, followed by an immediate rise in oxygen consumption that surpassed resting values. However, no numerical or statistical analysis was performed. Measurement's made during voluntary dives by (Kooyman et al., 1980) supports this. These measurements were made possible by taking 5 Weddell seals (*Leptonychotes weddelli*) to a secluded ice-covered area, with the exception of a man-made hole, in Antarctica. Mean resting rate of oxygen consumption was 309 millilitres oxygen kilogram^{-1} hour $^{-1}$ (mean weight 425 kilograms = 2189 309 millilitres oxygen · minute^{-1}) but whilst diving, oxygen consumption fell to 254 309 millilitres oxygen kilograms^{-1} hour $^{-1}$ (1799 millilitres oxygen · minute^{-1}).

Despite not reporting key values of oxygen consumption when breath-holding, Scholander (1940) measured a marked reduction in heart rate; the 'hallmark feature' of the diving response. Scholander (1940) found that seals (*Leptonychotes weddellii*) fell from 100 to 10 beats · minute^{-1} within one cardiac cycle. This reduction in heart rate was also present when the seal reacted to a threatening stimulus such as a loud noise (Scholander, 1940). This reduction was also present with breath-holding alone Kaczmarek et al. (2018), albeit a weaker response. Similarly, in freely diving seals (*Leptonychotes weddelli*), reductions in heart rates

are seen in the longest dives (> 5 minutes, heart rate fell as low as 16 beats · minute^{-1} even whilst swimming), though in shorter dives the reductions in heart rates were less severe (< 5 minute, heart rate averaged 36 beats · minute^{-1}) (Kooyman and Campbell, 1972).

Scholander also demonstrated that widespread peripheral vasoconstriction of skeletal muscle occurred during forced submersion, as when the seal's fin was cut it did not bleed. During forced submersion, Scholander (1940) also showed that blood flow to skeletal muscle was impaired by measuring lactate within the muscle and within the blood. Muscle lactate was measured to be 44 mM litre^{-1}, whilst blood lactate levels did not increase above 4 mM litre^{-1}. However immediately following resurfacing, perfusion to these tissues is restored and washout occurs, elevating blood lactate levels to 14 mM litre^{-1}.

Zapol and colleagues (1979) further examined the dive response in Weddell seals (*Leptonychotes weddelli*). They quantified the reduction in heart rate, which fell from 52 to 15 beats · minute^{-1}, and cardiac output, which fell from 40 to 6 litre · minute^{-1}. *Table 6* reports the reduction recorded by Zapol et al. (1979) in organ blood flow in these 6 Weddell seals (*Leptonychotes weddelli*). Blood flow was reduced to 0 millilitres gram · minute^{-1} (0.08 ± 0.06 to 0.0 ± 0.0 millilitres gram · minute^{-1}) in skeletal muscle (the psoas) as it had Scholander's (1940) experiment where the seal's fin did not bleed. Blood flow was also reduced in cardiac muscles, the spleen and other organs, such as the kidney and skin. In contrast, blood flow to the brain and central nervous system was maintained. Therefore, this indicates a selective gross redistribution of blood flow, prioritising oxygen rich blood to organs such as the brain and central nervous system which depend on continuous oxygen delivery.

Table 6 – Blood flow to organs at rest and during an 8 – 12 minute forced submersion in 6 Weddell seals as measured by (Zapol *et al.*, 1979)

	Blood flow (millilitres gram · minute^{-1})	
	At rest	During submersion
Brain		
Cerebral cortex	0.60 ± 0.15	0.65 ± 0.23
Cerebellum	0.60 ± 0.18	0.56 ± 0.19
Hypothalamus	0.52 ± 0.16	0.60 ± 0.20
Thalamus	0.44 ± 0.13	0.49 ± 0.14
Medulla	0.45 ± 0.08	0.60 ± 0.09 *
Skeletal muscle		
Psoas	0.08 ± 0.06	0.00 ± 0.00 *
Cardiac muscle		
Atria	0.29 ± 0.10	0.05 ± 0.04 *
Right ventricle	0.55 ± 0.22	0.06 ± 0.13 *
Left ventricle	0.8 ± 0.4	0.1 ± 0.1 *
Kidney	3.05 ± 1.60	0.35 ± 0.33 *
Spleen	1.83 ± 1.28	0.03 ± 0.03 *
Skin	0.03 ± 0.02	0.01 ± 0.01 *

Values are means ± SD for 6. * indicates $p < 0.05$ value differs from at rest.

4.8 Overall conclusion

This is the first study to show that metabolic rate falls over breath-holding. We believe the reduction in metabolic rate observed when breath-holding to be an accurate for the following reasons:

- ➢ We could successfully measure metabolic rate from one single breath
- ➢ We could detect an expected increase in metabolic rate following the act of maximal lung inflation
- ➢ The reduction in metabolic rate exceeded the net loss of the metabolic cost of breathing (n = 10)
- ➢ The reduction in metabolic rate was detectable:
  - In each of our 20 participants on all occasions it was measured
  - After allowance was made for arterial desaturation
  - After allowance was made for any increased oxygen availability from contraction of the spleen
- ➢ The reduction in metabolic rate was potentiated when breath-holds are prolonged with oxygen
- ➢ Metabolic rate was elevated immediately following breath-holding
- ➢ The immediate increase in metabolic rate to surpass resting values was greater and more prolonged when reductions in metabolic rate were accentuated with prolonged breath-holds with oxygen.

The most convenient explanation for the observed reduction in metabolic rate is that humans possess a dive response. However, we confirmed that heart rate does not fall when breath-holding and others have shown that cardiac output does not fall below resting values of ~ 6 litre · minute^{-1}. Whilst it is possible that some peripheral vasoconstriction may occur and account for the small reduction in metabolic rate that we observed, further investigation into organ blood flow is required.

Unlike diving mammals, humans do not rely on the dive response for survival, and subsequently it could be argued that the relative magnitude of such responses should not be expected to be equal. Confirming this, the close examination of diving mammals reveals that human's breath-hold capabilities are unremarkable. Humans are unable to store as much oxygen and do not appear to possess physiological reflexes of the same magnitude, if any at all, to limit metabolic rate. Whilst we would anticipate reductions in metabolic rate when breath-holding to be exaggerated with facial immersion, it is still unlikely to result in comparable responses to diving mammals. Even with facial immersion, humans cannot spend periods of up to 1 hour underwater, reduce their heart rate by a factor of 10, reduce their cardiac output by ~ 85%, or completely cease blood flow to non-essential organs. Therefore, conclusively defining observed reductions in metabolic rate when breath-holding as evidence of the dive response cannot be made without the exploration of further physiological mechanisms, namely organ blood flow, to establish similar origins.

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6 Appendix

6.1 Participant information sheet

Study title: Measurement of metabolic rate during breath-holding in healthy volunteers**.**

LREC reference 05/Q2708/53 RRK 3310

Investigators: Principal Investigator: Dr M J Parkes, School of Sports, Exercise and Rehabilitation Sciences, m.j.parkes@bham.ac.uk, 0121 414 6977.

Co- investigators: Chloe Davis (BSc), School of Sports, Exercise and Rehabilitation Sciences, [redacted].

Invitation:

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being carried out and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

It remains unclear what happens to metabolic rate during breath-holding in humans. We wish to investigate this with simple non-invasive experiments.

Why have you been chosen?

We invite a number of normal, healthy volunteers to take part to provide basic information on what measurements can be made successfully in normal subjects.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision

to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive nor the conduct of the University towards you nor your degree marks nor progress within the University.

What will happen to me if I take part? What do I have to do?

Our experiments take place in the Wellcome Trust Clinical Research Facility at the Queen Elizabeth Hospital. You may attend our experiments in the morning or afternoon. You can follow your normal lifestyle on the day of the experiment. You need not make any special preparations before arrival and wearing some loose, fitting clothes (so that the leads from ECG electrodes on your chest can be easily led to our equipment).

When you arrive, we will demonstrate the ventilation equipment to you. We will answer any questions that you may have about the experiment before we start.

Throughout the experiments you may listen to music via headphones and you may bring your own music if you wish. We will ask you to lie as still as comfortably possible, but it does not matter if you occasionally make some movements.

We will ask you to lie on a comfortable bed with your head propped up with pillows and when you are comfortable we will attach three self-adhesive ECG electrodes to your upper chest for heart rate analysis. We will attach a standard blood pressure cuff and a small clip to your finger to measure the saturation of your arterial blood (these are standard clinical monitors). At first, we will let you breathe through a mouthpiece, 3-way valve, airflow meter and carbon dioxide sensor and collecting your expired gas samples so that we can measure your normal breathing pattern and establish your resting metabolic rate.

What experimental procedure is involved?

We will ask you to wear a disposable facemask secured behind your head with a small strap. You may wear this mask for some time and we will take great care to make it as

comfortable as possible. The mask may make your mouth feel a little dry at first, but this is usually a very minor problem. You may remove the mask at any time if it is uncomfortable or you do not feel happy with the experiment. We would rather that you did not talk with the mask on and we will teach you to communicate with us with agreed and simple hand signals (e.g. thumbs up or down). We will attach the mask to a standard patient ventilator through a sterile bacterial and viral filter.

We will also ask you to hold your breath so that we can make the same measurements at rest. One some occasions we may ask you to breathe gas mixtures high in oxygen. We may also perform an experiment in which a mechanical ventilator breathes for you at your normal depth and frequency. This is a little strange and may take a few tries before you are comfortable. We will encourage you to practice this on a number of separate occasions until you are entirely comfortable with it. Most people find it a quite restful and not unpleasant nor uncomfortable experience.

What are the side effects of this procedure?

There are no associated side effects anticipated with this procedure.

What are the possible disadvantages and risks of taking part? What if something goes wrong?

To the best of our knowledge the experiment is entirely safe and the investigators themselves have taken part as experimental subjects on many previous occasions. There are no known risks of taking part.

If during the course of the experiment, we think we might have detected any medical condition of which you were not aware, a clinical doctor will speak to you about it.

If for any other reason you felt unwell during this study, there are nursing and medical staff available for you to talk to.

What are the possible benefits of taking part?

There are no obvious benefits in taking part in this study. Undergraduate students within the School will be awarded research credits for their contribution.

How much time will my involvement in this experiment take?

We anticipate that 4.5 hours of time will be necessary to complete this study: one training visit of approximately 1.5 hours and three experiments visits of approximately 1 hour each.

Will my participation be confidential?

Yes. The records we keep are your name, the dates of the experiments and the physiological data that we record. You will not be identified by name in any publication of the data.

What will happen to the results of this study?

We intend to publish these results in refereed scientific papers and you make have a copy of the papers when they are published.

Who is organising and funding this research?

This research is forms part of an MSc by Research project within The School of Sport Exercise and Rehabilitation Sciences at The University of Birmingham.

Who has reviewed this research?

This research has been reviewed by Walsall Local Research Ethics Committee. In addition to The University of Birmingham Ethics Committee (reference: ERN_16-0767).
At any time if you have any questions about this study you may contact Dr Parkes in the School of Sport & Exercise Sciences on 0121 414 6977 or Chloe Davis (BSc) at [redacted].

You may keep a copy of this information sheet and a copy of the signed consent form.

6.2 Participant health check form

Please answer the following questions:

Name:

Date of Birth:

Height (centimetres):

Weight (kilograms):

Are you now, or have you in the past ever suffered from any of the following?

1. Asthma
2. Epilepsy
3. Coronary Artery Disease (Angina etc.)
4. Hypertension (Raised blood pressure)
5. Diabetes
6. Renal failure
7. Morbid Obesity
8. Latex Allergy

NO: □

IF THE ANSWER TO ANY OF THE ABOVE IS YES THEN YOU MAY NOT TAKE PART IN THE STUDY.

Are you currently taking any medicines (other than the contraceptive pill)

NO: □

Have you had any of the following within the last 7 days?

1. A cold, ‘Flu, Sore Throat etc.
2. A blocked nose
3. A nose bleed
4. Earache

NO: □

IF THE ANSWER TO ANY OF THE ABOVE IS YES, A DOCTOR WILL CONSIDER YOUR ANSWERS MORE FULLY AND IN CONFIDENCE BEFORE YOU PROCEED WITH THIS STUDY.

Signature: ……..……Date:……..

6.3 Participant consent form

Title of Project: Measurement of metabolic rate during breath-holding in healthy volunteers.

Senior Investigator: Dr M.J.Parkes, School of Sport, Exercise and Rehabilitation Sciences

Co investigators Chloe Davis (BSc), School of Sport, Exercise and Rehabilitation Sciences

Please initial box

1. I confirm that I have read and understand the Participant information sheet for the above study, that I have had the opportunity to consider the information, ask questions and that I understand that mechanical ventilation, non-invasive measurements (including EEG) and venepuncture may be undertaken. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my hospital medical notes may be looked at by responsible individuals from the Wellcome Trust Clinical Research Facility or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature
_____	_____	_____
Researcher	Date	Signature