IMPACT OF HYDRATION ON PULMONARY FUNCTION AND VENTILATORY RESPONSES TO EXERCISE IN HEALTHY ADULTS

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By

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ABSTRACT

Water transport and airway hydration are vital for the normal physiological functioning of the lungs. Water supply to the airways stems primarily from the bronchial circulation, which arises from the systemic circulation. Little is currently known regarding the impact of systemic fluid loss upon pulmonary function. The over-arching aim of this thesis was to investigate the effects of systemic hydration on normal pulmonary function and the ventilatory response to exercise in healthy adults. The first experimental study (Chapter 4) aimed to assess the repeatability and reproducibility of pulmonary function testing (PFT) between and within days in healthy young adults. 'Excellent' repeatability and 'very good' reproducibility were shown for both spirometry and whole body plethysmography. In the second experiment (Chapter 5), these PFT were therefore used to evaluate the impact of mild systemic dehydration (2.5% body mass loss) and subsequent rehydration on pulmonary function in healthy young adults. Mild dehydration led to reductions in forced vital capacity and elevations in residual volume and functional residual capacity, indicative of impaired small airway function and potential gas trapping. Whilst systemic rehydration (fluid ingestion) restored pulmonary function, local rehydration (nebulised isotonic saline) had no effect, suggesting that increased plasma osmolality may contribute to small airway dysfunction during hypertonic-hypovolemia. In the third experimental chapter (Chapter 6), these findings were extended by demonstrating that *i)* a more severe state of dehydration negatively impacts small airway function of physically active young adults *ii)* ventilatory response to exercise is preserved and perceived breathing discomfort largely unaffected by moderate dehydration. Taken together, this thesis demonstrates the impact of systemic fluid loss upon the healthy human pulmonary system and provides insight into the potential mechanisms involved in dehydration-induced pulmonary alterations. In particular, these findings highlight a key role of hydration status upon small airway function, which could have particular relevance in older adults and those with pre-existing lung conditions.

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DEDICATION

Grandad- this thesis is dedicated to you. I'm sorry that it took so long, and that you are sadly not around to see me finish it (or sift through it to find some mistakes)… although you would have probably questioned why a thesis about hydration doesn't include any information regarding the importance of beer! Your support (both financial and emotional) helped me get to this point; knowing how proud you were has been a constant source of motivation to get me through, and for that I will be forever grateful.

PUBLICATIONS AND AWARDS

Publications arising from this thesis

- **Marshall, H.,** Gibson, O.R., Romer, L.M., Illidi, C., Hull, J.H., Kippelen, P. Systemic but not local rehydration restores dehydration-induced changes in pulmonary function in healthy adults (*Journal of Applied Physiology*; *130: 517–527; 2021).*

Conference Presentations

- **Marshall H.,** Gibson O.R., Romer L.M., Hull J.H., Kippelen P. (2019) Systemic but not local rehydration restores dehydration-induced alterations in lung function in healthy adults. *European College of Sport Sciences Annual Congress, Prague* (Oral Presentation).
- **Marshall H.,** Gibson O.R., Romer L.M., Hull J.H., Kippelen P (2018) Effects of systemic dehydration and subsequent systemic or local rehydration on lung function in healthy individuals (2018). *British Thoracic Society Winter Meeting, London* (Poster and Oral Presentation).
- **Marshall H.** (2018). Effects of whole-body dehydration on lung function in healthy adults. *Brunel Research student Poster Conference 2018.*
- **Marshall H.** (2018). Does dehydration impair lung function in healthy individuals? *3 Minute Thesis University Finals.*

Awards

- **Vice-Chancellor's Travel Prize for Postgraduate Research (Brunel University London; 2019).** A competitive prize awarded to support doctoral researchers in communicating and disseminating high quality research to wider audiences.
- **Third prize poster: Brunel Research student Poster Conference (2018)** Third place prize awarded for poster conference within the Brunel University Postgraduate school.

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LIST OF ABBREVIATIONS

°C	Degrees Celsius	**Min**	Minutes
ANOVA	Analysis of variance	**mL**	Millilitre
AQP	Aquaporin	**Mm**	Millimetre
ASL	Airway surface liquid	**mOsm**	Milliosmoles
ATS	American Thoracic Society	**MVV**	Maximal voluntary ventilation
A_x	Area of reactance	**Na^+**	Sodium
CFTR	Cystic fibrosis transmembrane conductance regulator	**O_2**	Oxygen
CI	Confidence interval	**DLCO**	Diffusing capacity of the lung for carbon monoxide
Cl^-	Chloride	**CV**	Coefficient of variation
CO_2	Carbon dioxide	**PaO_2**	Partial pressure of alveolar oxygen
COPD	Chronic obstructive pulmonary disease	**PEF**	Peak expiratory flow
CPET	Cardiopulmonary exercise testing	**PO_2**	Partial pressure of oxygen
ECF	Extracellular fluid	**PO_{max}**	Maximum power output
EELV	End expiratory lung volume	**P_{osm}**	Plasma osmolality
EFL	Expiratory flow limitation	**PV**	Plasma volume
EIB	Exercise induced bronchoconstriction	**R_{20}**	Respiratory resistance at 20 Hz
EILV	End inspiratory lung volume	**R_5**	Respiratory resistance at 5 Hz
ENAC	Epithelial sodium channel	**RER**	Respiratory exchange ratio
ERS	European Respiratory Society	**RH**	Relative humidity
ERV	Expiratory reserve volume	**RPE**	Rating of perceived exertion
FEF_{25}	Expiratory flow at 25% of forced vital capacity	**X_5**	Respiratory reactance at 5 Hz
FEF_{25-75}	Mid-expiratory flow	**SD**	Standard deviation
FEF_{50}	Expiratory flow at 50% of forced vital capacity	**$A\text{-}aDO_2$**	Alveolar-arterial oxygen difference
FEF_{75}	Expiratory flow at 75% of forced vital capacity	**SpO_2**	Oxygen saturation

FEV_1	Forced expiratory volume in one second	**RV**	Residual volume
FRC	Functional residual capacity	**sRaw**	Specific airway resistance
F_{res}	Resonant frequency	**T_{core}**	Core temperature
FVC	Forced vital capacity	**T_E**	Expiratory time
f_b	Breathing frequency	**T_i**	Inspiratory time
h	Hour	**T_i/T_{tot}**	Inspiratory duty cycle
H^+	Hydrogen	**TLC**	Total lung capacity
H_2O	Water	**T_{TOT}**	Total time of breath
Hb	Haemoglobin	**U_{osm}**	Urine osmolality
Hct	Haematocrit	**USG**	Urine specific gravity
HR	Heart rate	**VC**	Vital capacity
IC	Inspiratory capacity	**PFT**	Pulmonary function test
ICC	Intraclass correlation	**$\dot{V}CO_2$**	Volume carbon dioxide produced
ICF	Intracellular fluid	**$\dot{V}_E$**	Minute ventilation
IOS	Impulse oscillometry	**$\dot{V}O_2$**	Volume of oxygen uptake
IRV	Inspiratory reserve volume	**$\dot{V}O_{2max}$**	Maximal oxygen uptake
Kg	Kilogram	**V_T**	Tidal volume
L	Litre	**W**	Watts

Chapter 1 Introduction

Water, an essential nutrient for maintenance of physiological function, makes up ~60% of the human body (Jéquier and Constant, 2010). Dehydration defines a deficit in total body water and can occur as a result of various external and internal stressors (Cheuvront and Kenefick, 2014). Whilst total body water loss can fluctuate on a daily basis by <1%, deficits of >2% body mass can have a negative impact upon various physiological functions in humans. Amongst others, dehydration has been shown to impact cardiovascular (González-Alonso et al., 1997; Stöhr et al., 2011), thermoregulatory (Adams et al., 2018; Logan-Sprenger et al., 2015; Sawka et al., 2001), and cognitive (Bar-David et al., 2005; Ganio et al., 2011) functions, as well as to limit endurance exercise performance (Fallowfield et al., 1996; James et al., 2017; Sawka et al., 2015). In particular, dehydration appears to become detrimental in circumstances that challenge the cardiovascular system, such as in warm/hot environments (González-Alonso, 1998; Kenefick et al., 2010) and during prolonged physical activity (González-Alonso et al., 1997). There is evidence to suggest that the greater the severity of dehydration, the greater the negative alterations upon human physiological systems and functions (Baker et al., 2007; Logan-Sprenger et al., 2013; Sawka et al., 2007)

Many individuals are at risk of dehydration on a daily basis. Athletes (Sawka et al., 2007), firefighters (Horn et al., 2012; Walker et al., 2016), military personnel (Moore et al., 2016; Stacey et al., 2015), and older adults (Lavizzo-Mourey, 1987; Lavizzo-Mourey et al., 1988; Rolls and Phillips, 1990) often experience dehydration, and are therefore commonly exposed to the negative consequences associated with systemic fluid loss. Dehydration in hot conditions has been consistently associated with impaired aerobic performance (Armstrong et al., 1985; Ebert et al., 2007; McConell et al., 1997; Stearns et al., 2009), and has been cited as a key contributor to physical collapse in marathon runners (Kenefick and Sawka, 2007). In addition to the aforementioned physical performance impairments, Carter *et al.*, (2005) demonstrated that 17% of 5,246 US Army soldiers hospitalised with heat illness from 1980–2002 were suffering from dehydration. Further, in a cohort study of 42,553 elderly patients, 9% were dehydrated upon admission to hospital (El-Sharkawy *et al.*, 2017). Dehydration in elderly patients admitted to hospital was associated with an increased length of stay (ranging from 4-19 days with, and 1-8 days without dehydration)

and higher risk of death, whereby patients diagnosed with dehydration were twice as likely to die in hospital compared to patients without dehydration (El-Sharkawy et al., 2017).
The pulmonary system is one of the most important physiological systems in the human body and is vital for human function. However, in contrast to other physiological systems including cardiovascular, cerebral, and skeletal muscle function (Trangmar and González-Alonso, 2019), limited research has yet been conducted on the effects of whole body water loss on pulmonary function. The only three studies currently available (Govindaraj, 1972; Javaheri et al., 1987; Simpson et al., 2017) presented contrasting findings (i.e., improvements *vs* impairments in pulmonary function) following induced dehydration in healthy and clinical populations.

Water is fundamental to the function of the pulmonary system and influences the composition and content of the airway surface liquid (ASL, i.e. a thin layer of fluid involved in maintaining airway homeostasis (Haq et al., 2016) and the removal of inhaled foreign particles (Boucher, 2003; Knowles and Boucher, 2002), and thereby contributes to maintenance of airway stability (Chen et al., 2019). Whether maintaining systemic water balance is essential to preserve pulmonary function remains controversial. Further, optimal functioning of the lungs ensures adequate oxygen (O_2) delivery, which is vital for performing physical activity; yet no research thus far has established whether the exercise ventilatory response is compromised in a state of dehydration. Understanding this response would be relevant to athletic and clinical populations (particularly, in those with pre-existing lung conditions), as ventilatory limitations to exercise have been observed in both these populations (Phillips and Stickland, 2019). In particular, it could lead to: more specific research interventions; contribute to novel clinical treatment and disease management; and assist coaches and practitioners with understanding and designing optimal hydration strategies.

Therefore, the main aims of this thesis were to:

i) determine the effect of mild and moderate systemic dehydration upon resting pulmonary function in healthy young adults;
ii) compare the efficacy of local *versus* systemic rehydration in restoring baseline pulmonary function;

iii) evaluate the functional and perceptual implications of dehydration-induced pulmonary changes during exercise in young trained adults.

By implementing various dehydration and rehydration methods, the objective of this thesis was to provide novel mechanistic insights into the role and regulation of water within the healthy airways.

Chapter 2 Literature review

2.1 Dehydration and body fluid balance

2.1.1 Body fluid balance

Water is the primary chemical constituent of the human body – accounting for 45-70% of total body mass in the average adult (Edelman and Leibman, 1959). Water plays an essential role in physiological functioning, including temperature regulation (Montain, Latzka and Sawka, 1995), and cardiovascular function (Roy et al., 2000). Total body fluid consists of intracellular (ICF) and extracellular fluid (ECF) compartments, which make up ~55% and ~45% of total body water, respectively (Edelman and Leibman, 1959). These fluid compartments govern the distribution of water around the body, and alterations in the water content of any of these compartments results in the redistribution of fluids and an adjustment to the compartmental solute concentrations. The normal distribution of body water across the various fluid compartments in an average adult (~70kg) is demonstrated in Figure 2.1; however, it is important to note that the specific distribution of water varies with: age, total body mass, lean body mass, and sex (Mack and Nadel, 2010), as well as training status (Senay Jr., 1979) and heat acclimation (Gibson et al., 2015; Patterson et al., 2014).

The ECF refers to all fluid outside of the cells and makes up ~20% of total body mass. The ECF consists of both intravascular and extravascular fluid spaces and is divided into five main subsections: a) plasma, b) rapidly equilibrating interstitial and lymph fluid, c) slow equilibrating interstitial fluid and dense connective tissue and cartilage, d) interstitial fluid in bone (inaccessible), and e) transcellular fluid. The interstitial fluid (~75% of ECF) and the plasma (~25% of ECF) are the two largest compartments of the ECF. Plasma is the non-cellular component of the blood and constantly exchanges substances with the interstitial fluid through pores of the capillary membrane. These pores are highly permeable to almost all solutes within the ECF, except for proteins. As a result, all compartments of the ECF have approximately the same composition, whilst the plasma has a higher protein concentration (Edelman and Leibman, 1959; Mack and Nadel, 2010). Water moves freely between compartments via an osmotic gradient (from areas of high solute concentration to areas of low solute concentration) in a process known as osmosis; this process is constant to ensure adequate fluid balance is maintained (Hall and Guyton, 2011).

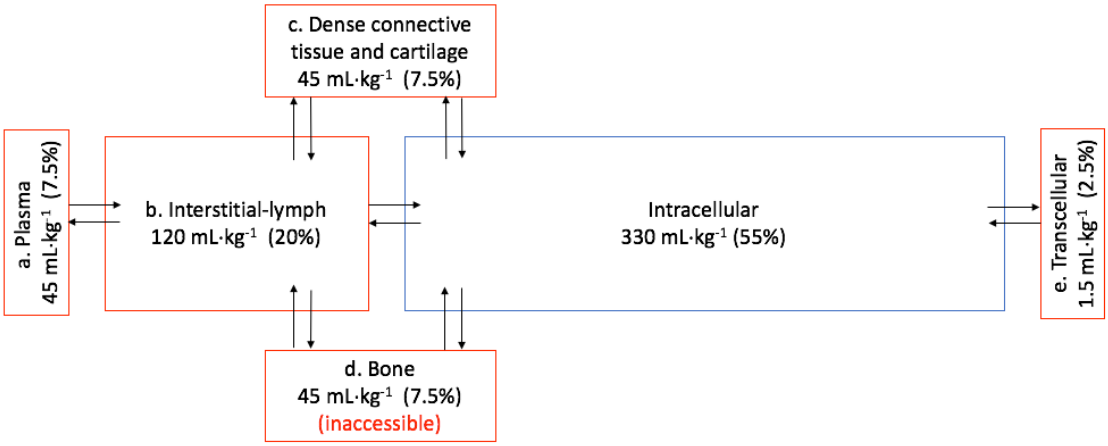

Figure 2.1. Distribution of total body water between the intracellular (blue outline) and the extracellular (red outline) compartments. The extracellular fluid compartment is divided into 5 subsections [a) plasma, b) rapidly equilibrating interstitial and lymph fluid, c) slow equilibrating interstitial fluid and dense connective tissue and cartilage, d) interstitial fluid in bone (inaccessible), and e) transcellular fluid], and makes up ~55% of total body water [redrawn from Edelman and Leibman (1959)].

The ECF contains large concentrations of sodium, chloride, and bicarbonate ions, as well as nutrients for the cells, such as oxygen, glucose, fatty acids, and amino acids (Hall and Guyton, 2011). The ECF also contains carbon dioxide (CO_2) that is being transported from the cells to the lungs for removal (Arthurs and Sudhakar, 2008), and waste products, such as creatinine, nitrogen, urea, and uric acid (McCorry, 2008) that are transported to the kidneys for excretion (Kurts et al., 2013). The ions and nutrients found in ECF are integral for the maintenance of cell life. The ECF moves continuously around the body, is transported rapidly in the circulating blood, and diffuses through the capillary walls to enter the tissues.

The ICF differs substantially to the ECF in terms of its content. The ICF contains large amounts of potassium, magnesium, and phosphate ions (Porth, 2007), and its volume remains relatively stable, as cells require water for normal cellular function (Bray, 1993). If the volume of ICF reduces significantly, the cytosol can become too concentrated with

solutes to continue with normal cellular function. In contrast, if too much water enters the ICF, the cells can burst or become destroyed, which is known as cell cytolysis. The maintenance of stable ICF volume is therefore key for normal cellular function in humans. Numerous mechanisms involving the major organs in the body (i.e., lungs, kidneys, and gastrointestinal system) work constantly to maintain ion concentrations and fluid balance within the body. The movement of ECF and ICF ensures the maintenance of homeostasis, and thus the mechanisms controlling these movements are vital. The primary mechanism regulating the movement of ECF and ICF is the blood passing through the capillaries, allowing a continual exchange of ECF between the plasma portion of the blood, and the interstitial fluid that fills the intercellular spaces. The ECF is transported to all parts of the body in two stages: firstly, via the blood vessels, and secondly via the blood capillaries and intracellular spaces between the tissues cells (Gauer et al., 1970). The permeability of the capillary walls allows this movement, and aids the diffusion of large amounts of fluid back and forth between the blood and the tissue spaces (Pappenheimer, 1953). Water is the solvent in which organic and inorganic solutes are dissolved and made available for transportation between sites (Haussinger, 1996; Jéquier and Constant, 2010). The organic solutes consist mainly of nutrients and by-products of metabolism, whereas the inorganic solutes include O_2, CO_2, and electrolytes (i.e., sodium, chloride, phosphate, potassium and magnesium) (Mack and Nadel, 2010).

Approximately 5-10% of total body water is turned over daily via obligatory non-exercise fluid loss avenues (Raman et al., 2004). Body water is lost continuously via various mechanisms. Insensible water loss describes water lost from mechanisms that are not precisely regulated and occur unconsciously to the individual; this includes evaporative water loss (the rate of loss via this mechanism is highly variable and dependent upon the environmental conditions, activity undertaken and clothing worn); diffusion of water through the skin (~300-400 $mL \cdot day^{-1}$); and respiratory water loss [~ 300-400 $mL \cdot day^{-1}$ (Bossingham et al., 2005)]. The volume of water lost from the respiratory tract can vary based upon: physical activity, air temperature and humidity, and changes in minute ventilation ($\dot{V}_E$) (Daviskas et al., 1990). A substantial increase in the volume of respiratory water loss may not only affect fluid homeostasis, but has also been shown to trigger episodes of bronchoconstriction (Anderson and Daviskas, 2000; Sheppard and Eschenbacher, 1984), highlighting the importance of adequate fluid balance. Sweating also contributes to daily water loss; this will average ~100 $mL \cdot day^{-1}$ during normal temperate

environmental conditions at rest. The volume of water loss through sweating can rise to ~1.5 L·hour^{-1} in a healthy unacclimatised male during exercise, but may reach 2-3 L·hour^{-1} in a highly trained acclimatised individual exercising in hot humid conditions (Gisolfi, 1993). To the author's knowledge, the highest recorded sweat rate in man was 3.71 L·hour^{-1} (~13% body mass), which occurred during the 1984 Olympic marathon in the USA (Armstrong et al., 1986). Exposure to extreme heat and/or strenuous exercise will lead to an increase in sweat rate (Bergeron, 2003; Maughan et al., 2005; Speedy et al., 2001). Normal daily faecal water loss is relatively low and remains relatively constant at ~100 mL·day^{-1} (Newburgh et al., 1930). The remaining water loss is controlled by urine excretion through the kidneys, which ensure that the correct amount of water and electrolytes are excreted, and thus adequate fluid balance and overall hydration are maintained (Bankir et al., 1989).

To ensure that the water lost is adequately replaced, two main sources contribute to adding water to the body. Firstly, water is ingested as liquid, or as water in food; this accounts for ~90% of daily water intake (a desired volume of ~2100 mL water·day^{-1}). Secondly, water is synthesised in the body from oxidation of carbohydrates – accounting for ~200 mL·day^{-1} (Buskirk and Puhl, 1996; Sawka et al., 2005). However, these values can vary greatly between and within individuals, depending on environmental temperature, physical characteristics, and physical activity levels (Bergeron, 2003; Broad et al., 1996; Sawka et al., 2007).

2.1.2 Changes in total body water

Changes in total body water (excess loss *and* excess gain), and subsequent changes in fluid balance can occur under various circumstances and can involve various external stressors. The process of losing body water is known as dehydration, whereas the state of excess water loss is known as hypohydration – both terms are used interchangeably throughout the literature and from this point forward, will be referred to as either 'dehydration' or 'dehydrated'. The type of dehydration induced can vary in terms of the magnitude of fluid loss (i.e., mild – severe dehydration) and type of fluid loss (i.e., movement from the intracellular and/or extracellular fluid compartments). The two primary types of dehydration are hypertonic-hypovolemia and isotonic-hypovolemia. Hypertonic-hypovolemia occurs as a result of sweat loss (i.e. during exercise or from a fever) or prolonged fluid restriction leading to >2% body mass loss (Bartok et al., 2004; Costill et

al., 1976; Popowski et al., 2001), and leads to a significant increase in plasma osmolality (P_{osm}) and small ratio of plasma to total body water loss (~1:10) (Cheuvront et al., 2013; Nose et al., 1988b). Isotonic-hypovolemia, on the other hand, occurs as a result of diuretic administration (Kimura et al., 1976; Roy et al., 2000), and cold and/or altitude exposure (Hoyt and Honig, 2011; Young et al., 1986). This type of dehydration leads to significant solute losses (Cheuvront et al., 2013), and therefore leads to little change in P_{osm} but large alterations in plasma volume.

Slight changes in body water content and total body water can play an important role in the normal physiological functioning of the human body. Dehydration is commonly categorised into mild (1-3% body mass loss), moderate (3-5% body mass loss), and severe (>5% body mass loss) (Cheuvront and Kenefick, 2014). With progressive fluid loss, relative changes occur between the fluid compartments, and there are alterations to the volumes of the interstitial, intracellular and plasma compartments. Figure 2.2, taken from Costill, Cote and Fink, (1976), demonstrates the fractional losses of water from those three compartments with progressive levels of dehydration. It is shown that from mild (-2.2% total body water loss) to moderate (-4.1% total body water loss) dehydration induced via exercise in a warm environment, the fluid shift from the intracellular compartment is significant: intracellular fluid loss accounts for 30% of total fluid loss at mild dehydration, but increases to ~50% at moderate and severe dehydration. These relative changes to fluid compartments are important to consider when examining the physiological responses to dehydration, and when assessing how movement from specific fluid compartments might contribute to observed changes.

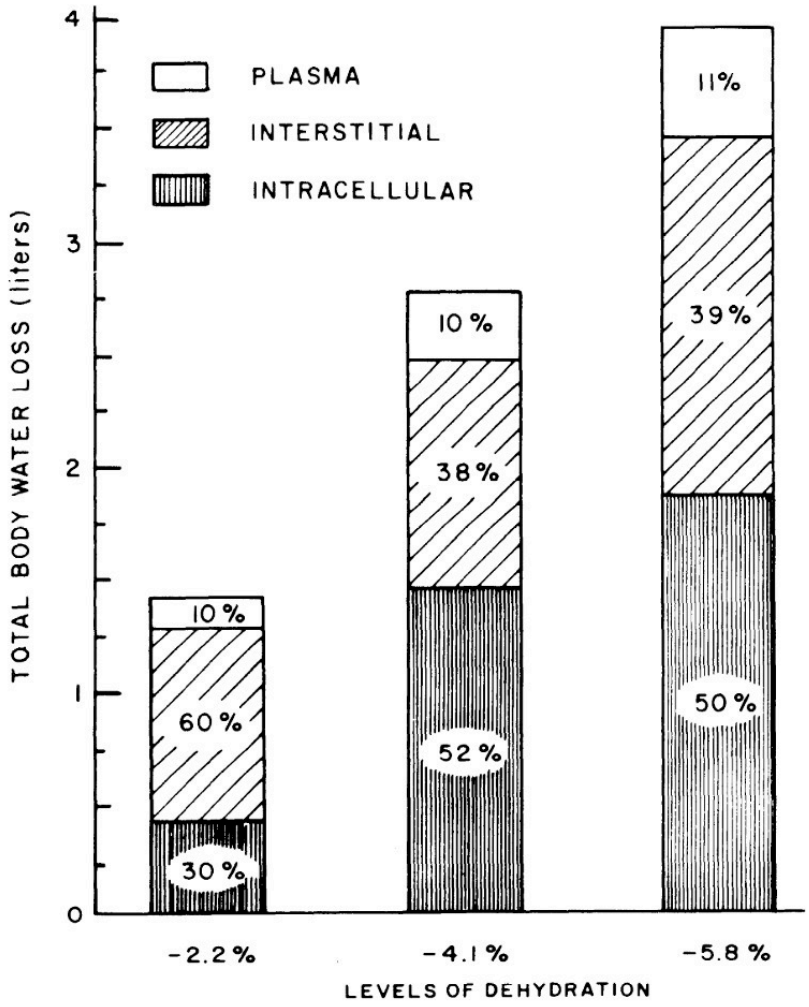

Figure 2.2. Fractional losses of water from plasma, interstitial and intracellular fluid compartments at progressive levels of dehydration. Taken from (Costill et al., 1976).

A reduction of >2% body mass (~3% total body water) can begin to impact upon important biological processes [including, changes in cardiac output (Callegaro et al., 2007; González-Alonso et al., 1995), gastric emptying (Van Nieuwenhoven et al., 2000), and thermoregulatory function (Sawka et al., 2001)]. Further, body water loss has also been shown to impair physical performance (Casa et al., 2010), though this is often contingent on the intensity and duration of the exercise performed (Carlton and Orr, 2015), as well as the environmental conditions the task is performed in (Cheuvront et al., 2010b; Kenefick et al., 2010). Physiological responses occur to compensate for the reduction in total body water: blood and plasma volume slowly decline (Jimenez et al., 1999), and a subsequent prioritisation of blood-flow distribution occurs (González-Alonso et al., 1998). Blood flow is then primarily directed towards the active muscles and organs, to ensure they can

continue to work effectively (González-Alonso et al., 2008). Consequently, blood flow to the skin and sweat glands will be reduced (González-Alonso, Calbet and Nielsen, 1998), which can impair sweating, and thus, impair heat dissipation (Sawka *et al.*, 1985; Montain, Latzka and Sawka, 1995). The increased cardiovascular demand can lead to tachycardia, and the combination of these responses can ultimately culminate in an increase in core temperature [T_{core}; (Armstrong *et al.*, 1997; González-Alonso, 1998)]. In extreme conditions, dehydration puts individuals at an increased risk of heat-related illness, or even death, due to compromised cardiovascular function and gut barrier permeability when the increase in T_{core} cannot be tolerated by the individual (Armstrong, 2007; Casa et al., 2010; Logan-Sprenger et al., 2015; Sawka et al., 2015).

The assessment of absolute or relative changes in plasma/serum markers (P_{osm} and plasma volume) and/or urinary markers (urine osmolality; U_{osm}, and urine specific gravity) can be implemented to help monitor dehydration (Sawka et al., 2007). Whilst there is currently no official gold standard marker of hydration status, these biological markers - in combination with absolute water loss (measured by body mass change) - can provide an accurate indication of dehydration severity (Armstrong, 2007).

2.1.2.1 *Thermal dehydration*

In laboratory-based experiments, the most common method of inducing dehydration is by exposing individuals to elevated environmental temperatures, either at rest (Gutierrez et al., 2003; Melin et al., 2001) or during exercise (González-Alonso *et al.*, 1997; Hillman *et al.*, 2011). Understanding the physiological response to these conditions in an artificial but controlled environment (i.e., laboratory-based studies) is important, as passive and active heat stress are two of the most common causes of dehydration. Outside of the laboratory, individuals may be exposed to prolonged and/or unfamiliar elevations in environmental temperature, this is particularly the case during heat-waves (Baccini et al., 2008; D'Ippoliti et al., 2010), in occupational settings (Horn et al., 2012; Tawatsupa et al., 2013), or during exercise (Kenefick and Sawka, 2007). Prolonged exposure to elevated environmental temperatures may lead to an increased risk of dehydration.

The response of the body to thermal discomfort is one which aims to defend against an increase in T_{core}. Humans are homeothermic and attempt to maintain an optimal T_{core} of ~37°C through mechanisms of heat loss and heat gain (Benzinger, 1969). Exposure to

elevated temperatures elicits physiological responses that work to protect the body against severe heat gain and aim to regulate T_{core} at the optimal level. When an individual is exposed to extreme heat, or exercises under cool conditions, T_{core} begins to rise (Galloway and Maughan, 1997). If T_{core} exceeds 37.2°C, the brain's thermoregulatory control centre (i.e. the preoptic anterior hypothalamus) is activated to promote heat loss (Bazett, 1933). Blood flow is redirected to the skin to aid vasodilation and to dissipate heat (Rowell et al., 1969), and the preoptic anterior hypothalamus signals the activation of sweat glands to increase sweat rate, and evaporative heat loss (Saper and Lowell, 2014; Shibasaki and Crandall, 2010). As a result of this increased sweat rate, a consequential reduction in total body water occurs (Sawka et al., 2011), alongside a concurrent increase in P_{osm}. In order to preserve body fluid balance, two homeostatic reflex mechanisms work in conjunction: the anti-diuretic hormone (ADH) system, and the thirst response (Zerbe and Robertson, 1983). The primary stimulus for activation of these two mechanisms is the change in P_{osm} that is detected by receptors in the hypothalamus (Robertson and Athar, 1976; Thompson et al., 1986). When the increase in P_{osm} is detected, the sensation of thirst and the release of ADH occur – ADH release means the kidneys will retain more water, and the sensation of thirst encourages the individual to ingest fluid, leading P_{osm} to return to normal euhydrated levels. If the individual does not consume enough fluid to satisfy the thirst response, or if fluid is not available, the individual may not be able to match fluid loss with fluid replacement, and dehydration can subsequently occur.

Passive exposure to extreme heat-stress, combined with reduced fluid intake, can lead to a mild level of dehydration in a short period of time (Table 2.1). Schoffstall et al. (2001) recorded a 1.5% reduction in body mass in power lifters following 60 min of intermittent sauna exposure [60°C, relative humidity (RH) not reported], which was coupled with a significant 8.5% reduction in plasma volume. Similarly, Gutierrez et al. (2003) found a similar degree of dehydration (1.4–1.8% body mass loss) following 60 min of intermittent sauna exposure at 70°C (RH not reported). Although these conditions lack ecological validity for field-based research (due to the extreme temperatures), the direct effects of extreme heat upon hydration status are displayed and provide important information regarding the rate of water loss that can occur in humans.

2.1.2.2 *Exercise-induced dehydration – with and without heat stress*

More ecologically valid conditions (representative of typical fluid loss in the real world) have been utilised in laboratory-based studies investigating the severity of dehydration induced by exercise in the heat. Dehydration severity is largely variable between individuals and heavily influenced by the duration and intensity of exercise (Cramer and Jay, 2016; Notley et al., 2015; Sawka et al., 2007), as well as environmental temperature and humidity (Galloway and Maughan, 1997; Gibson et al., 2014; Maughan et al., 2012). For example, Hillman et al. (2011) explored the effects of exercise, with or without environmental heat stress, on hydration status. Seven male cyclists performed 90 min cycling at 95% lactate threshold, in conditions of 33.9 ± 0.9°C *vs* 23.0 ± 0.1°C. Exercise-induced dehydration combined with heat stress led to a significant reduction in body mass and plasma volume of 3.8% and 6.3%, respectively. Exercise-induced dehydration without environmental heat stress also led to a reduction in body mass (3.0%) and plasma volume (7.1%), whereas exercise-heat stress with adequate rehydration induced only a 0.2% loss of body mass and 2.7% reduction in plasma volume. These findings highlight the importance of adequate fluid replacement during exercise in hot environments and indicate that combining exercise with heat stress may increase the magnitude of dehydration. Furthermore, commencing exercise in an already dehydrated state also presents a common risk, and can amplify the magnitude of body mass loss to a more severe state (~5%) (Armstrong et al., 1997; Buono and Wall, 2000; Judelson et al., 2007). Table 2.1 summarises the varying magnitudes of dehydration that can be induced via hyperthermia, exercise, and exercise heat-stress.

When heat cannot be dissipated efficiently, the individual is unable to regulate T_{core} at a safe level. The addition of dehydration to this increased T_{core} subsequently increases the rate of heat storage, elevates thermoregulatory strain, and ultimately increases the risk of heat-related illness (Sawka et al., 1985). Montain and Coyle (1992) demonstrated that hyperthermia is directly related to the level of dehydration induced during exercise in a warm environment: after 120 min of moderate intensity exercise (62% of maximal volume of oxygen uptake; $\dot{V}O_{2max}$), T_{core} reached 39.2 ± 0.1°C in the 'no fluid' condition, in comparison to only 38.4 ± 0.1°C when 82% of fluid loss was replaced. These differences in T_{core} highlight the importance of adequate hydration for successful thermoregulation and normal physiological functioning. Thermal dehydration can occur in recreational athletes (Kenefick and Sawka, 2007) and occupational workers (Carter et al., 2005) who are not

provided with the correct provision (i.e., protective clothing, fluid intake, rest breaks, etc.), and/or knowledge to ensure they are adequately prepared for, or protected against, the conditions. Occupational heat stress and associated dehydration is experienced regularly, particularly in military personnel and firefighters (Lucas et al., 2014). Carter et al. (2005) showed that from 1980 to 2002, dehydration was associated with 17% of the 5,246 hospitalisations and 37 deaths from heat-illness in US Army soldiers, highlighting the risks of insufficient fluid replacement.

2.1.2.3 *Fluid restriction*

Whilst exposure to extreme heat and/or vigorous/prolonged physical activity are often considered the most common methods of dehydration in humans, chronic dehydration can also be caused by prolonged periods of fluid restriction/inadequate fluid replacement. Chronic dehydration is defined by a persistent water deficiency, generally lasting over 24 hours, usually caused by insufficient fluid intake (Bennett et al., 2004). Chronic dehydration can occur in individuals of all ages, with the threat from this form of dehydration heightened in elderly individuals (particularly in nursing homes and hospitals), and children (Bar-David et al., 2005). Bennett et al. (2004) demonstrated that chronic dehydration was present in 89 of the 185 (48%) older individuals admitted to an American hospital in June 2000. It is known that physiological function is compromised in elderly individuals, and aging has been shown to cause, amongst others: a reduced sensation of thirst, decreased activity of renin [an enzyme secreted from kidney cells to help balance sodium and potassium levels in the blood, and fluid levels in the body (Persson, 2003)], and reduced ability of the kidneys to concentrate urine (Kenney and Chiu, 2001; Rowe et al., 1976; Sands, 2009). Consequently, elderly individuals may go for prolonged periods of time without fluid intake and/or may not consume an adequate amount of fluid to replace the volume that is lost daily; this may subsequently put them at increased risk of chronic dehydration. Bar-David, Urkin & Kozminsky, (2005) showed that 32 of 51 (63%) studied school children (age 10-12 years) were dehydrated (U_{osm} >800 mOsm·kg H_2O^{-1}) in the morning, with the children's noon-time U_{osm} highly related to morning U_{osm}. The poor drinking habits of children can contribute to inducing dehydration and highlights the vulnerability of children to unfavourable changes in body fluid balance.

Research investigating the influence of prolonged fluid restriction (commonly between 24–37 h) has been shown to induce a comparable level of dehydration (~1.5–2.0% body mass

loss) to acute heat/exercise stress (Szinnai et al., 2005). The risk of dehydration induced by prolonged fluid restriction is heightened during summer and/or during episodes of heat waves. As water is lost at a faster rate during periods of heat exposure, the combination of these stressors will ultimately increase the likelihood of dehydration occurring.

Fluid restriction for 16 h has been shown to reduce total body mass by up to 1.2 ± 0.9% (Govindaraj, 1972), whilst 28 h of restriction led to a loss of >2.5% of total mass (Szinnai et al., 2005). In the latter study, the total body mass loss was gradual, with losses of 1.1 ± 0.5% and 2.6 ± 0.7% at 24 h and 28 h, respectively. Shirreffs et al. (2004) exposed 15 healthy males to 37 h of fluid restriction, which led to a 1.9 ± 0.4% reduction in total body mass and a 6.2% reduction in plasma volume. These findings demonstrate that the total loss of body mass over a prolonged period of fluid restriction can be similar to the body mass loss induced by exercising in hot conditions. Though it is important to note that studies investigating prolonged periods of fluid restriction (>24 h in duration) are often unable to avoid the confounding influence of circadian rhythms – whether this influences physiological response to fluid restriction induced dehydration remains unknown, but should be considered when interpreting the reproducibility and repeatability of dehydration-induced alterations in physiological function. Table 2.1 shows a comparison of the dehydration severity induced by fluid restriction, alongside the aforementioned dehydration methods. Whilst the overall change in body mass is similar between methods, it is now important to understand whether the various methods of dehydration and their subsequent impact upon various fluid compartments induce similar changes on pulmonary function in humans.

Table 2.1. Representative comparison of severity of dehydration induced via multiple fluid loss methods

Author	Activity	Participants	Duration	Intensity	Temperature (°C)	Relative Humidity (%)	Dehydration rate (%.hour^{-1})	Δ Body mass (%)	Dehydration severity
Exercise-induced dehydration									
Bachle et al. (2001)	Cycling	4 males 6 females	60 min	122 ± 9 W	20.6 ± 1.0	72 ± 1	1.0	-1.0	Mild
Cian et al. (2001)	Treadmill running	7 males	2 h	65% $\dot{V}O_{2max}$	25.0 - 26.0	35-45	1.4	-2.7 ± 0.3	Mild
Fallowfield et al. (1996)	Treadmill running	4 males 4 females	77.7 ± 7 min	70% $\dot{V}O_{2max}$	20.0	-	1.6	-2.0 ± 0.2	Mild
Hillman et al. (2011)	Cycling	7 males	90 min	95% LT	23.0 ± 1.0	-	2.0	-3.0 ± 0.3	Moderate
Melin et al. (2001)	Treadmill running	8 males	120 min	60% $\dot{V}O_{2max}$	25.0 - 26.0	30-50	1.4	-2.8	Mild
Passive heat stress									
Cian et al. (2001)	Passive heat (chamber)	7 males	2 h	-	45.0 50.0	70 20	1.3	-2.6 ± 0.3	Mild
Melin et al. (2001)	Passive heat (chamber)	8 males	120 min	-	45.0	70	1.4	-2.8	Mild
Gutierrez et al. (2003)	Passive heat (sauna)	6 males	3 x 20 min	-	70.0	-	1.8	-1.8 ± 0.5	Mild

Gutierrez et al. (2003)	Passive heat (sauna)	6 females	3 x 20 min	-	70.0	-	1.4	-1.4 ± 0.6	Mild
Schoffstall et al. (2001)	Passive heat (Sauna)	10 males	120 min	-	60.0	-	0.9	-1.7	Mild
Exercise-heat stress									
Gonzalez-Alonso (1998)	Cycling	7 males	135 ± 4 min	70% $\dot{V}O_{2max}$	35.0		2.5	-4.0	Moderate
González-Alonso et al. (1995)	Cycling	7 males	120 min	62 ± 2% $\dot{V}O_{2max}$	35.4 ± 0.2	48 ± 2	2.5	-4.9 ± 0.2	Moderate
González-Alonso et al. (1997)	Cycling	7 males	100-120 min	60% $\dot{V}O_{2max\ x}$	35.0	50	2.6	-4.4 ± 0.2	Moderate
Hillman et al. (2011)	Cycling	7 males	90 min	95% LT	33.9 ± 0.9	-	2.5	-3.8 ± 0.3	Moderate
Judelson et al. (2007)	Fluid restriction + Treadmill walking	7 males	24 h 184 ± 4 min	- 1.5 $m.s^{-1}$, 3% incline	- 36.0 - 37.0	- 40-50		-4.8 ± 0.4	Moderate
Trangmar et al. (2014)	Semi-recumbent cycling	10 males	120 min	55% WR_{max}	35.0	50	1.6	-3.1 ± 0.3	Moderate

Simpson et al. (2017)	Cycling Stepping	6 males 4 females	4 x 20 min 4 x 10 min	25% EPP 45 steps.min^{-1}	37.0	50	1.2	-2.3 ± 0.8	Mild
Fluid restriction									
Szinnai et al. (2005)	Fluid restriction	8 males 8 females	28 h	-	22.0	-	0.1	-2.6 ± 0.7	Mild
Govindaraj (1972)	Fluid restriction	20 males	16 h	-	-	-	0.1	-1.2 ± 0.9	Mild
Shirreffs et al. (2004)	Fluid restriction	9 males 6 females	37 h	-	-	-	0.1	-1.9	Mild

W: Watts, $\dot{V}O_{2max}$: Maximal volume of oxygen uptake, LT: Lactate threshold, WR_{max}: Maximum work rate, Δ: Change, EPP: Estimated peak power.

2.1.3 Physiological and health responses to dehydration in humans

Research investigating the influence of dehydration upon various physiological and biological functions has demonstrated how thermoregulatory (Sawka et al., 2001), cardiovascular (González-Alonso et al., 2008; Montain and Coyle, 1992), cognitive (Lindseth et al., 2013), and, more recently, cerebrovascular functions (Trangmar et al., 2014) can be compromised by a change in total body water. However, the direct consequences of whole-body dehydration upon pulmonary function have been largely overlooked. This is somewhat surprising given the growing evidence that the pulmonary system of vulnerable populations can be severely compromised in environments that increase the risk of dehydration, such as during heat-waves (Witt et al., 2015).

Whilst there is currently no standard definition of a heat-wave, it is commonly referred to as a prolonged period (> 2-3 days) of abnormally hot weather (McGregor et al., 2015). Exposure to elevated temperatures for a prolonged period has been shown to contribute to increased mortality rates. The 2003 heat-wave in Western Europe led to a significant rise in mortality, with an estimated 2,139 excess deaths in the United Kingdom and Wales (Johnson et al., 2005), 1,316 in Portugal (Nogueira, 2005), 3,134 in Italy (Conti et al., 2005) and a substantial increase of ~14,000 excess deaths in France (Vandentorren et al., 2006). These deaths have been strongly associated with complications associated with cardiovascular, cerebrovascular, and respiratory-related illnesses (D'Ippoliti et al., 2010). Data presented by D'Ippoliti et al. (2010) highlighted that 16.2% of all deaths during heatwaves from 1990-2004 in London were attributable to respiratory causes. Yet, the mechanisms underpinning those respiratory-related deaths remain largely unknown and have received little attention. Epidemiological data has demonstrated how deteriorations in cardiovascular and respiratory related illnesses are two of the primary causes of hospitalisations and deaths during heat waves (D'Ippoliti et al., 2010).

These hospitalisations and deaths most commonly occur towards the end of the heat wave and are likely the result of the cumulative effect of prolonged heat stress upon vulnerable populations. One common side effect of prolonged heat stress is significant fluid loss – i.e. dehydration. Fouillet *et al.*, (2006) detailed that dehydration was the primary cause of 1,628 of the 14,539 excess deaths during the France heat wave of 2003. Since body water is a fundamental component of all physiological function in the human body, it is possible that the dehydration commonly experienced as a result of prolonged heat exposure during heat-

waves may have contributed to the deterioration of pulmonary function, particularly in those individuals with pre-existing medical conditions and/or already compromised organ systems.

The cardiovascular response to dehydration is well-documented (Montain and Coyle, 1992; González-Alonso et al., 1997; González-Alonso, 1998; González-Alonso, Calbet and Nielsen, 1998) – human cardiovascular control can be severely impaired as a result of exercise-induced thermal dehydration. González-Alonso et al. (1995) demonstrated reductions in stroke volume and cardiac output following fluid restricted exercise in the heat (35°C) that caused 4.9 ± 0.2% loss of body mass. Furthermore, during exercise-induced dehydration and hyperthermia, circulatory function is blunted, as reflected by reductions in blood flow to skeletal muscle, skin, brain and visceral organs. González-Alonso et al. (1998) showed that exercise in the heat (35°C, 40-50% RH) at 61 ± 2% $\dot{V}O_{2max}$ led to a reduction in forearm blood flow by 39 ± 8% when fluid was restricted, in comparison to the euhydrated exercise condition. The resultant physiological strain can subsequently contribute to the elevation in cardiovascular-related hospital admissions and mortality during heat-waves (D'Ippoliti et al., 2010).

In contrast, the effect of dehydration upon pulmonary function, and the mechanisms of any observed response, remain unclear and thus require further investigation. Research is now required to aid the development of a greater understanding regarding *i)* the role of dehydration upon pulmonary function, and *ii)* the mechanisms associated with the pulmonary response to body fluid loss.

2.2 The human pulmonary system

To understand how dehydration may impact upon pulmonary function, it is first of interest to review the importance of the pulmonary system in the human body. Understanding the anatomy and function of this system will help to develop our understanding of the potential consequences of water loss.

2.2.1 Anatomy of the pulmonary system

The human pulmonary system contains all organs involved in breathing and can be divided into the upper and lower respiratory tract. This includes: the mouth, nose, pharynx, and

larynx (upper respiratory tract), and the trachea, bronchi, bronchiole, alveolar duct and alveoli (lower respiratory tract) (Figure 2.3). Inspired air travels down the airway generations (Figure 2.4), from the 'conducting zone' (where no gas exchange takes place) to the 'transitional and respiratory zones' (where gas exchange occurs) (Weibel, 1963; West, 2013).

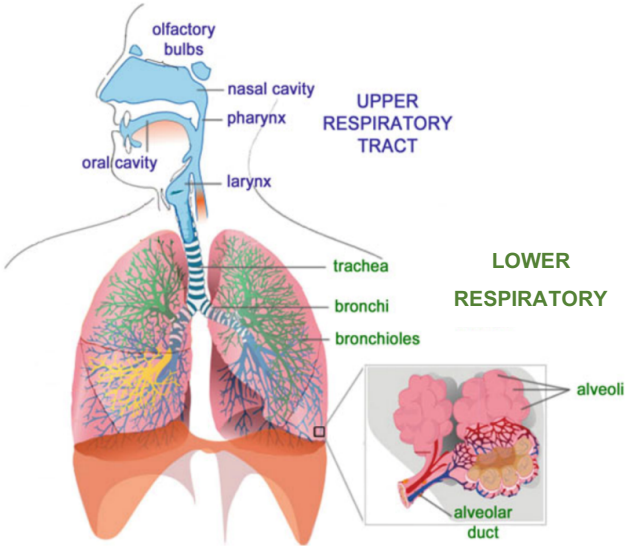

Figure 2.3. A schematic representation of the major organs of the pulmonary system, displayed as the upper and lower respiratory tracts (Tu et al., 2013).

The pulmonary system has three main functions: to act as a channel for gases moving to and from the lungs (West, 2013); to protect the lungs against inhaled foreign matter (Schlesinger, 1982); and to control the heat and humidity of the inspired and expired gases (Negus, 1952; Tu et al., 2013). To ensure these functions are met, air is supplied to the lungs by two circulations: the pulmonary and the bronchial circulations (Baile, 1996; West, 2013).

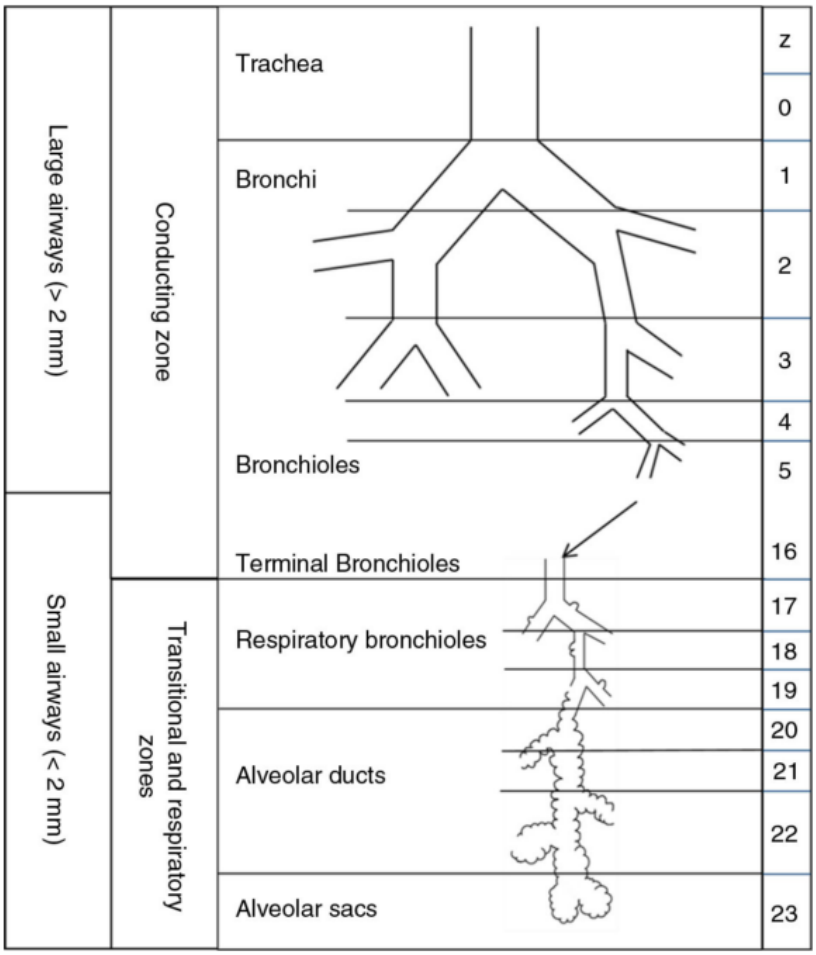

Figure 2.4. Generations of the human airways – the first sixteen generations make up the conducting zone, and the last seven make up the transitional and respiratory zones (also referred to as the respiratory zone). Taken from McNulty & Usmani (2014).

The pulmonary circulation, a low-pressure high-flow circulation, provides blood flow from the heart to the lungs. Deoxygenated blood is carried from the pulmonary artery to the alveolar capillaries, where gas exchanges occurs [with O_2 added and CO_2 removed from the blood, (Hall and Guyton, 2011)]. The oxygenated blood returns to the left atrium of the heart via the pulmonary veins, to be distributed to the rest of the body by the systemic circulation (Hall and Guyton 2011). Whilst the main role of the pulmonary circulation is gas exchange, the bronchial circulation (a high-pressure, low-flow circulation) is responsible for supplying blood to the cells of the lungs (Flieder, 2018). The bronchial circulation, which works complementary to the pulmonary circulation, carries systemic arterial blood to: the trachea, the bronchial tree, the supporting tissue of the lung, and the outer coats of the pulmonary arteries and veins via the bronchial arteries (Figure 2.5) (Hall

and Guyton 2011). The bronchial circulation also carries waste products (such as CO_2) away from the lungs, and despite only receiving ~1% of total cardiac output, is critical to the maintenance of airway and pulmonary function, as well as respiratory fluid balance (McCullagh et al., 2010).

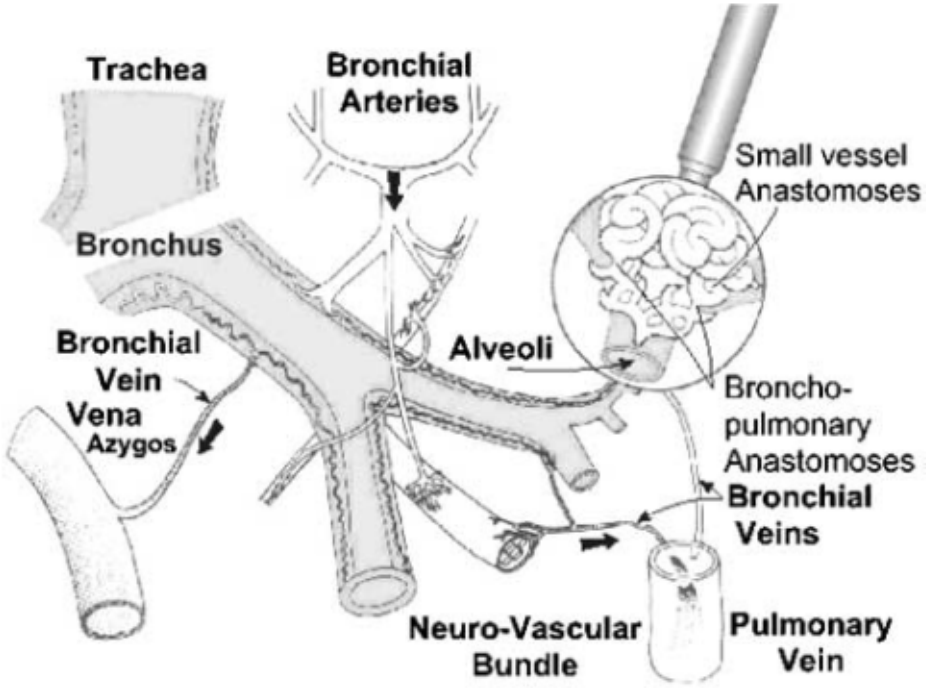

Figure 2.5. A schematic representation of the bronchial circulation (Kalhoff, 2003).

2.2.2 Functions of the pulmonary system

The primary function of the pulmonary system is gas exchange, which takes place in the alveoli and the surrounding capillaries. Air is inhaled through the nose or the mouth, and travels through the pulmonary system until it reaches the alveoli (Figure 2.3). The partial pressure of oxygen (PO_2) is the driving force behind the diffusion of the oxygen-rich air through the alveolar/capillary wall and into the bloodstream. This gas exchange works in line with the law of diffusion – whereby gas exchanges from an area of high concentration (or pressure) to an area of low concentration (or pressure) (Pappenheimer, 1953). The partial pressure of oxygen in the alveoli (PAO_2) is high, and is low in the blood of the pulmonary capillaries, which results in the diffusion of O_2 across the respiratory membrane from the alveoli and into the bloodstream (West, 2013), where the O_2 binds to haemoglobin within red blood cells to form oxyhaemoglobin, which circulates around the rest of the

body (Lumb, 2012). – the CO_2 from the blood is subsequently exchanged into the alveoli and removed via respiration. The small airways are vital in this process, ensuring the gas can be exchanged and the O_2 can be delivered (Lumb, 2012).

The pulmonary system also works to protect the lungs against foreign matter and infections (Schlesinger, 1982). Mucociliary transport and mucus clearance are the primary form of innate airway defence against inhaled particles (Boucher, 2003; Knowles and Boucher, 2002). Individuals are constantly exposed to potentially harmful foreign particles (such as dust, pollen, bacteria, and viruses), which must be removed from the airways before reaching the lungs. The mucociliary transport mechanism, also referred to as mucociliary clearance, ensures that these particles do not enter the lower airways (Eliezer et al., 1970; Hilding, 1963).

The mucociliary apparatus consist of three major components: the mucus layer, the cilia, and the airway surface liquid [ASL; (Figure 2.6)], which work together to remove inhaled particles from the airways and lungs (Mall, 2008). The ASL consists of two layers: the 'sol phase', i.e., the watery periciliary layer, which contains electrolytes, in particular sodium and chloride, which allow the cilia to move freely between each power stroke (described below), and the 'gel phase', i.e., the portion proximal to the air (Figure 2.6). Mucus is secreted from three main cell types throughout the airways; these cells have varied distribution across the airway generations (Widdicombe and Wine, 2015). First, a 10-15 μm wet mucus blanket covers the nasal cavity and trachea, whereby submucosal glands are the predominant secretory cell type for airway generations 0-10 (see Figure 2.3) (Sahin-Yilmaz and Naclerio, 2011; Wilson and Allansmith, 1976). Next, the bronchi and conducting bronchioles (up to airway generation ~15) receive mucus secretion from goblet cells (Davis and Dickey, 2008). At this level, the depth of the mucus blanket is reduced to ~5 μm, and this depth continues to reduce as the airway generations progress (Widdicombe and Widdicombe, 1995). The third secretory cell type, providing mucus for the respiratory and smaller conducting bronchioles (i.e., generations 10-20) are the Clara cells (Boers et al., 1999). The Clara cells have the lowest mucus secreting capacity of the three cell types – the progressive decline in secreting capacity from glands > goblet cells > Clara cells reflects the decline in the need for mucus at each of the levels of the airways (Widdicombe and Wine, 2015). Across the airway generations, the concentration of particles in the airway declines, resulting in a reduced need for mucus to assist particle removal, and thus

decreased mucus secretion. The hydration of the mucus gel has a strong influence upon the clearance of foreign particles and plays a key role on its viscous and elastic properties; the more hydrated the mucus is, the less viscous and elastic it becomes, making it easier to clear. Dehydration of the mucus layer can increase its viscosity, and may ultimately impair its ability to clear foreign particles from the airways, which can culminate in airway obstruction (Fahy and Dickey, 2010) (this is described in more detail in section 2.2.3). The depth of the ASL reflects the balance between the mucus secretion and absorption of the liquid across the surface epithelium, and the rate at which ASL moves upwards from the distal regions. For this defence mechanism to function correctly, mucus must be adequately hydrated (Wanner et al., 1996).

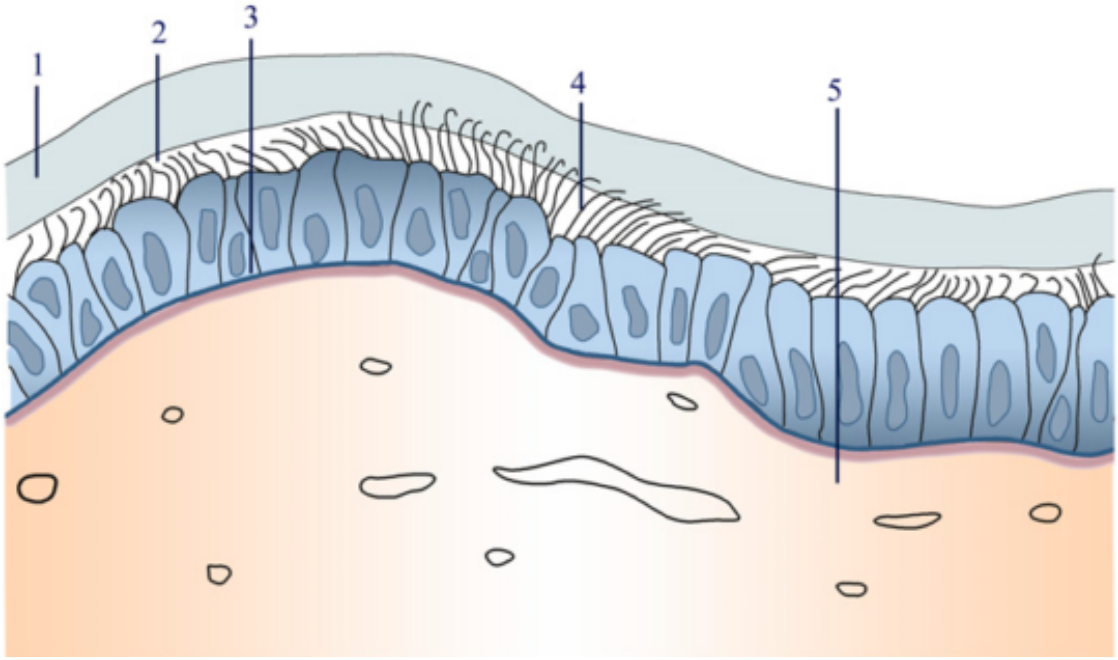

Figure 2.6. Two phases of nasal airway mucus layer. 1. Gel Phase, 2. Sol Phase, 3. Basement membrane, 4. Cilia, 5. Submucosal layer. Adapted by Sahin-Yilmaz and Naclerio (2011).

The mucus layer serves as the first line of defence against inhaled particles and bacteria by trapping these foreign particles before they are able to enter the lower airways and the lungs (Boucher, 2003). The cilia, a layer of tiny, hair-like projections that line the airways, then assist with the clearance of these trapped particles (Mall, 2008). Cilia beat in a fixed direction using a two-stroke pattern: the power stroke, i.e., a fast movement whereby the cilia straighten, contact the gel phase, and remove the mucus; followed by the recovery

stroke during which the cilia bends laterally to return to its original position and restart a new cycle of ciliary beating (Macchione et al., 1995; Trindade et al., 2007). The cilia beat >1000 times *per* minute, sweeping the mucus and its trapped particles upwards in the trachea, and towards the nasal cavity to be expelled (Gizurarson, 2015), or to the nasopharynx where it is swallowed (Lund, 1996). These hair-like projections are covered with an ASL, which lubricates the airway surface and assists ciliary beating and efficient mucus clearance (Wanner et al., 1996). Dehydration or depletion of the ASL volume can impair this clearance function, and may place the individual at a greater risk of respiratory infection or dysfunction (Randell and Boucher, 2006).

The third key role of the pulmonary system is the conditioning of inspired air; a process that, at rest, predominantly occurs at the level of the upper respiratory tract (see Figure 2.3) and ensures air is warmed and humidified before reaching the alveoli (Negus, 1952). When air is inspired, rapid heat transfer and humidification occur from the nasal mucosa (Doorly et al., 2008), causing ambient air to be warmed to ~34°C by the time it reaches the nasopharynx (during resting respiration). The anterior nasal segment causes the greatest increase in temperature, and humidifies the air to ensure a humidity approaching 100% in the nasopharynx (Lindemann et al., 2004). Further down the tract, ASL also contributes to heat and moisture exchange, to regulate the inhaled air to body conditions, i.e., 37ºC, 100% RH (Lumb, 2012). Once conditioned, this air travels to the alveoli to commence gas exchange (Weibel, 1973).

Failure to fully condition inspired air can lead to local airway drying/dehydration, particularly at the level of the small airways (those with a diameter < 2mm), as fluid availability is greatly reduced at this level (Daviskas et al., 1990; Weibel, 1964). Water loss from the airway generations is dependent upon ventilation, inspired air conditions, and return of water to the airways (Daviskas et al., 1990, 1991). Since fluid availability at the level of the small airways (i.e. below generation 9) is low, small volumes of water loss from these generations could be significant. Whilst dehydration of the airways via ventilation is unlikely to occur in a resting state (when $\dot{V}_E$ is low; i.e., ~5-8 $L \cdot min^{-1}$), in situations involving hyperventilation and/or hyperpnoea (such as during exercise) the conditioning process could be overwhelmed and transient dehydration could occur (Daviskas et al., 1995). Relative to the small volume of water in the ASL, water loss during hyperpnea of dry air can be very high. Daviskas, Gonda and Anderson, (1991) calculated that water loss

at a ventilation of 60 L·min^{-1} in temperate conditions can exceed 1 mL·min^{-1} (Figure 2.7). Thus, it is likely that, during more strenuous exercise (i.e. when ventilatory rates can exceed 200 L·min^{-1}), the volume of water loss at the airways is substantial. Dehydration of the airways could then: *i*) prevent the alveoli from performing gas exchange correctly (Negus, 1952; Shelly et al., 1988); *ii*) cause damage to the airway epithelium and peripheral airway mucosa (Kalhoff, 2003; Kippelen et al., 2012); and *iii*) trigger exercise-induced bronchoconstriction in susceptible individuals (Kippelen, Anderson, & Hallstrand, 2018). Further, even small volumes of fluid loss are enough to alter the osmolarity of the ASL (Daviskas et al., 1991). Changes to ASL osmolarity is discussed in greater detail in Section 2.2.3.2, and could play a key role in fluid balance at the airway surface (Anderson and Daviskas, 1999).

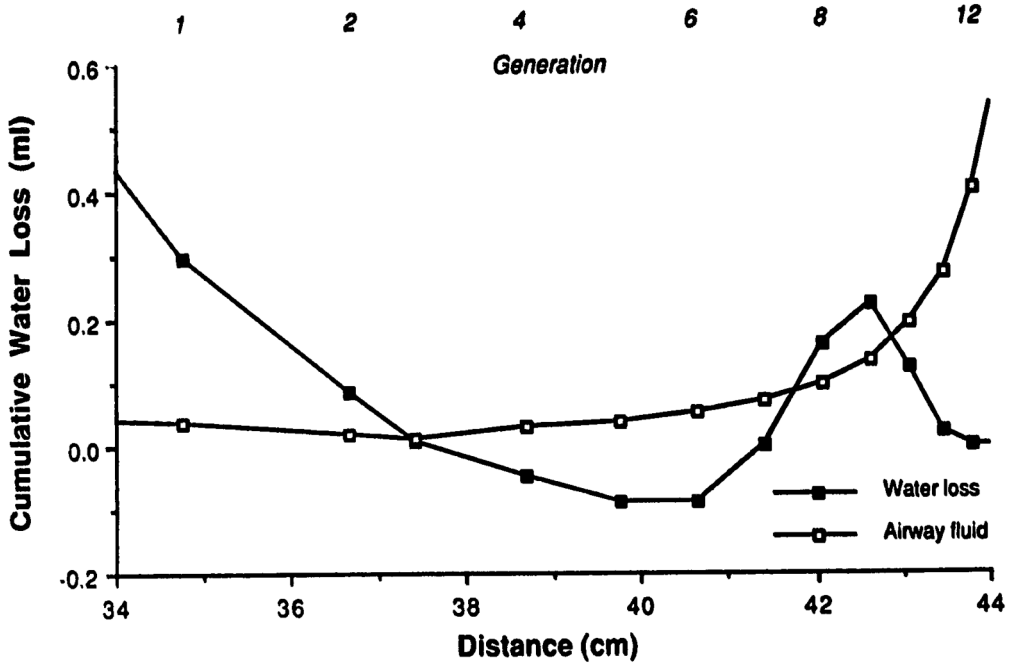

Figure 2.7. Cumulative water loss past the trachea after 4 min of exercise at a ventilation rate of 60 L·min^{-1} in temperate (26.7°C, ~36.5% relative humidity); from Daviskas et al., (1991).

2.2.3 Movement of water in the pulmonary system: molecular and cellular influences

Water is a fundamental component of the pulmonary system. As described above, adequate hydration of the airways is a key contributor to normal pulmonary functioning. Movement of water within the pulmonary system is a constant cycle; the lung loses ~700 mL of water per day from its proximal airway surfaces (Boucher, 1999). Water lost must subsequently be replenished to maintain adequate airway hydration at all times. Depletion or dehydration of the ASL could lead to possible airway epithelial damage (Kalhoff, 2003), and could severely inhibit mucociliary transport by impairing ciliary beating (Widdicombe and Widdicombe, 1995). Consequently, tight regulation of ASL volume and composition is vital.

Maintenance of ASL volume is dependent upon: the rate at which liquid enters and leaves the lung (i.e., via mucociliary clearance); the evaporation rate; and the rate at which liquid can cross the airway epithelium (Widdicombe and Widdicombe, 1995). The mechanisms underpinning this regulation are extremely difficult to identify. Quinton (1994) highlighted the difficulty of collecting sufficient volumes of uncontaminated and unchanged fluid for analysis *in vivo*, due to the very thin layer of liquid (even smaller than the 50 μm depth in the normal airway). Further Boucher (1999) indicated the challenge of generating accurate models to simulate the functions of the very thin fluid layer *in vitro*. As a result, the exact mechanisms supporting ASL regulation remain in question. As outlined below, the role of aquaporins and active ion transport have been reported as key regulators of ASL volume.

2.2.3.1 *The role of aquaporins*

Firstly, water influx from capillaries and the airway interstitium into the ASL is thought to be driven by osmotic gradients created by evaporative water loss (Song et al., 2001). Aquaporins (AQP) are ‘water channels’ that assist the movement of water between cells at various membranes by facilitating the dissipation of osmotic gradients and increasing water permeability across cell membranes (Verkman, 2007, 2013). They are responsible for the high water permeability between cells in multiple organ systems, and are involved in fluid transport in many cell types, including: airway epithelial cells, epithelia and endothelia in the kidney, eye, gastrointestinal organs, and exocrine glands (Verkman, 2013). At present, at least thirteen sub-types of AQP have been identified in humans (Zhu et al., 2016), of these thirteen, at least four AQPs are expressed in the lung: AQP1 is expressed throughout

the microvascular endothelia in airways and lungs (Hasegawa et al., 1993), AQP3 and AQP4 are expressed in airway epithelia (Frigeri et al., 1995; Nielsen et al., 1997), whilst AQP5 is expressed in the luminal membrane of type I alveolar epithelial cells (Funaki et al., 1998).

Expression of these AQP throughout the airways would indicate an important role of this protein in airway hydration and ASL volume regulation. However, phenotype analysis of transgenic knockout mice has indicated a less significant role than expected: whilst AQP1 and AQP5 contribute to water transport, the deletion of these AQP in mouse models led to a >30-fold reduction in osmotic water permeability, but had no physiologically significant impact upon alveolar fluid clearance (Ma et al., 2000). In addition, AQP4 deletion in AQP1-null mice led to a reduction in water permeability, indicating that AQP4 may play a role in water movement within the lung (Song et al., 2000a). These findings suggest that, whilst AQP contribute to osmotically-driven water transport within the airways, their role upon airway humidification, ASL hydration, and upper airway fluid absorption is of lesser importance. Whilst Song et al., (2000) concluded that AQP are not required for physiologically important pulmonary functions, Song and Verkman, (2001) later presented evidence that the deletion of AQP5 in submucosal glands of mouse models led to a reduction in fluid secretion of >50%, suggesting that AQP play a (potentially minor) role within airway hydration. It remains therefore possible that these water channels make at least some contribution to water transport at various levels of the airways.

2.2.3.2 *The role of active ion transport*

As AQP are not the main driver of changes in airway hydration, alternative modes of ASL regulation have also been investigated. Ion channels have been proposed as key contributors to ASL hydration (Ballard et al., 2002; Button and Button, 2013; Tarran, 2004) and are responsible for regulating the mass of salt and water on the airway surfaces (Button et al., 2007). The highly water permeable airway epithelia can absorb and secrete fluid, thus, by modifying the activity of apical ion channels, the epithelia is able to optimise the hydration status of the ASL. This regulation is enabled by the epithelia ability to switch these ion channels between secretory and absorptive phenotypes (Button et al., 2007). For example, if high levels of salt are secreted by the epithelium into the ASL, water will subsequently follow to maintain isotonicity of the airway (Button and Button, 2013). Chloride (Cl^-) secretion, mediated by the cystic fibrosis transmembrane conductance

regulator (CFTR), is the primary driver of fluid secretion into the airways. In support of the importance of this regulatory mechanism, mutations of the CFTR gene coding [associated with cystic fibrosis; i.e. a genetic lung disorder, characterised by a defective CFTR gene (Ratjen, 2009)] are associated with substantial reductions in epithelial Cl^- secretions and excess sodium (Na^+) absorption (Hobbs et al., 2013), which results in elevated mucus layer concentration and increased risk of infection and inflammation (Boucher, 2007a; Garland et al., 2013; Haq et al., 2016). Normal function of the CFTR gene to mediate Cl^- secretion is thus a vital component of the ASL hydration process, and ion transport is therefore essential for the maintenance of ASL hydration.

The alteration to ion concentrations within the airways directly influences the osmolarity of the airway fluid. Anderson and Daviskas, (1999) previously described the importance of the osmotic changes as a mechanism of fluid movement within the airways. When water is lost from the airway surface, the increased ion concentration leads to increased osmolarity of ASL. The osmotic gradient caused by airway water loss is thought to stimulate the movement of water from the surrounding cells to the airway lumen. This is demonstrated in Figure 2.8, whereby a comparison is made between normal basal conditions *vs* conditions of airway water loss (in this instance, stimulated by dry air hyperpnea). In normal conditions, the osmotic gradient created by the movement of Na^+ and Cl^- causes the movement of water into the epithelial cells and the submucosa. In contrast, the elevated water loss induced by hyperpnea leads to increased ion concentration, and thus an osmotic gradient that causes water to move out of the epithelial cells – causing them to shrink. The role of the epithelial cell for water movement at the airway therefore appears fundamental.

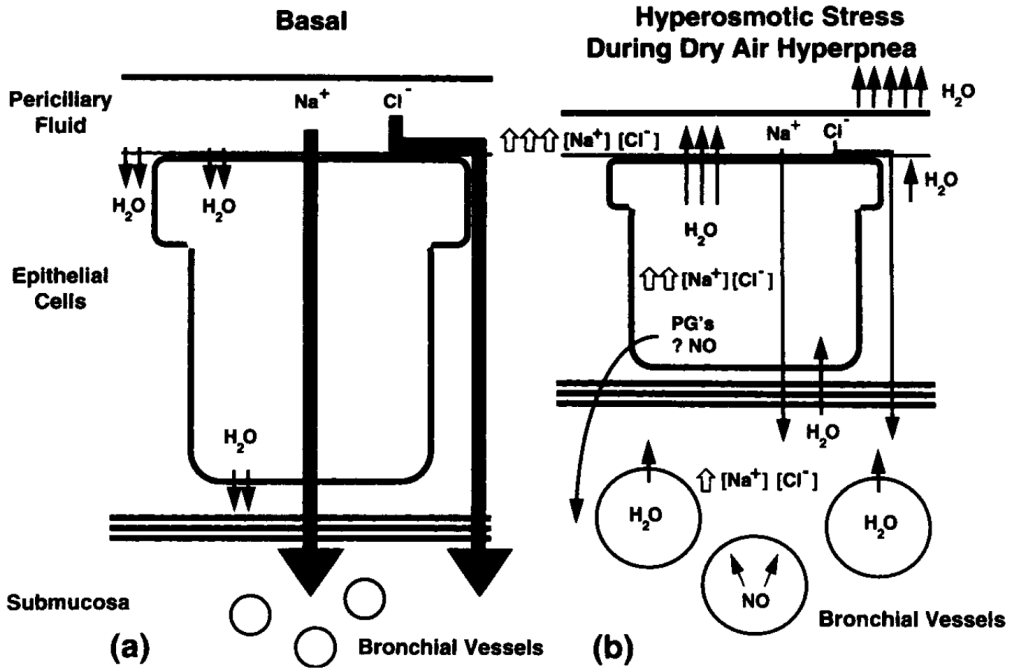

Figure 2.8. Ion transport and their influence upon epithelial cells and water movement under a) basal conditions and b) conditions of hyperpnea leading to hyperosmotic stress [from Anderson and Daviskas, (1999)].

If sodium chloride is absorbed – a process mediated by the amiloride sensitive epithelial sodium channel (ENaC) – water will follow, which can result in dehydration of the ASL (Button and Button, 2013). This fluid regulation ensures that ASL height is adjusted to remain close to the optimal level – i.e. when ASL height approaches ciliary height, the rate of Na^+ absorption decreases, and Cl^- secretion increases, which causes adjustment in ASL depth (Spring 1999).

The role of ion channel activity to control absorption and secretion is vital in the maintenance of ASL hydration. Tarran et al., (2005) demonstrated the regulation of ASL height using an *in vitro* normal lung epithelia model, assessing periciliary layer volume. Findings showed that when periciliary layer volume excess (i.e. volume above homeostasis) was high, Na^+ absorption was at its greatest. Next, as periciliary layer volume approached its optimal height (7 μm), Na^+ absorption was inhibited, whilst Cl^- secretion increased. Although Na^+ absorption under static conditions appeared to be regulated by the activation of CFTR, control of Na^+ transport under phasic motion conditions was associated

with the release of adenosine and adenosine triphosphate. Secretion of Cl^- was also primarily controlled by CFTR during the phasic motion condition. Figure 2.9a-b [from Tarran et al. (2005)] demonstrates the role of active ion transport on changes in periciliary layer height in a normal, healthy population. Figure 2.9a shows that under normal static conditions, the airway epithelium regulates the rates of Na^+ absorption, and that Cl^- secretion regulates periciliary layer to the optimal height (7µm) over time. Conversely, in Figure 2.9b, the phasic motion leads to an increase in CFTR Cl^- secretion, alongside increases in adenosine and adenosine triphosphate release. These findings indicate the importance of active ion transport on ASL volume regulation, specifically within the periciliary layer.

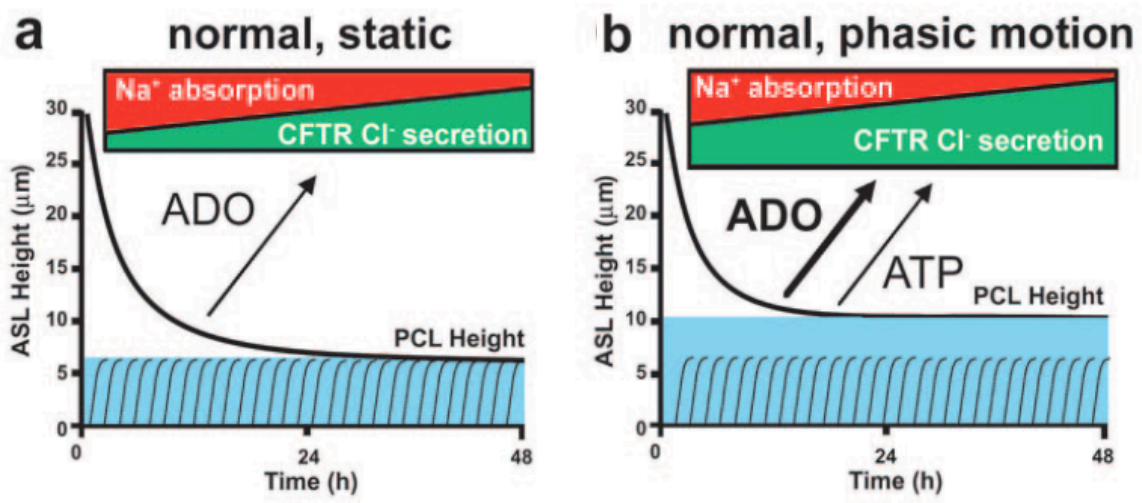

Figure 2.9 a-b: Periciliary layer (PCL) height regulation by active ion transport: a) Normal airway epithelia under static conditions, b) normal airway epithelia under conditions of phasic motion. The blue colour depicts PCL height as a reference to extended cilia. [From Tarran et al., (2005)]. Na^+: Sodium; Cl^-: Chloride; CFTR: Cystic Fibrosis Transmembrane Regulator; ADO: Adenosine; ATP: Adenosine triphosphate.

2.2.4 Changes in airway surface liquid hydration: the consequences

Adequate hydration of the ASL is vital for normal pulmonary function (Boucher, 1999; Widdicombe, 2002). Reductions of the ASL, and of airway hydration in general, may cause detrimental effects. In particular, mucociliary clearance rate is dependent upon adequate

airway surface hydration (Figure 2.10). In the normal hydrated airway, there is an adequate volume of water to hydrate the periciliary layer and mucus layer, allowing mucus transport to proceed at its normal rate of ~60 $\mu m \cdot s^{-1}$. However, loss of water and salt from the airway surface causes the periciliary layer and mucus layer to collapse, leading to adhesion of mucus to cell surface, and preventing cilia from clearing the mucus efficiently (Randell and Boucher, 2006).

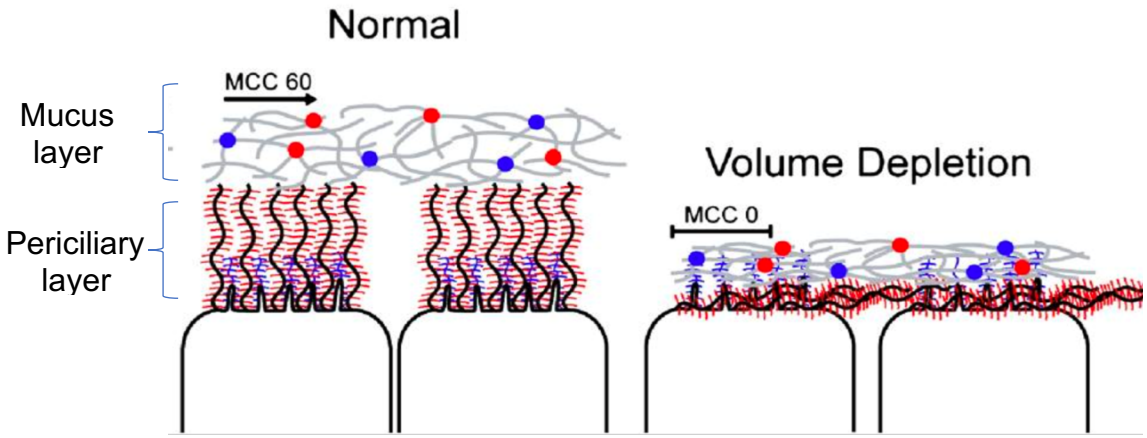

Figure 2.10. Changes in mucociliary transport rates as a result of ASL volume depletion. Normal hydration of the ASL allows mucociliary transport to continue at its normal rate (left), however, dehydration of the ASL causes adhesion of mucus to the airway surface, and subsequently prevents mucociliary clearance from occurring (Randell and Boucher, 2006).

The reduction, or dysfunction of mucus clearance can impair airway function and overall lung health. In mouse models, mucus adhesion to the cell surface has been shown to cause the formation of mucus plugs that can be severe enough to cause suffocation (Hogg et al., 2004). Whilst it is unlikely that this level of obstruction could occur in the healthy human lung, a build-up of mucus could lead to: an increased risk of infection, abnormal lung function, and pulmonary symptoms, including coughing and breathing discomfort (Fahy and Dickey, 2010).

The dehydrated airway with increased mucus levels, may also lead to a higher concentration of pro-inflammatory stimuli, which would subsequently lead to an increased risk of infection (Randell and Boucher, 2006). This is reflective of the pathophysiology of cystic fibrosis. As discussed in section 2.2.3.2, the CFTR gene plays a key role in ion

transport across the airway epithelium, thus the impairment in ion transport in individuals with cystic fibrosis indicates that this mechanism is significantly impaired. The resultant ASL dehydration means that mucus clearance is also impaired (Figure 2.10), leading to a build-up of particles on the airway surface, and ultimately to an increased incidence of airway infection and inflammation (Ratjen, 2009). Whilst the severity of the infection and inflammation may be reduced in those without this genetic condition, airway dehydration likely causes disruption to the normal clearance mechanisms and thus the normal functioning of the airway. This detriment to the airways primary defence mechanism highlights the importance of adequate airway hydration.

The extent of the negative effects associated with airway dehydration shows the importance of the ASL for pulmonary function and, ultimately, for human survival. Gaseous exchange, lung defence, and airway conditioning may all be compromised by localised airway dehydration, but it is important to consider that dehydration in humans commonly occurs in a more systemic manner (i.e., whole-body dehydration as discussed in 2.1.2). Whether dehydration at systemic level can compromise pulmonary function through alterations in local airway hydration remains unclear.

2.2.5 Influence of systemic and airway hydration on pulmonary function

As established earlier, the supply of water to the airways stems primarily from the bronchial circulation. Subsequent movement of water from the bronchial vessels to the surface of the airways is then governed by osmotic changes across the airway epithelia. Whilst it has been shown that direct dehydration of the ASL can impair certain key physiological functions of the respiratory system (i.e. mucociliary clearance), it remains unknown whether whole-body (systemic) dehydration is directly associated with changes in airway hydration, and how this might influence the function of the pulmonary system.

At present, only three research studies have been conducted to specifically investigate the role of dehydration upon pulmonary function in human subjects. First, Govindaraj (1972) investigated the effect of 16 h of complete fluid restriction upon pulmonary function (using spirometry pre- and post-dehydration) in twenty healthy males with no history of respiratory illness. A reduction in body mass ranging from 0.5 to 2.5% (0.25–1.50 kg) occurred. Govindaraj (1972) found an average reduction in forced expiratory volume in one second (FEV_1) of 7.3% (~ 180 mL) from baseline (which indicates possible airway

narrowing), with sixteen of the twenty participants experiencing a significant drop in FEV_1 (~ -180 ± 210 mL). Whilst airway calibre appeared affected by the change in hydration status, lung volume [as assessed by forced vital capacity (FVC)] showed little change (with post-dehydration FVC within 5% of baseline levels for fourteen participants). Govindaraj (1972) concluded that mild dehydration may cause small, but significant increases in airway obstruction.

The findings from Govindaraj (1972) were the first step towards establishing a relationship between systemic dehydration and pulmonary function. However, follow-up work did not confirm those original findings. In 1987, Javaheri et al. explored the impact of diuretic-induced dehydration upon pulmonary function. Six healthy male participants (aged 25-40 years), with no history of respiratory illness undertook a two-day dehydration protocol. To induce a moderate-to-severe level of dehydration, participants were required to take a diuretic drug (chlorthalidone) for two days (50 mg every 12 h, total 200 mg), whilst fluid intake was restricted; this led to an average body mass loss of 4.0–4.5% (~3.3–3.7 kg) from baseline. Pulmonary function parameters were measured via spirometry twice during each experimental day (morning and evening) and were compared to baseline (euhydrated) values. Participants were rehydrated following the diuretic administration protocol (via ad libitum water intake), and spirometry was performed on three further days to assess the effect of rehydration. Surprisingly, FEV_1 significantly increased from an average (± standard deviation; SD) of 4.46 ± 0.43 L at baseline to 4.66 ± 0.48 L at dehydration (+ 4.48%). Whilst FVC remained unchanged during the dehydration period, maximal voluntary ventilation (MVV) and peak expiratory flow rate (PEFR) increased by 4.30% and 2.54%, respectively. These values (FEV_1, MVV, PEFR) returned to baseline levels following rehydration via ad-libitum water ingestion. Contradictory to the findings from Govindaraj (1972), these results were therefore indicative of a positive effect of dehydration upon pulmonary function.

A direct comparison of the findings between these two studies is difficult due to major differences in the study designs, including severity and mode of dehydration. Smaller reductions in body mass (ranging from 0.0–2.5% over 16 h) were seen in the study conducted by Govindaraj, (1972) which may have impacted the severity of the pulmonary function impairment. Whilst Javaheri et al., (1987) demonstrated an increase in pulmonary function at a moderate-to-severe level of dehydration (4-4.5% body mass loss over 48 h),

it is important to note that the extracellular dehydration (hypertonic-hypovolemia) that is induced by diuretic administration (Cheuvront and Kenefick, 2014) may have contributed to the recorded changes in pulmonary function. Extracellular dehydration may have resulted in different osmotic gradients across the airway epithelium, and thus may have caused less water loss directly from airway surface in comparison to the intracellular dehydration induced via 16 h fluid restriction. Thus, whilst the severity of systemic dehydration may have been higher, it is possible that ASL properties were not modified, consequently causing no impairment to airway function.

More recent work has since been conducted to explore the effects of exercise-induced dehydration [which also induces intracellular dehydration (Cheuvront and Kenefick, 2014)] upon pulmonary function. In contrast to the previous investigations examining healthy individuals, Simpson et al., (2017) recruited ten recreational athletes (four females) with a prior diagnosis of asthma/exercise induced bronchoconstriction (EIB) – a population that may be more susceptible to the negative effects of dehydration. All participants attended three experimental visits involving: *i*) exercise in the heat (37°C, 50% RH) with no fluid intake (dehydration) to induce mild dehydration (~2.5% body mass loss), *ii*) exercise in the heat with ad libitum fluid intake (control), and *iii*) a time-matched resting period (rest). Pulmonary function was assessed pre-exercise (baseline) and at 10 and 120 min post-exercise using spirometry, whole body plethysmography, and diffusing capacity of the lung for carbon monoxide (DLCO). Pulmonary lung volumes of patients with asthma were significantly impaired following mild dehydration, with reductions in FVC (~ 300 mL) and elevations in residual volume (RV; ~ 260 mL) and functional residual capacity (FRC; ~ 260 mL) recorded following fluid restricted exercise in the heat. Table 2.2 compares the observed changes in pulmonary function following fluid restriction (Govindaraj, 1972), diuretic-intake (Javaheri et al., 1987) and exercise-induced dehydration (Simpson et al., 2017).

Table 2.2. Overview of previous studies investigating the effect of dehydration on pulmonary function, and the magnitude of change.

Author	Participants	Mode of Dehydration	Dehydration Severity: % body mass loss (rate)	Primary type of dehydration	Pulmonary function: Euhydrated	Pulmonary function: Dehydrated	Δ Pulmonary function
Govindaraj (1972)	20 healthy males	Fluid deprivation: 16 h	Mild: 0.5 – 2.5% (~0.04 $L \cdot h^{-1}$)	Intracellular	FEV_1: 2.43 ± 0.43 L VC: 2.70 ± 0.40 L	FEV_1 ↓: 2.25 ± 0.50 L* VC ↔: 2.70 ± 0.44 L	FEV_1: -178 ± 206 mL VC: - 8 ± 159 mL
Javaheri et al. (1987)	6 healthy males (aged 25-40 years)	Diuretic administration (chlorthalidone): 2 days	Moderate: 4.5% (~0.07 $L \cdot h^{-1}$)	Extracellular	FEV_1: 4.46 ± 0.43 L FVC: 5.88 ± 0.77 L PEFR: 687.7 ± 43.8 $L \cdot sec^{-1}$ MVV: 227.7 ± 7.67 L	FEV_1 ↑: 4.66 ± 0.48 L* FVC ↑: 5.91 ± 0.78 L PEF ↔: 705.2 ± 31.9 $L \cdot min^{-1}$ MVV ↑: 237.5 ± 8.0 L*	FEV_1: + 200 mL FVC: + 30 mL PEF: + 17 $mL \cdot sec^{-1}$ MVV: + 10 mL
Simpson et al. (2017)	10 recreational athletes (4 female, 6 male): diagnosed asthma/EIB.	Exercise in the heat (37°C, 50% RH) + fluid restriction: 2 h	Mild: 2.3 ± 0.8% (0.75 $L \cdot h^{-1}$)	Intracellular	FEV_1: 4.21 ± 0.89 L FVC: 5.09 ± 1.22 L FRC: 3.40 ± 0.99 L RV: 1.73 ± 0.46 L	FEV_1 ↔: 4.24 ± 0.90 L FVC ↓: 4.79 ± 1.10 L* FRC ↑: 3.65 ± 0.90 L* RV ↑: 1.99 ± 0.57 L*	FEV_1: +3 mL FVC: - 300 ± 190 mL FRC: +260 ± 250 mL RV: +260 ± 182 mL

EIB: Exercise induced bronchoconstriction; FEV_1: Forced expiratory volume in one second, VC: Vital capacity; FVC: forced vital capacity; PEFR: Peak expiratory flow rate; MVV: Maximal voluntary ventilation; FRC: Functional residual capacity; RH: relative humidity; RV: Residual volume. Δ: delta change, ↓ = decrease from baseline, ↑ = increased from baseline, ↔ = no change from baseline. *Significant change ($p < 0.05$) *vs* euhydrated.

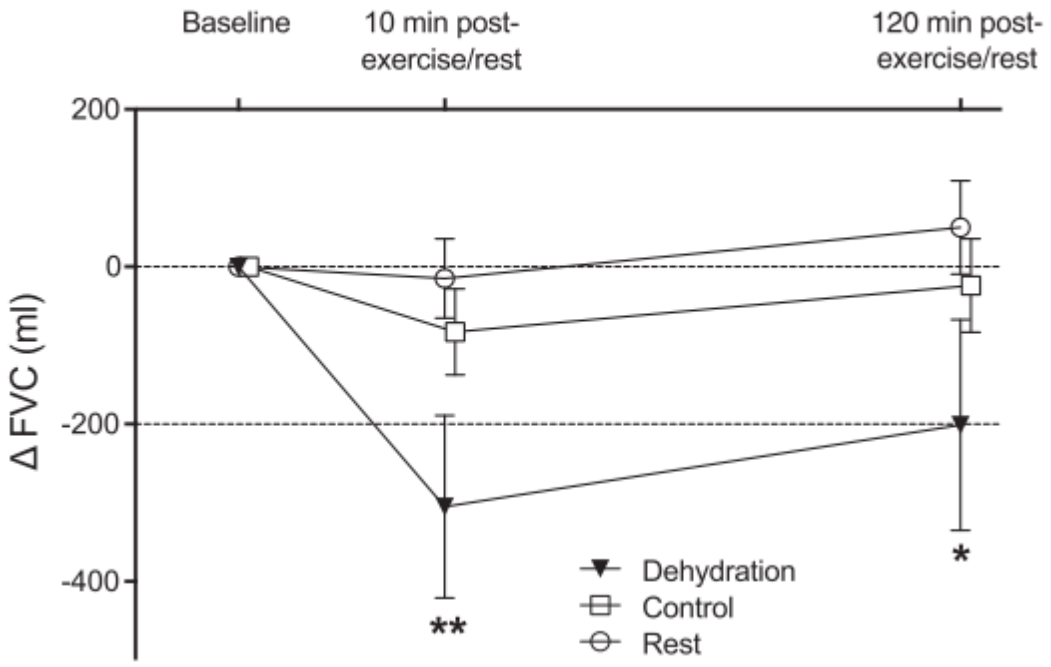

Figure 2.11. Changes in FVC following exercise in a dehydrated state (dehydration), exercise in a euhydrated state (control) and a time-matched rest period (rest). Values are shown as means ± 95% confidence interval. *p<0.05; **p<0.01 *vs* control and rest conditions. The dashed line represents a clinically meaningful reduction in FVC (>200ml). Figure from Simpson et al. (2017).

The impairment to pulmonary function noted following exercise-induced dehydration in asthmatic individuals is contradictory to the improvement in pulmonary function shown by Javaheri et al., (1987). Of those studies, an impairment in pulmonary function was observed when intracellular dehydration (hypertonic-hypovolemia; i.e. via exercise and fluid-restriction), but not extracellular (isotonic-hypovolemia; i.e. diuretic administration) was induced. It is possible that extracellular dehydration would result in lesser water loss from the airway surface compared to intracellular dehydration and could explain the observed pulmonary response.

Whilst Govindaraj, (1972) and Simpson et al., (2017) saw an overall detrimental pulmonary function response, there remain inconsistencies between these studies. Govindaraj (1972) demonstrated a reduction in expiratory flow rate (i.e. FEV_1), with no associated change in lung volume (FVC), in healthy participants; whilst Simpson et al., (2017) recorded changes in pulmonary volumes only (FVC, FRC and RV; change in FVC presented in Figure 2.11)

in individuals with pre-existing lung conditions. The findings from Simpson et al., (2017) are the first to highlight the possibility of compromised small airway function following exercise-induced systemic dehydration. However, it is not to exclude that the pre-existing respiratory condition (i.e., asthma) has influenced these findings – especially as asthma has previously been associated with impaired fluid secretion at epithelial level (Park et al., 2008). Whilst it is possible that the perturbations in small airway function found by Simpson et al., (2017) will also occur in healthy individuals, there is currently no research to support or refute this proposal.

2.3 Exercise and the pulmonary system

Exercise can induce significant fluid loss (see section 2.1.2.2), and is a common cause of dehydration in recreational and elite athletes (Sawka et al., 2007). Given the proposed importance of water in maintaining normal pulmonary function at rest, it is critical to also understand the impact of dehydration on the ventilatory response during physical activity and exercise. Whilst the normal ventilatory response to exercise ensures appropriate pulmonary gas exchange via increased delivery of O_2 and removal of excess CO_2 in most healthy adults, ventilatory limitation during exercise can occur in both clinical and healthy populations. This section will first discuss the normal ventilatory response to exercise from the perspective of the healthy individual (i.e., with no pre-existing respiratory disease), and will discuss the causes and consequences of ventilatory limitation during exercise and review methods of assessing ventilatory limitation. Finally, the impact of external factors (such as environmental conditions and the hydration status of the individual) on ventilatory limitation will be considered.

The pulmonary system functions to facilitate the exchange of gases into and out of the pulmonary circulation. At the onset of exercise, demand for O_2 and production of CO_2 are significantly increased. In order to compensate for these changes, tidal volume (V_T; i.e., the volume of air displaced in one full breathing cycle) and breathing frequency (f_b; i.e., the number of breaths taken per minute) rapidly increase, producing an elevation in $\dot{V}_E$ (Forster et al., 2012). The increase in V_T occurs due to an increase in end inspiratory lung volume (EILV) and a reduction in end expiratory lung volume (EELV). The reduction in EELV is a beneficial mechanism, which occurs due to expiratory muscle recruitment, and allows for a more optimal inspiratory muscle length (Smith et al., 2017). To meet the requirements

for elevated O_2 demand and removal of excess CO_2 and hydrogen (H^+), alveolar ventilation ($\dot{V}_A$; the exchange of gas between the alveoli and the external environment) and alveolar-capillary diffusion must increase in proportion to the increase in metabolic rate (Forster et al., 2012), so that respiratory homeostasis can be maintained.

The exact mechanism(s) by which the ventilatory response to exercise occurs remains incompletely understood. The elevation in $\dot{V}_E$ at the onset of exercise is exactly matched with the elevation in metabolism, which, when first reported by Haldane and Priestly (1905), was proposed to be caused by a stimulation of chemoreceptors. The exercise-induced increase in alveolar partial pressure of CO_2 (P_aCO_2) and arterial partial pressure of O_2 (P_aO_2) was thought to induce the increase in $\dot{V}_E$, and to provide the link between $\dot{V}_E$ and $\dot{V}CO_2$. However, since $\dot{V}_E$ and $\dot{V}CO_2$ are so precisely matched, doubts regarding the feasibility of a 'feedback' mechanism have arisen. The potential mechanisms that underpin the exercise-induced elevation in $\dot{V}_E$ are often categorised as either neural (i.e. feed-forward and feed-back mechanisms) or humoral (blood-borne) mechanisms and are associated with signals sent to the respiratory neurons of the brain that regulate breathing. The neurohumoral response, a theory first set out by Dejours (1964), proposed that the ventilatory response to exercise can generally be categorised into three phases (Figure 2.12): phase one (I) sees an immediate elevation in $\dot{V}_E$ at the onset of exercise to compensate for the sudden increase in O_2 demand. In phase two (II), a slow exponential increase in $\dot{V}_E$ occurs (with a time constant of ~1 min), which is followed by phase three (III), a steady state of $\dot{V}_E$. During severe intensity exercise, the steady state (phase three) will not be reached, and $\dot{V}_E$ will continue to increase until either exhaustion occurs and/or exercise is terminated. During steady state exercise, the elevation in $\dot{V}_E$ is proportional to the increase in CO_2 production and O_2 consumption (Haverkamp et al., 2005).

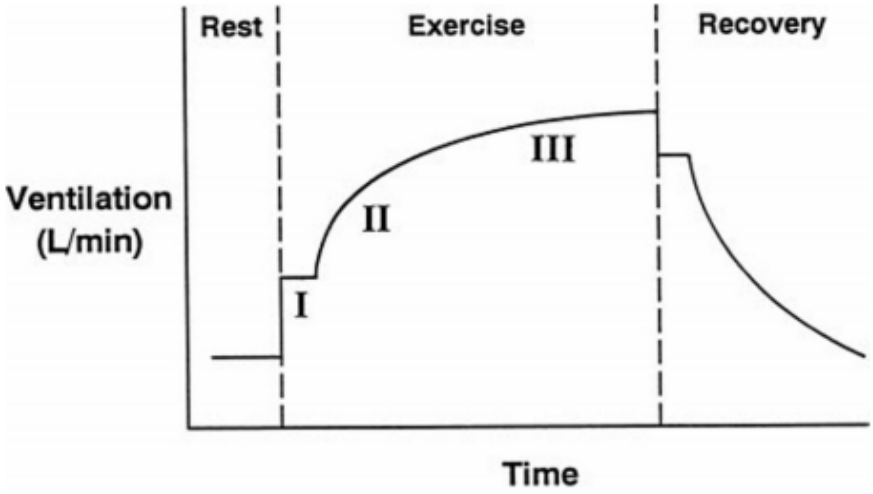

Figure 2.12. The normal ventilatory response to exercise, representing the three-phase response: I) the rapid increase in $\dot{V}_E$ at the onset of exercise, II) a slow exponential increase in $\dot{V}_E$, and III) a steady state of $\dot{V}_E$. From Caruana-Montaldo et al., (2000).

It is thought that a feed-forward neural control underpins the rapid ventilatory response at the onset of exercise (phase I). The neural processes involved with the control of breathing are thought to begin in the medulla, where breathing rhythms begin (Mitchell et al., 2009). A signal is generated at the brain that initiates the hyperpnea stimulus, either with, or in advance of locomotion (Forster et al., 2012). The first evidence to support this mechanism was presented by Krogh and Lindhard (1913), who investigated the $\dot{V}_E$ response to high intensity bicycle exercise in six healthy adults (*n=3* trained, *n=3* untrained). With the onset of exercise, a rapid increase in $\dot{V}_E$ was observed in all participants. Based on the rapid respiratory changes at the onset of exercise (with a latent period of less than 1 s), the authors concluded that the changes must be neurally-mediated. Ventilatory changes associated with chemical regulation from processes in the working muscles would indeed not be able to occur as quickly, and thus feed-forward control must contribute to the immediate ventilatory response to exercise.

These early findings have since been supported and understanding of the neurally-mediated rise in $\dot{V}_E$ in response to exercise has been enhanced. The proposal of a 'feed-forward' command to elevate $\dot{V}_E$ in anticipation of exercise was supported by Asmussen and Nielsen (1964), who occluded circulation at the upper thigh in healthy males prior to exercise and found that the rapid $\dot{V}_E$ response to cycling remained present. This finding implied that the

$\dot{V}_E$ response is most likely mediated by a centrally-driven neural activation, as the occlusion at the legs blocked the signal from the muscle chemoreflex. The neural feed-forward mechanism is further supported by evidence that show an increase in $\dot{V}_E$ at rest when exercise is imagined (Decety et al., 1993) and following passive limb movement (Bell and Duffin, 2006).

In contrast to the rapid and immediate rise in $\dot{V}_E$ experienced during phase one, the phase two response occurs when $\dot{V}_E$ has reached a steady-state and is sufficient to meet the demands for metabolic gas exchange (West, 2013). Finally, the phase three response is independent of feed-forward control and uses peripheral sensory feedback mechanisms to refine $\dot{V}_E$ to meet demand. Both phase two and phase three of the ventilatory response to exercise are governed by central neural command and metabolic control (West, 2013).

The $\dot{V}_E$ response to exercise may be altered by the duration, mode, and intensity of exercise. The $\dot{V}_E$ response may be further altered as a result of: external factors, such as the sex of the individual (Sheel et al., 2004); and environmental factors, such as hypoxia (Flenley et al., 1979), heat stress (Hayashi, 2015), and its associated side effects [i.e., elevated body temperature] (Tipton et al., 2017a). Interestingly, there are still many unknowns in regard to the ventilatory response to exercise. For example, whilst dehydration is commonly experienced during exercise in the heat, there is little information regarding how the ventilatory system responds to fluid loss, and whether dehydration may have negative implications for ventilatory function during exercise.

2.4 Ventilatory limitation during exercise

Whilst a normal ventilatory response to exercise is commonly experienced by healthy individuals (as described above), it has also been noted that ventilatory limitation can occur within this population. It has been proposed that the healthy respiratory system may be 'underbuilt' for the demands imposed during exercise in elite athletes and in those undertaking highly demanding endurance-based exercise (Dempsey et al., 2020). Three key factors may contribute to ventilatory limitation during exercise in healthy individuals: *i)* impairment of pulmonary gas exchange (Stickland et al., 2013) (see section 2.4.1); *ii)* respiratory muscle fatigue (Johnson et al., 1996) and sympathetic-mediated blood flow distribution (Dempsey et al., 2006; Harms et al., 1997) (see section 2.4.2); and *iii)*

expiratory flow limitation (EFL) and dynamic hyperinflation (Babb, 2013) (see section 2.4.3).

2.4.1 Pulmonary gas exchange impairment

Pulmonary gas exchange is the first step of the oxygen-transport chain: the lungs are required to optimise the exchange of respiratory gases to maintain the delivery of O_2 and removal of CO_2 (Stickland et al., 2013). Impairment to pulmonary gas exchange can therefore result in ventilatory limitation. The alveolar-arterial difference (A-aDO_2) provides a measure of the alveolar concentration of O_2 and is often relatively low (~5-10 mmHg) (Stickland et al., 2013). A widening of the A-aDO_2 [from 5-10 Torr at rest to 20-25 Torr at maximal exercise (Dempsey and Wagner, 1999)] can indicate exercise-induced arterial hypoxemia (EIAH) and is a sign of impaired pulmonary gas exchange (Phillips and Stickland, 2019). Whilst arterial O_2 concentration is well-maintained in healthy untrained to moderately trained individuals, healthy well-trained athletes may experience EIAH when undertaking exercise (Constantini et al., 2017). A-aDO_2 widens linearly with $\dot{V}O_2$, and is therefore indicative of alterations to pulmonary gas exchange during exercise (Prefaut et al., 2000). Wagner, (2015) has previously described the six key mechanisms that primarily contribute to this pulmonary gas exchange impairment, including *i)* relative hypoventilation (i.e., when alveolar ventilation is below the rate required for normal values of arterial blood gases); *ii)* mismatching of alveolar ventilation and pulmonary perfusion, and *iii)* diffusion limitation (leading to widened A-aDO_2), *iv)* reduced inspired PO_2, *v*) shunting of blood flow from the right to left sides of the heart, and *vi)* a reduction in pulmonary arterial PO_2.

The first recorded report of EIAH in a healthy population was presented by Dempsey, Hanson and Henderson, (1984), who showed that many highly trained men could not maintain arterial blood gas homeostasis during whole body exercise (quantified by a PaO_2 < 75 mmHg), suggesting that the large reserves for gas exchange are insufficient to meet the metabolic requirements achievable by trained individuals. Through inadequate supply of O_2 to the locomotor muscles, the occurrence of EIAH during exercise may limit performance. Indeed, an association has been previously been noted between EIAH and reduced $\dot{V}O_{2max}$ (Powers et al., 1993). Severe EIAH [SpO_2 < 88% (Dempsey and Wagner, 1999)] has been associated with a reduction in endurance exercise performance. Harms *et al.*, (2000) reported an association between EIAH and alterations in $\dot{V}O_{2max}$ in twenty-five healthy females, whereby preventing EIAH led to an improvement in $\dot{V}O_{2max}$ from 115%

to 200% of normal predicted values. The prevalence of EIAH has previously been reported at ~50 % in male endurance athletes (Powers et al., 1988), which might be slightly under-estimated. Constantini *et al.*, (2017) suggested that the prevalence of EIAH in healthy male athletes is more likely >70%: in a sample of 124 athletes, 84% experienced moderate or severe EIAH (when EIAH was defined as an oxygen saturation, $SpO_2 \leq 93\%$). When a more strict criteria was implemented (SpO_2 of $\leq 91\%$), 70% of athletes experienced EIAH.

Further, it has been observed that EIAH is more common in male athletes with a high $\dot{V}O_{2max}$ (>60 $mL \cdot kg \cdot min^{-1}$) (Powers et al., 1988; Powers and Williams, 1987). In females, however, the training status of the individual does not appear to influence the risk of developing EIAH in the same way as in males. Richards *et al.*, (2004) reported a prevalence rate of 67% in 52 healthy young females undertaking an incremental test to exhaustion. Dominelli *et al.*, (2013) assessed pulmonary gas exchange and EIAH in thirty healthy females ($\dot{V}O_{2max}$ ranging from 28 – 62 $mL \cdot kg \cdot min^{-1}$) during a treadmill exercise test. EIAH was found to occur in 65% of all participants, and in 93% of the trained individuals. These findings therefore suggests that EIAH is more common in females than males; a finding that has been attributed to the ventilatory constraints (i.e., EFL; see Section 2.4.3) experienced by females during exercise (Sheel et al., 2004).

Whether athletes performing in a dehydrated state may be at increased risk of EIAH has not yet been fully investigated. However, González-Alonso et al., (1998) reported average group values for PaO_2 in hydrated and dehydrated endurance-trained males. After 135 min of cycling in a dehydrated state (-3.9 ± 0.3% body mass), PaO_2 was significantly higher (108.8 ± 0.6 mmHg) compared to the euhydrated state (102.2 ± 0.8 mmHg). This suggests that dehydration may not significantly compromise gas exchange and blood gas homeostasis during prolonged exercise in endurance-trained male athletes.

2.4.1.1 *Assessing pulmonary gas exchange impairment*

Direct measurement of pulmonary gas exchange involves highly invasive procedures (i.e., arterial blood sampling for assessment of blood gases – largely focused upon PaO_2, $PaCO_2$, and $A\text{-}aDO_2$), requiring specialist medical training. As an alternative, SpO_2, via pulse oximetry, can be used as an indirect measurement of EIAH. This simple, non-invasive technique (which measures blood oxygen saturation), requires the placement of a pulse

oximeter onto the earlobe, finger-tip, or forehead of the participant, with a drop in SpO_2 of >4% from resting levels considered as EIAH (Prefaut et al., 2000). This method has been used to identify EIAH in a variety of populations and settings, including: competitive cyclists at altitude (Siegler *et al.*, 2007), swimmers (Spanoudaki et al., 2004) and children (Nourry et al., 2004). Further, SpO_2 has been shown to correlate strongly with arterial blood gases in both clinical- (Fanconi et al., 1985) and research-based settings (Ross et al., 2013), which adds confidence that this technique can be implemented for assessment of EIAH without the need for arterial blood sampling. The simplicity, reliability and availability of indirect EIAH assessment renders it a valuable tool for understanding ventilatory limitation during exercise.

2.4.2 Respiratory muscle fatigue

The second key contributing factor to ventilatory limitation in healthy adults during exercise is the onset of respiratory muscle fatigue. The diaphragm, a skeletal muscle, is the primary muscle involved in inspiration (Poole et al., 1997), and is supported by the other respiratory muscles, including the intercostal, ribcage and abdominal muscles (Welch et al., 2019). These muscles are vital to normal pulmonary function; activation of these muscles helps EELV to reduce below resting levels during exercise (Henke et al., 1988). The reduction in EELV means the diaphragm is lengthened and can operate and near optimal length to generate an adequate amount of force to meet the demands of exercise (Smith and Bellemare, 1987).

In healthy untrained individuals, the O_2 cost of breathing at maximal effort is ~8-10% of total $\dot{V}O_{2max}$ (Aaron et al., 1992); however, in the highly trained athlete this demand can be up to ~16% (Aaron et al., 1992). It is possible, therefore, that the increased O_2 demand during exercise cannot be met, particularly if the respiratory muscle work needs to be sustained for longer durations. Harms et al., (1997) and Dominelli et al., (2017) have previously reported a significant inverse relationship between work of breathing and locomotor blood flow. This infers that the diaphragm must compete for its allocation of cardiac output [which is estimated to be ~14-16% of overall cardiac output during maximal exercise (Harms et al., 1998)]. If a reduction in O_2 supply to the diaphragm occurs, the likelihood of respiratory muscle fatigue is increased (Romer and Polkey, 2008). Previous work by Vogiatzis *et al.*, (2009) in ten trained cyclists showed that intercostal muscle blood flow increases linearly with the work of breathing at rest during hyperpnea; however,

during exercise at intensities >80% WR_{max}, a plateau in muscle blood flow occurs, with blood flow lower than during resting hyperpnea (despite a matched $\dot{V}_E$). During exercise, the circulatory system may therefore not be able to meet the increased demands for O_2 of both the locomotor muscles and the respiratory muscles, thereby contributing to respiratory muscle fatigue. Dehydration has been shown to lead to alterations in the distribution of blood flow under specific exercising conditions. González-Alonso et al., (1998) showed a reduction in locomotor blood flow by ~2 $L \cdot min^{-1}$ during prolonged submaximal cycling exercise (~60% $\dot{V}O_{2max}$) until volitional exhaustion (135 ± 4 min) in healthy endurance-trained adults. During one-legged knee extensor exercise (23 ± 1 W at ~65rpm for 6 min), Pearson et al., (2013) however demonstrated that dehydration did not reduce leg blood flow and cardiac output at rest in healthy male participants. Therefore, factors causing alterations to muscle blood flow, such as systemic fluid loss, may only contribute to the onset of respiratory muscle fatigue and subsequently lead to ventilatory limitation when large muscle mass are recruited during exercise.

2.4.3 Expiratory flow limitation and dynamic hyperinflation

The third key contributor to ventilatory limitation in healthy exercising adults is the onset of EFL and dynamic hyperinflation. EFL is defined as an increase in transpulmonary pressure with no increase in expiratory flow (Calverley and Koulouris, 2005). EFL is a sign of intrathoracic airflow obstruction and, as well as being shown to limit ventilation (Tantucci, 2013), has been associated with increased perceived difficulty of breathing (Iandelli et al., 2002; Kayser et al., 1997), known as dyspnea, and increased work of breathing (O'Donnell et al., 2001). EFL, dyspnea and an increased work of breathing, alone or in combination, can ultimately lead to impaired performance and/or premature termination of exercise (Abdel Kafi et al., 2002). During high intensity exercise, the increased ventilatory demand is met via elevations of V_T, which is achieved by reducing EELV below FRC and increasing EILV (McClaran et al., 1999). When EELV increases above resting values during exercise, dynamic hyperinflation occurs (O'Donnell and Laveneziana, 2006). During dynamic hyperinflation, expiration is terminated prematurely, and the individual breathes at higher lung volumes. Working at higher lung volumes reduces ventilatory capacity by preventing the required increase in V_T. This can lead to an increased work of breathing (Loring et al., 2009), breathing discomfort (Iandelli et al., 2002), and may ultimately induce premature termination of exercise, particularly in individuals with pre-existing pulmonary disease (O'Donnell et al., 2001; O'Donnell and

Webb, 2008). Dynamic hyperinflation is often used as an effective compensatory strategy to facilitate greater airflow and avoid EFL.

Lung compliance refers to the lungs ability to stretch and expand (Kraman, 2007), and is directly proportional to lung volume (i.e., with increased lung compliance, lung volume increases). Elasticity refers to the resistance to the stretch and is inversely proportional to compliance; the more compliant the lung, the better the elastic recoil. The onset of EFL or dynamic hyperinflation has been associated with impaired elastic recoil function of the lung, which reduces its ability to produce high flow rates (O'Donnell and Laveneziana, 2006). Elastic recoil of the lung is highly dependent upon airway surface tension, which is mediated by ASL (Widdicombe and Widdicombe, 1995). Since ASL is an important contributor to surface tension, it can be postulated that reductions to this surface liquid volume may contribute to changes in lung compliance, and thus, contributes to EFL and dynamic hyperinflation. At present, no research has been conducted to assess the impact of systemic fluid loss upon EFL and dynamic hyperinflation. A greater understanding of the role of systemic water balance on ventilatory limitation is therefore required.

Whilst EFL and dynamic hyperinflation are more commonly experienced in clinical populations, such as in individuals with airway obstruction (i.e., chronic obstructive pulmonary disease; COPD, and asthma), it has also been shown to occur in healthy individuals with normal pulmonary function (Johnson et al., 1992). Guenette et al., (2007) reported significant EFL (assessed using the negative expiratory pressure technique; detailed in Section 2.4.3.1) in twelve out of seventeen endurance-trained cyclists at maximal exercise. Of the seventeen participants involved in this study, nine out of ten females and three out of seven males (>70% of the cohort) experienced EFL, demonstrating the risk of EFL to healthy individuals, and reinforcing that females may be more prone to EFL than males. Dominelli et al., (2013) also reported that fourteen out of thirty female participants (ranging from sedentary to elite athletes) experienced EFL (measured via oesophageal balloon-tipped catheter) during an incremental cycling test to exhaustion. Alongside this finding, eighteen of the participants also experienced EIAH at submaximal intensities during the test. These findings highlight how more than one factor may contribute to ventilatory limitation during exercise in healthy individuals.

2.4.3.1 *Assessing expiratory flow limitation and dynamic hyperinflation*

Assessment of EFL and dynamic hyperinflation has previously been somewhat challenging. However, in recent years numerous methods of detection have been developed. The direct assessment of EFL requires mostly invasive procedures with expensive equipment. To confirm the changes in inspiratory and expiratory pleural or oesophageal pressures for the measurement of EFL and dynamic hyperinflation, an oesophageal balloon is often inserted, which can assess flow, volume and transpulmonary pressure simultaneously whilst the participant performs repeated expiratory vital capacity efforts (Grieco et al., 2017). The oesophageal balloon methodology is complex and time consuming for both participant and researcher, and the invasive nature of the method renders it unfavourable with participant populations (Koulouris et al., 2012).

Alternatively, the negative expiratory pressure (NEP) technique has been implemented, which involves applying negative pressure at the mouth during tidal breathing. The principle of the NEP technique is that when flow-limitation is absent, an elevated pressure gradient between the alveoli and the airway opening caused by the negative pressure should lead to increased expiratory flow. On the other hand, when the participant is experiencing EFL, the application of negative pressure should not influence their expiratory flow (Koulouris et al., 1995). The NEP method is often considered the 'gold standard' for detection of flow limitation; however, alternative methods exist if the NEP equipment is unavailable.

Assessment of EFL without invasive procedures and specialist equipment is also possible, with two commonly used methods available. Manual compression of the abdomen is the first non-invasive technique, which follows a similar principle to the NEP method. The researcher applies a forceful pressure to the abdomen of the participant at the onset of expiration, and failure of the participant to increase expiratory flow is indicative of EFL (Koulouris et al., 2012). This method has been implemented during exercise (Abdel Kafi et al., 2002); however it has been less commonly used in research, likely due to the difficulty of squeezing the abdomen at the precise point of expiration, thus leaving potential for inaccurate results.

Another non-invasive but effective method for use at rest and during exercise is the inspiratory capacity (IC) manoeuvre to determine EELV (Hyatt, 1961). The IC is the

maximal volume of air that can be inhaled after a quiet breath out. This simple measurement allows accurate determination of EELV and EILV, without the need of specialist equipment (as all metabolic analysers are capable of measuring lung volumes). This measurement can provide information regarding changes to operating lung volumes, thus assisting with the assessment of dynamic hyperinflation. To establish whether EFL is present, the flow volume loop of a tidal breath needs to be superimposed within the maximum flow-volume curve (Figure 2.13). When the tidal breath is below the maximal expiratory flow-volume curve (Figure 2.13, panel A), EFL is not present. However, when the tidal breath appears to impinge or overlap the maximal expiratory flow-volume curve (Figure 2.13, panel B), EFL has occurred. The degree of EFL is quantified by the percent of the tidal flow-volume loop that exceeds the expiratory portion of the maximal expiratory flow-volume curve (Johnson et al., 1999). This technique relies on TLC remaining constant, which is known to occur in healthy adults (Stubbing et al., 1980). It also relies heavily on participant cooperation, which may be difficult in clinical populations. Whilst this conventional method does not provide direct measurement of inspiratory pressures, it is a simple, effective method for the assessment of both EFL and dynamic hyperinflation (Dolmage and Goldstein, 2002), which requires minimal specialist equipment and is non-invasive. An advantage of this method is that the assessment of operating lung volumes can be performed concurrently to the exercise test. The measurement of operating lung volumes (i.e., EELV, EILV) and degree of EFL can be useful indicators of ventilatory constraint, particularly during exercise (Guenette et al., 2013). When combined with breathing pattern responses (i.e., f_b and V_T), a comprehensive evaluation of ventilatory limitation during exercise can therefore be obtained.

Utilising one or more of the above-mentioned methods for the assessment of ventilatory limitation is needed when one wants to thoroughly evaluate the ventilatory response to exercise. Whilst it is apparent that ventilatory limitations can occur in both, clinical and healthy populations, external/environmental factors may also contribute to the development of such limitations.

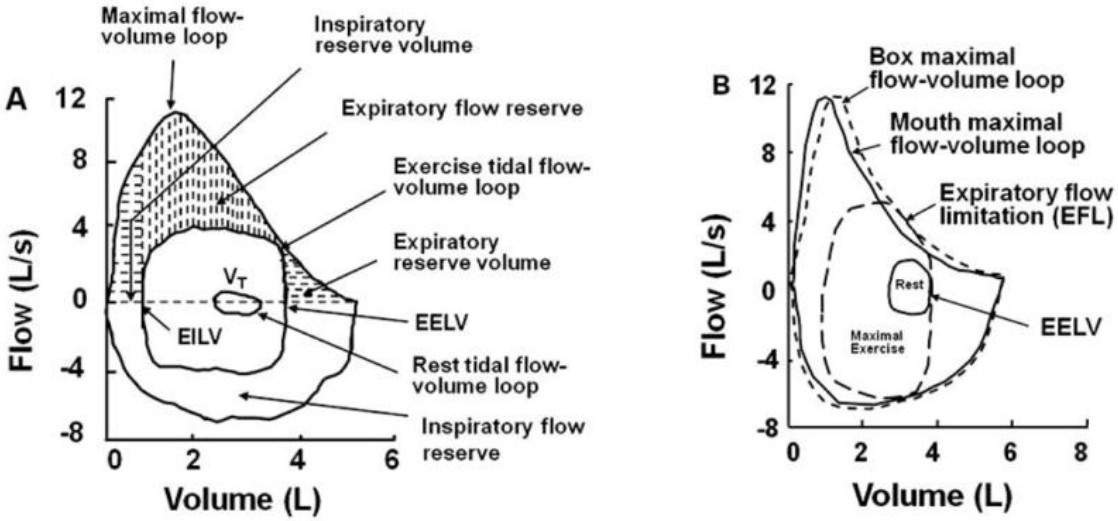

Figure 2.13. Examples of two expiratory flow volume loops a) without expiratory flow limitation (EFL) and b) with EFL. When tidal expiratory flow approaches or impinges on maximal expiratory flow (as shown in panel B), this is referred to as EFL. Taken from Babb (2013).

2.4.4 Factors influencing ventilatory limitation

2.4.4.1 *Ventilatory responses to heat exposure*

External (i.e., heat stress) and internal (i.e., elevated T_{core}) factors can influence the response of the ventilatory system at rest and during exercise. Early work by Haldane (1905) demonstrated that elevated T_{core} led to significant elevations of $\dot{V}_E$. This response has now been well-established (Tsuji et al., 2016) and is referred to as hyperthermia- (or heat-) induced hyperventilation. Hyperthermia-induced hyperventilation in humans is accompanied by elevations in alveolar ventilation; consequently, blood gases are altered (PaO_2 is increased and $PaCO_2$ decreased) when body temperature raises by 1.5ºC (Saxton, 1975). For core temperatures above 38.0–38.5ºC, increases of ~20–30 $L \cdot min^{-1}$ in $\dot{V}_E$ are commonly noted (Fujii *et al.*, 2008; Tsuji *et al.*, 2012).

The ventilatory response to heat exposure has been shown both at rest and during exercise. Tsuji *et al.*, (2019) demonstrated significant elevations in $\dot{V}_E$ following passive heating (via water immersion at 41ºC) until T_{core} reached 39.0ºC in ten healthy male participants. An increase in $\dot{V}_E$ from 9.3 ± 0.8 $L \cdot min^{-1}$ to 26.7 ± 6.8 $L \cdot min^{-1}$ was noted after 30 min of passive heating. These elevations in $\dot{V}_E$ were also associated with an increased work of

breathing (from 13.0 ± 8.9 J·min^{-1} at rest to 59.4 ± 49.5 J·min^{-1} at termination of heating). This increased work of breathing was likely due to the fact that $\dot{V}_E$ was elevated with no change in EELV. The authors discussed that these changes in ventilatory responses are involuntary and likely associated with increased brain temperature. Further investigation using passive heating showed that pulmonary function can also be influenced by changes in T_{core} (Tipton *et al.*, 2017). Following 30 min of water immersion at 40ºC, nine healthy males displayed improvements in FEV_1, expiratory flow at 50% (FEF_{50}) and 75% (FEF_{75}) of FVC, indicative of a bronchodilator effect of elevated T_{core} (no breathing pattern data were recorded in that study). The authors proposed that an increase in T_{core}, independent of exercise, may contribute to airway smooth muscle compliance, and thus caused bronchodilation.

Alterations to the ventilatory response to exercise during heat stress have also been displayed during sub-maximal exercise. A linear relationship between T_{core} and $\dot{V}_E$ (mediated by increased f_b) was demonstrated by Hayashi *et al.*, (2006) in thirteen healthy males exercising at sub-maximal intensity (50% of peak O_2 uptake; $\dot{V}O_{2peak}$). Throughout the 60 min cycling bout, $\dot{V}_E$ increased at a rate of 5-6 L·min^{-1} for every 1ºC increase in T_{core}. In a study by Tsuji *et al.,* (2012b), the T_{core} of ten healthy males was elevated to 37.5 ± 0.35ºC (via pre-heating with water immersion at 40ºC) prior to cycling at 50% of $\dot{V}O_{2peak}$ in hot conditions (37°C and 50% relative humidity), and rose to 38.6 ºC following exercise. Significant elevations in $\dot{V}_E$, f_b and $\dot{V}CO_2$ were noted, whilst V_T significantly decreased, and $\dot{V}O_2$ remained unchanged. Further, a linear relationship was reported between increasing T_{core} and increasing $\dot{V}_E$. The reduction in V_T , alongside an elevation of f_b, demonstrates that, during exercise in the heat, hyperventilation is modulated by the rate, rather than the depth of the ventilatory response. The findings from Tsuji *et al.,* (2012b) were confirmed by further investigations into the ventilatory response to exercise heat-stress. Tsuji *et al.*, (2015) demonstrated that hyperthermia-induced hyperventilation can be supressed by controlled breathing during exercise. Twelve healthy males followed the same protocol as described above (Tsuji *et al.,* 2012). In one condition, participants breathed freely during exercise, and in the other, participants were required to control their own breathing by timing their f_b using a metronome. In the normal breathing trial, $\dot{V}_E$ increased from 9.9 ± 1.5 L·min^{-1} at rest to 55.1 ± 8.9 L·min^{-1} at termination of the 60 min exercise bout. The rate of increase in $\dot{V}_E$ of 8 ± 4 L·min^{-1} for every 1ºC increase in T_{core} was comparable to that reported by Hayashi *et al.*, (2006). After the initial increases in V_T and

f_b at the start of exercise, V_T gradually reduced, whilst f_b increased, demonstrating that the depth of the ventilatory response may play a major role in hyperthermia-induced hyperventilation. In contrast, $\dot{V}_E$ and f_b in the controlled-breathing trial were lower and V_T was higher, indicating that the ventilatory response to hyperthermia can be voluntarily supressed. Altogether this information suggest that it is important to consider the impact of heat-stress upon the ventilatory response to exercise when designing research studies involving exercise in which T_{core} is likely to be elevated.

As an elevated T_{core} can alter the ventilatory response to exercise, and as dehydration is a common occurrence during exercise in the heat (Galloway and Maughan, 1997; Gibson et al., 2014), exploring the independent and combined effects of dehydration and heat stress on ventilatory response during exercise is of relevance.

2.4.4.2 *Ventilatory responses to dehydration*

Little work has been conducted so far to directly investigate the impact of dehydration upon the ventilatory response at rest and during exercise in humans. Of the research currently available, conflicting results have been reported, with studies reporting increases (Fan et al., 2008; Senay and Christensen, 1967), decreases (Caldwell et al., 1984), and no change (Dengel et al., 1992; England et al., 1984; Fujii et al., 2008a) in $\dot{V}_E$ in a state of dehydration.

Early work by Senay and Christensen (1967) investigated the impact of heat-induced dehydration on the resting ventilatory response. Five males were exposed to 43ºC for 12 h, resulting in a dehydration severity of 5% body mass loss. In the dehydrated trial, elevations in $\dot{V}_E$ (from 8.3 ± 2.0 L·min^{-1} to 10.6 ± 3.8 L·min^{-1} at rest) and P_{osm} (from 289 ± 3 mOsm·kg^{-1} to 300 ± 4 mOsm·kg^{-1}) were noted. Similar changes did not occur in the control condition, in which participants' body mass loss was replenished hourly with 0.1% saline throughout the 12h exposure period. Notably, f_b was not altered, suggesting that the observed changes in $\dot{V}_E$ were mediated by changes in V_T. These findings also suggested an association between increased P_{osm} and elevated $\dot{V}_E$. One limitation to this study is that the elevations in body temperature were not the same between trials: in the dehydration condition, body temperature was elevated by ~1ºC (from 36.6 ± 0.3ºC to 37.7 ± 0.2 ºC), whereas in the control trial, the increase was only ~0.5 ºC (from 36.5 ± 0.2 ºC to 37.1 ± 0.4ºC). Based on information presented in section 2.4.4.1, it is likely that the change in body temperature acted as a confounder and contributed to the changes in ventilatory response. Based on

these data, it is therefore difficult to isolate the effects of dehydration (and the associated change in P_{osm}) on ventilatory response at rest. Future work should aim to avoid significant changes in T_{core} during dehydration to fully understand the role of dehydration upon ventilatory function. Follow-up work by the same group investigated the impact of elevated blood osmolarity on ventilation in dehydrated males independent of changes in body temperature (Senay, 1969). In this study, eight males undertook 2 h of heat exposure (at 43ºC), with 10 min of stair stepping undertaken at 30 min intervals (at 0, 30, 60, and 90 min) on two occasions, with 60-90 min break between exposures. Participants were fluid restricted throughout the trials, and thus hypertonic-hypovolemia was induced (quantified by elevations in P_{osm} from 301 ± 9 mOsm·L^{-1} H_2O to 317 ± 8 mOsm·L^{-1} H_2O). The authors reported findings that contradicted their previous work and showed that the elevated P_{osm} reduced the respiratory response (quantified by reductions in alveolar ventilation) to elevated body temperatures. These findings suggest that increased T_{core} may alter the ventilatory response to dehydration, and again highlights the importance of controlling this confounding variable in future research investigating the impact of changes to body fluid balance upon the pulmonary system.

More recently, Fujii *et al.*, (2008) investigated the impact of dehydration upon hyperthermic-hyperventilation. Thirteen healthy adult males undertook two bouts of exercise in the heat (35 ºC, 50% RH) at 50% $\dot{V}O_{2peak}$ (50-60 min in bout one, and 30-50 min in bout two). The exercise bouts were separated by a period of either fluid replacement with sodium water (150% of body mass loss during bout one was replaced) or no fluid replacement. By the end of exercise bout two in the fluid replacement trial, participants were dehydrated by ~2% body mass (no change in P_{osm} from pre-exercise: 290 ± 4 mOsm·kg^{-1} H_2O to post-exercise: 291 ± 3 mOsm·kg^{-1} H_2O) and by ~4% body mass (increase in P_{osm} from pre-exercise: 290 ± 3 mOsm·kg^{-1} H_2O to post-exercise: 299 ± 4 mOsm·kg^{-1} H_2O) in the no fluid trial. The authors reported no difference in the rate of change in $\dot{V}_E$ following exercise in the heat with no fluid replacement (i.e., in a dehydrated state; increase in $\dot{V}_E$ of 9.9 ± 7.6 L·min^{-1} per 1ºC increase in T_{core}) compared to with fluid replacement (i.e., a euhydrated state; increase in $\dot{V}_E$ of 8.8 ± 6.1 L·min^{-1} per 1ºC increase in T_{core}). These findings suggested that it is the change in T_{core}, rather than the change in hydration status, that primarily influenced the ventilatory response to sub-maximal exercise in the heat. However, it is important to consider that whilst Fujii *et al.*, (2008) did provide fluid to their participants between exercise bouts in their control condition, participants still

completed the trial in a state of 2% body mass loss, which would be classified as a state of mild dehydration (Cheuvront and Kenefick, 2014). It therefore difficult to confirm that the observed responses were not, at least in part, attributable to the change in hydration status from baseline to post-exercise. Further, since exercise was performed at moderate intensity and in a steady-state, it remains unknown how the ventilatory response to incremental and maximal exercise is impacted by hydration status.

Alongside the ventilatory response to exercise-induced dehydration, concomitant perceptual responses associated with respiratory comfort may also be of interest. Endurance-trained individuals exercising at high intensities develop extremely high ventilatory rates (Wells and Norris, 2009). Elite athletes working at such intensities commonly report respiratory symptoms during exercise, such as difficulty breathing, breathlessness and feelings of breathing discomfort (Smoliga et al., 2016; Turcotte et al., 2003). Respiratory symptoms are particularly common in athletes competing in cold/dry environments (Lennelöv et al., 2019) who ventilate high volumes of cold/dry air. The high volumes of cold/dry air that is ventilated in those environments must be warmed and humified upon inspiration, a process which may promote local airway dehydration (Kippelen et al., 2012). Respiratory symptoms may impair an athlete's performance in training and/or competition. Understanding whether systemic dehydration may contribute to the onset of breathing discomfort and other respiratory symptoms would be beneficial to understanding the overall ventilatory response to exercise.

Whilst components (i.e. V_T, $\dot{V}_E$, and f_b) of the ventilatory response to exercise in dehydrated individuals have been reported in a limited number of studies (Table 2.3), a direct and comprehensive analysis of this response, including a combination of breathing pattern, operating lung volumes, and respiratory symptoms is still warranted.

Table 2.3. The impact of dehydration upon ventilatory parameters at rest and during exercise

Author (year)	Participants	Dehydration method	Dehydration severity (%)	Exercise test	T_{core}	Ventilatory response
Senay and Christensen, (1967)	5 males (health / training status not included)	12 h resting heat exposure (43ºC)	-5 % body mass	None	Rest: 36.67ºC Dehy: 37.69ºC	$\dot{V}_E$ ↑*: Rest: 8.3 ± 2.0 L·min^{-1} Dehy: 10.6 ± 3.8 L·min^{-1} f_b↔: Rest: 17.3 ± 6.3 breaths·min^{-1} Dehy: 18.3 ± 6.6 breaths·min^{-1}
Caldwell, Ahonen and Nousiainen, (1984)	62 male athletes	1) Diuretic (furosemide) 2) Sauna (80ºC, 50% RH) 3) Exercise (running / swimming) 4) Control (*n=15)*	1) -3.1 ± 0.8 kg 2) -3.5 ± 0.8 kg 3) -2.3 ± 0.8 kg 4) -0.8 ± 1.0 kg	Incremental cycle test to exhaustion	Not reported	Submaximal intensities: 1) $\dot{V}_E$ ↓*: 2) -5.1 ± 8.8 L·min^{-1} V_T ↔: -0.2 ± 0.4 L f_b↔: -0.1 ± 0.6 breaths·min^{-1} 3) $\dot{V}_E$ ↓: -0.4 ± 9.8 L·min^{-1} V_T ↔: 0.2 ± 0.3 L f_b↔: 0.1 ± 0.6 breaths·min^{-1} 4) $\dot{V}_E$ ↓: 1.0 ± 6.2 L·min^{-1} V_T ↔: 0.0 ± 0.2 L f_b↔:0.1 ± 0.8 breaths·min^{-1} 5) $\dot{V}_E$↔: 1.9 ± 7.0 L·min^{-1} V_T ↔: 0.0 ± 0.3 L f_b↔: 0.2 ± 0.7 breaths·min^{-1} Peak exercise: 1) $\dot{V}_E$ ↓*: -9.4 ± 17.3 L·min^{-1} V_T ↔: -0.3 ± 0.2 L

						f_b↔: 1.3 ± 6.0 breaths·min^{-1} 2) $\dot{V}_E$ ↓*: -9.1 ± 12.9 L·min^{-1} V_T ↔: -0.3 ± 0.2 L f_b↔: 1.8 ± 6.3 breaths·min^{-1} 3) $\dot{V}_E$ ↓: -1.5 ± 10.4 L·min^{-1} V_T ↔: -0.1 ± 0.2 L f_b↔: 2.7 ± 5.5 breaths·min^{-1} 4) $\dot{V}_E$ ↑: 12.0 ± 12.3 L·min^{-1} V_T ↔: 0.0 ± 0.1 L f_b↔: 5.1 ± 6.**0** breaths·min^{-1}
England *et al.*, (1984)	6 trained males	Intermittent sauna exposure (65ºC)	-5% mass	Incremental cycle test to exhaustion	Dehydration: ~38.2ºC Euhydration: ~ 37.5ºC	Submaximal: $\dot{V}_E$ ↔ *vs* euhydration Peak: $\dot{V}_E$ ↔ *vs* euhydration
Dengel *et al.*, (1992)	9 healthy males	Exercise at 50% $\dot{V}O_{2max}$ for 1.5h (38ºC), repeated x2 in a 36 h period	1) - 3% mass 2) - 6% mass	Incremental cycling test to exhaustion	Not reported	Submaximal intensities: 1) $\dot{V}_E$ ↔ *vs* euhydration 2) $\dot{V}_E$ ↔ *vs* euhydration Peak exercise: 1) $\dot{V}_E$ ↔ *vs* euhydration 2) $\dot{V}_E$ ↔ *vs* euhydration 3)
Fan *et al.*, (2008)	10 healthy males	Passive heating in water perfused suit (48°C)	40°C, 20% RH	None	↑ by 2°C *vs* rest	$\dot{V}_E$ ↑*: Rest: 10 ± 3 L·min^{-1} End: 18 ± 5 L·min^{-1} V_T ↔:

						Rest: 0.7 ± 0.3 L End: 0.9 ± 0.4 L $\boldsymbol{f_b}$↑*: Rest: 15 ± 3 breaths·min^{-1} End: 20 ± 5 breaths·min^{-1}
Fujii *et al.*, (2008)	13 healthy males	2x bouts cycle exercise of 30-60 min at 50% $\dot{V}O_{2peak}$ in 35°C with no fluid	-2.5% mass	Steady state cycling exercise at 50% $\dot{V}O_{2peak}$	Dehydration: ↑ 2.1 ± 0.3°C Euhydration: ↑ 1.8 ± 0.2°C	$\boldsymbol{\dot{V}_E}$ ↔ *vs* euhydration $\boldsymbol{V_T}$ ↔ *vs* euhydration $\boldsymbol{f_b}$ ↔ *vs* euhydration

$\dot{V}_E$: minute ventilation; V_T: tidal volume; $\dot{V}O_{2max}$: maximal volume of oxygen consumption; f_b: breathing frequency; kg: kilograms; RH: relative humidity. *p<0.05: dehydration vs euhydration

2.5 Overall summary of the literature

Water is fundamental for normal physiological function in humans and is an essential component of the pulmonary system. It has been shown that body fluid loss (i.e. dehydration) during exercise negatively influences a multitude of physiological systems (including the cardiovascular, renal, metabolic, neurohumoral, and gastrointestinal systems), whereas the function of these physiological systems during prolonged exercise heat stress in a euhydrated state are well maintained (Travers et al., 2020). The impact of dehydration upon the pulmonary system however, remains unclear. Since optimal function of the pulmonary system in humans is vital both at rest and during exercise, it is of growing importance to further our understanding of *i)* the pulmonary response to systemic fluid loss, and *ii)* potential mechanisms associated with the observed response. During large muscle mass exercise (such as long duration cycling), the pulmonary system is placed under extreme stress, and ventilatory limitation (i.e. impaired gas exchange, respiratory muscle fatigue, and/or EFL) can occur in both healthy and clinical populations. However, the functional implications of fluid loss (commonly experienced during exercise – particularly in hot conditions) remains to be fully elucidated.

2.6 Thesis aims and hypotheses

The overarching aim of this thesis was to determine the effects of systemic hydration on normal pulmonary function and ventilatory response to exercise in healthy adults. Following an in-depth review of the literature, the following objectives, which were addressed by specific experimental chapters, were developed:

1. To investigate the inter-day repeatability and intra-day reproducibility of pulmonary function in healthy young adults (Chapter 4).
    a. It was hypothesised that spirometry and whole body plethysmography would show good repeatability when performed on multiple days (inter-day), and good reproducibility when performed at multiple times in the same day (intra-day).
2. To investigate the impact of mild systemic dehydration on resting pulmonary function in healthy young adults (Chapter 5).
    a. It was hypothesised that pulmonary function would be impaired following systemic dehydration induced via both, exercise in the heat, and a prolonged period of fluid restriction.
3. To establish whether dehydration-induced changes in pulmonary function at rest are reversible with immediate systemic and/or local airway rehydration (Chapter 5).
    a. It was hypothesised that immediate rehydration by oral fluid intake and isotonic saline nebulisation would successfully reverse dehydration-induced pulmonary function impairments.
4. To investigate the impact of moderate dehydration induced by exercise on resting pulmonary function in trained young adults (Chapter 6).
    a. It was hypothesised that moderate dehydration would lead to negative alterations in pulmonary function in trained young adults.
5. To establish the effect of dehydration on ventilatory and perceptual responses to exercise.
    a. It was hypothesised that ventilatory parameters would be negatively altered and breathing discomfort would increase when healthy trained individuals perform an incremental exercise test to exhaustion in a state of moderate dehydration.

Chapter 3 General methods

Within this thesis, three experimental studies are presented. This chapter details the general materials and methods that were implemented throughout the experimental studies. Details of additional (experiment-specific) protocols are presented in the relevant chapters, although some specific techniques requiring detailed explanation are also outlined in this chapter.

3.1 Ethical approval

The University Research Ethics Committee (UREC) approved the protocols and procedures of all experimental studies presented in this thesis. Ethical approval codes are provided in the 'ethical approval' sections of each experimental chapter. Evidence of ethical approval is presented in Appendix A.

3.2 Participants

Healthy adults (male and female) aged between 18 and 50 years were recruited for each experimental study. The age range was capped at 50 years to avoid confounding issues of age on pulmonary function (Thomas et al., 2019). Participants were deemed healthy to take part prior to participation following completion of a health questionnaire (Appendix B). Individuals that reported adverse health issues were excluded from study participation. Smokers were not permitted to take part in the experiments due to the well-established negative impact of smoking on pulmonary health and function (Comstock et al., 1970; Xu et al., 1994). Individuals with a history of respiratory illness or disease (i.e., asthma), or any other chronic illness that may have impacted participation, were also excluded. A detailed description of the participants recruited to each study is provided in the relevant experimental chapters.

3.2.1 Participant recruitment

Participants were recruited via posters placed around Brunel University London and the surrounding areas. Announcements were made in lectures, and the research was posted on the University Intranet site (under *'Call for Participants'*) and on social media (including

Facebook and Twitter). For study 3 (Chapter 6), information was also distributed to local cycling and triathlon clubs, and announcements were made at local sporting events.

3.2.2 Pre-participation

Before agreeing to participate, volunteers were provided with a detailed participant information sheet, which described: the requirements of the study; precise experimental procedures; benefits and risks associated with participation. Willing volunteers were encouraged to ask any questions regarding the studies before deciding whether or not to take part. All participants signed written informed consent (Appendix C) prior to taking part.

3.3 Participant characteristics

3.3.1 Anthropometry: stature

Participants' standing stature (cm) was recorded at the first experimental visit of each study. Stature was measured using a stadiometer (SECA model 798, Hamburg, Germany), with shoes and socks removed. Participants were required to stand vertically, facing away from the stadiometer. The stadiometer measurement arm was lowered until rested upon the top of the head, participants were instructed to take one deep inspiration, followed by a relaxed expiration to ensure correct posture. Stature was recorded to the nearest 0.5 cm.

3.3.2 Anthropometry: body mass

Participants self-reported nude body mass (to the nearest 0.1 kg) in the privacy of a lockable bathroom using electronic scales (SECA model 798, Hamburg, Germany) at each experimental visit (specific time points of measurement are detailed in the relevant experimental chapters). Participants were instructed to remove all clothing, dry themselves with a towel (to remove any excess sweat), and step onto the zeroed scales until the digital display was stable.

3.4 Control measures and standardisation

The following control measures were adhered to within each experiment to reduce the risk of confounding factors influencing the dependent variables.

3.4.1 Pre-trial restrictions

Participants were required to avoid alcohol, caffeine, diuretics, and strenuous exercise in the 24 h prior to each experimental trial to avoid any potential confounding impact upon: pulmonary function (Chapman and Mickleborough, 2009), T_{core} (McHill et al., 2014), hydration status (Roberts, 1963), and exercise effort (Ganio et al., 2009b). Participants recorded a food diary in the 24 h prior to the first experimental trial and replicated this food diary prior to each visit. Participants were required to arrive to the laboratory in a euhydrated state on each visit, by maintaining normal drinking and consuming a minimum of 500 mL the night before and the morning of each experimental trial.

3.4.2 Diurnal variation

Unless necessitated by experimental design, comparable trials were performed at the same time of day in order to reduce the impact of diurnal variation on pulmonary function (Spengler and Shea, 2000), T_{core} and exercise performance (Waterhouse et al., 2005). Each experimental trial began between 08:00 h – 10:00 h to ensure inter-individual standardisation. All participants completed their individual trials at the same start time to ensure intra-individual standardisation. Chapter 4 provides a detailed explanation of control data collected to assess reproducibility of pulmonary function tests performed at multiple times on the same day (intra-day reproducibility).

3.5 Dehydration protocols

3.5.1 Exercise

Exercise was used as a method of inducing dehydration in experiments 2 and 3 (Chapters 5 and 6). Specific exercise protocols are detailed in the relevant experimental chapters.

3.5.2 Cycle ergometry

All cycling tests were performed on a cycle ergometer (Lode Excalibur, Lode, B.V, Groningen, The Netherlands). Participants were required to maintain a self-selected steady cadence of 70-90 rpm for the duration of the exercise trials and remained seated on the cycle ergometer at the same saddle height at all times during exercise. The ergometer provides a workload accuracy of ± 2 W when operated at workloads below 100 W, and ±

2 % when operated at workloads from 100 to 1,500 W (Lode, 2016). Power output was controlled by the electro-magnetic braking mechanism of the ergometer using the following equation:

$$\text{Power output (W)} = \text{Resistance} \times \text{Cadence}$$

Note. Where resistance is measured in kilograms (kg) and cadence recorded in revolutions per minute (rpm).

Workload was determined specifically for each individual study. For study 2 (Chapter 5), participants cycled at 25% of estimated peak power, in line with the protocol used by Simpson et al., (2017). Estimated peak power was determined using the following equations (Hansen et al., 1984):

$$\text{Male estimated peak power} = [(\text{stature} - \text{age}) \times 20] / 10$$

$$\text{Female estimated peak power} = [(\text{stature} - \text{age}) \times 14] / 10$$

Note. Where stature is measured in centimetres (cm), and age is measured in years.

To induce a moderate severity of dehydration in study 3 (Chapter 6), participants were required to work at 40% of their peak power (determined via an incremental test to exhaustion - see section 3.5.4). This power output was adapted from previous work (Trangmar et al., 2014), in which 3.1 ± 0.3% of body mass loss was induced via semi-recumbent cycling at 55% maximal work rate. Semi-recumbent cycling is associated with reduced maximal work rate and $\dot{V}O_{2max}$ in comparison to upright cycling (Scott et al., 2006); therefore the relative workload applied to induce moderate dehydration in study 3 via upright cycling was set at 40 % of their peak power.

3.5.3 Exercise in the heat

Exercise in the heat was used in studies 2 and 3 (Chapters 5 and 6) as a mode of inducing dehydration via elevated sweat loss. Environmental conditions were controlled by a purpose-built environmental chamber (Procema Ltd., Twickenham, UK). Conditions were set at 37°C and 50% relative humidity (RH) for the duration of exercise. These environmental conditions were based on previous work investigating the impact of dehydration upon pulmonary function of individuals with asthma (Simpson et al., 2017).

Temperature and humidity were monitored and recorded every 15 min using a hygrometer (RH32, Omega, Manchester, UK) to ensure stability at the desired levels.

3.5.4 Incremental exercise test

A maximal incremental exercise test was used as a means to *i)* determine peak power output of participants in study 3, and *ii)* to establish the ventilatory response to incremental exercise in a dehydrated state. Participants performed a standardised warm up for 3 min at 50 W, followed by increments of 50 W every 2 min until exhaustion. To ensure similarity in test duration between participants, females began the first stage at 50 W, whilst males began at 100 W. Cadence was maintained between 70 and 90 rpm throughout the test. Participants were verbally encouraged throughout the test by the experimenter to ensure maximal effort was obtained. The test was terminated when the participant reached volitional exhaustion, or when the cadence dropped below 60 rpm for > 5 s.

Criteria for determining whether $\dot{V}O_{2max}$ had been reached was conducted in accordance with the guidelines from the British Association of Sport and Exercise Sciences (Winter et al., 2006). The end-point criteria were as follow: achievement of a plateau in $\dot{V}O_2$ despite an increase in workload; heart rate within 10 beats·min^{-1} of age related maximum (see section 3.9.1); a respiratory exchange ratio (RER) of ≥ 1.15; and a perceived exertion of ≥ 19 (see section 3.10.2).

3.6 Ventilatory parameters during exercise

3.6.1 Calibration of breath-by-breath gas analyser

During the exercise trials, ventilatory parameters were recorded via breath-by-breath analysis (Vyntus CPX, Carefusion, Hoechberg, Germany) using the SentrySuite 360º ® software (Carefusion, Hoechberg, Germany). The system was calibrated for volume and gas concentration prior to each test. The gas calibration was performed first using the SentrySuite 360º ® automated gas calibration function, and the gas analyser was calibrated using certified gas concentrations (CO_2 = 5%, O_2 = 15%; balanced with N_2). Volume calibration was performed used a 3 L calibration pump (Jaeger Calibration Pump, Jaeger, Hoechberg, Germany), with the pump piston pulled back and forth at a steady speed to ensure the pump was completely filled and emptied with each stroke. The SentrySuite 360º

® software provided live feedback throughout the calibration, and calibration was repeated if volume deviated from an acceptable range (± 10%).

3.6.2 Breath-by-breath analysis

Table 3.1. Names and definitions of ventilatory parameters presented within this thesis.

Parameter	Units	Definition
Minute ventilation ($\dot{V}_E$)	$L \cdot min^{-1}$	Total volume of gas inhaled or exhaled from the lungs per minute
Breathing frequency (f_b)	$breaths \cdot min^{-1}$	The number of breathing cycles *per* minute
Tidal volume (V_T)	L	The volume of gas which is inhaled and exhaled during one ventilatory cycle
Rate of oxygen uptake ($\dot{V}O_2$)	$L \cdot min^{-1}$ / $mL \cdot kg \cdot min^{-1}$	Volume of oxygen utilised *per* minute / Volume of oxygen utilised *per* minute, relative to body mass
Rate of carbon dioxide produced ($\dot{V}CO_2$)	$L \cdot min^{-1}$	Volume of carbon dioxide exhaled per minute
Inspiratory time (T_i)	s	Time taken for inhalation
Total time of breath (T_{tot})	s	Total time taken for whole breath

Participants breathed into a small mouthpiece with a nose-clip on for breath-by-breath analysis. During exercise in the heat (studies 2 and 3), participants breathed into the mouthpiece for 5 min at each data collection point (both at rest and during exercise). Data from the final minute of recorded breathing was taken for analysis to allow stabilisation of breathing rate when using the mouthpiece. Table 3.1 details the specific ventilatory parameters recorded. Analysing multiple ventilatory parameters during exercise provides a comprehensive analysis of the breathing pattern and the ventilatory response of the individual tested (Neder et al., 2003). Parameters recorded and analysed within each study are detailed in the relevant experimental chapters.

3.6.3 Operating lung volumes

In study 3 (Chapter 6), the ventilatory response to exercise was assessed by measuring dynamic changes in operating lung volumes, i.e., EELV (L) and EILV (L). Analysis of operating lung volumes, alongside breathing pattern (see section 3.6.2) and perceptions of breathing discomfort (see section 3.10.1) allowed for a detailed evaluation of ventilatory abnormalities during exercise (Guenette et al., 2013).

Assessment of operating lung volumes was performed using IC manoeuvres. During the incremental exercise test (see section 3.5.4), participants performed IC manoeuvres in duplicate at rest, during the last 30 s of each incremental exercise stage, and at maximal exertion. The manoeuvre was clearly explained to the participant using the following instruction, as recommended by Guenette et al., (2013): *"Take a deep breath in until you are completely full. First finish your normal breath out, and then proceed to fill your lungs quickly and without hesitation until you are as full as possible. When you can get no more air in, then you can return to normal breathing."* Following this explanation, an example of how the manoeuvre should be performed was demonstrated by the experimenter. Participants performed practice manoeuvres at rest until the experimenter was satisfied and the participants were comfortable with the technique. Prior to the test, participants were instructed to raise their hand when they felt they had approximately 10 s until exhaustion, to allow the final IC manoeuvre to be performed. During the test, approximately 10 s prior to each IC manoeuvre, participants were instructed as follow: *"at the end of a normal breath out, take a fast maximal breath in until your lungs are completely full."* Participants then repeated the manoeuvre following 5–10 normal breaths. Verbal encouragement was provided throughout each manoeuvre to ensure a maximal inspiratory effort was achieved.

Stability of EELV prior to IC manoeuvres was checked by the experimenter via visual inspection of real-time breath-by-breath recordings throughout each manoeuvre. The experimenter was able to ensure the breathing pattern of the participant was not altered in anticipation of the deep inspiration. Figure 3.1 shows examples of a correctly performed IC manoeuvre (panel a), alongside two incorrectly performed manoeuvres that would lead to either b) underestimated or c) overestimated IC values. When the experimenter deemed EELV to be unstable, or when IC was performed incorrectly, the participant was asked to repeat the manoeuvre.

As TLC is considered to remain stable throughout exercise (Stubbing et al., 1980), operating lung volumes can be calculated using the following equations (operating lung volumes were presented as a percentage of TLC for analysis):

$$EELV\ (L) = TLC\ (L) - IC\ (L)$$

$$EILV\ (L) = EELV\ (L) + V_T\ (L)$$

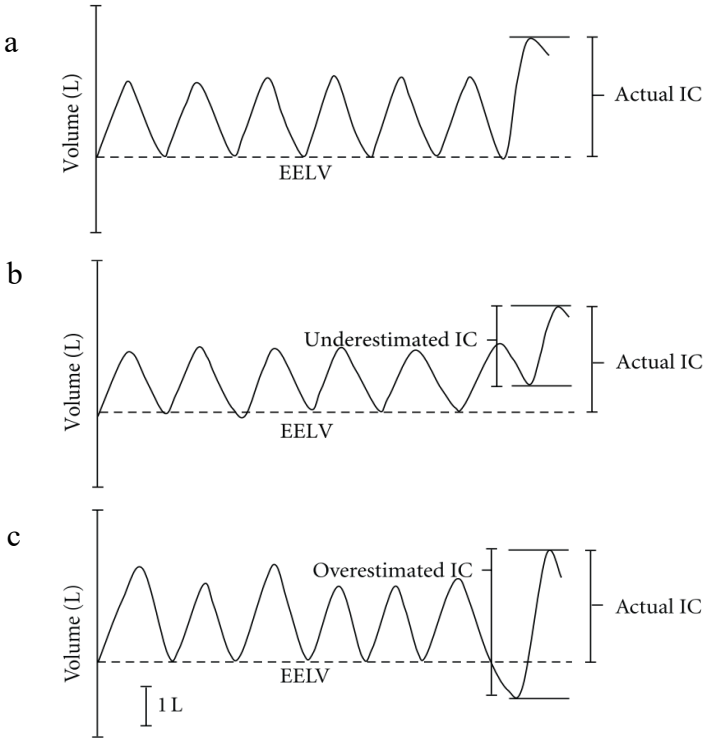

Figure 3.1. Examples of inspiratory capacity (IC) manoeuvres performed during exercise. A) Represents a correctly performed manoeuvre, with IC initiated at the correct end-expiratory lung volume (EELV), B) represents an example of an underestimated IC, characterised by the IC being initiated before the EELV and C) demonstrates an overestimated IC as a result of the manoeuvre being initiated after surpassing EELV [from Guenette *et al.*, (2013)].

3.7 Hydration status

3.7.1 Capillary blood samples

In all experiments, capillary blood samples were collected (in triplicate) from the participants' finger-tip at each time point and analysed for haemoglobin content and

haematocrit (for the calculation of plasma volume), and P_{osm}. The mean value of the three recorded measures was calculated and used for analysis.

Participants submerged their arm (up to the elbow) in warm water (~50 °C) for 5 min prior to the collection of the sample to increase arterialisation of capillary samples. The fingertip was cleaned using an alcohol wipe. A single-use disposable lancet (Accu-Check ®, Safe-T Pro Plus, Roche, Basel, Switzerland) was inserted ~2.3 mm into the fingertip, the first drop of blood was wiped away with a tissue, as this drop may be contaminated with tissue fluid (World Health Organization, 2010). Blood was drawn into the appropriate tube for each sample type (described below); if blood flow ceased before all samples were collected, a second fingertip puncture was made on an alternative finger.

3.7.1.1 *Haemoglobin*

For haemoglobin ($g \cdot L^{-1}$) determination, 10 µL of whole blood was collected into three HemoCue® Hb 201 microcuvettes (HemoCue® Ltd., Dronfield, Derbyshire, UK) and placed into a HemoCue® Hb 201^{+} (HemoCue® Ltd., Dronfield, Derbyshire, UK) for analysis. The average of the three samples was calculated and used for analysis. If one sample presented a large deviation, the average from the two other samples was taken.

3.7.1.2 *Haematocrit*

For haematocrit, 75 mm whole blood samples were collected into three microhaematocrit capillary tubes (Hawksley, Lancing, Sussex, UK), and were then centrifuged for 3 min at 12,000 g (Hawksley HaematoSpin 1400, Hawksley, Lancing, Sussex, UK). Following this, capillary tubes were placed under a microscope (173582, Vickers Instruments, York, UK), on a 150 mm ruler. Haematocrit (%) was calculated using the following equation, with the mean of the three samples used for analysis:

$$\text{Haematocrit (\%)} = (\text{blood total length} / \text{total sample length}) \times 100$$

Note. Where total blood length and total sample length are measured in millimetres (mm).

3.7.1.3 *Plasma volume*

Haemoglobin and haematocrit concentration were used to measure changes in plasma volume using the following equation (Dill and Costill, 1974; Strauss et al., 1952):

$$\Delta \text{ plasma volume} =$$

$$((100 \times \text{Hb pre/Hb post}) \times (1- (\text{Hct post} - 100) / (1- (\text{Hct pre} - 100)) - 100$$

Note. Hb: haemoglobin, Hct: haematocrit

3.7.1.4 *Plasma osmolality*

For P_{osm}, three 200 µL whole blood samples were collected into lithium heparin microvette tubes (CB 300 LH) and centrifuged for 3 min at 12,000 g (1-15k microfuge, Sigma, SciQuip Ltd. UK). A sample of plasma (20 µL) was extracted from the centrifuged sample using the Ease-Eject Sampler pipette (3M0825 20 µL Ease-Eject Sampler, Advanced Instruments, Massachusetts, USA) for analysis of P_{osm}. The osmolality ($mOsm \cdot kg^{-1}$) of the plasma was analysed via freezing point depression (3320 micro-osmometer, Vitech Scientific Ltd., West Sussex, UK). The freezing point depression osmometer cools the plasma to a temperature below its expected freezing point, with a wire rotating to stir the plasma and form ice crystals. When ice crystals are formed, thermal energy is released, which raises the temperature of the sample. Freezing point osmometry is the temperature at which the solid and liquid phases will co-exist in equilibrium (Koumantakis and Wyndham, 1989). A standard freezing point curve is then generated, with the osmolality of the plasma sample calculated from the freezing point depression. The mean osmolality of the three samples was calculated and used for analysis.

3.7.1.5 *Measurement error of blood sample analysis*

To confirm reproducibility and repeatability of blood sample analysis for hydration status markers, measurement error was assessed and is presented in Table 3.2. A coefficient of variation (CV), defined as the ratio of the standard deviation (SD) to the mean, was calculated for each variable; a CV of <5% is deemed to indicate 'excellent' repeatability (David et al., 2006). The intraclass correlation (ICC), a common statistical measure of reliability (Atkinson and Nevill, 1998), was also calculated. ICC is a measure of agreement to determine how closely data within the same group agrees with each other. An ICC greater

than 0.800 or greater than 0.900 indicates 'good' or 'excellent' agreement between measurements, respectively (Liljequist et al., 2019).

The technical error of measurement (TEM), i.e., the variability encountered when the same specimen is measured at multiple sessions, was also calculated to provide an indication of the precision associated with the measure, where error due to both biological and technical factors is considered (Harris and Smith, 2009). The following equation is used to calculate TEM (Arroyo et al., 2010):

$$TEM = \sqrt{(\Sigma d^2)} / 2n$$

Note. Where d is the difference between repeated measures, and n is the number of individuals measured.

Table 3.2. Measurement error for blood sample analysis at baseline (euhydrated) across four experimental visits Chapter 5.

Variable	ICC	ICC 95 % CI	CV (%)	TEM
Haematocrit (%)	0.911	0.771 – 0.975	0.98	1.14
Haemoglobin ($g \cdot L^{-1}$)	0.973	0.930 – 0.992	3.27	4
P_{osm} ($mOsm \cdot kg^{-1}$)	0.973	0.930 – 0.993	2.92	2
Plasma volume (%)	0.973	0.930 – 0.993	2.34	1.1

$P_{osm:}$ plasma osmolality; ICC: intraclass correlation; 95 % CI: 95 % confidence interval; CV: coefficient of variation; TEM: technical error of measurement.

3.7.2 Urine osmolality

Participants provided a urine sample for the analysis of urine osmolality (U_{osm}) – Note. This sample was not their first void of the day. Urine osmolality was measured using a portable refractometer (Pocket Pal-Osmo, Atago Vitech Scientific, UK). Euhydration was accepted as U_{osm} < 700 $mOsmol \cdot kgH_2O^{-1}$ (Sawka et al., 2007). Individuals that exceeded this threshold upon arrival to the laboratory were excluded from participation on that day and were asked to return on an alternative day.

3.7.3 Fluid replacement

In studies 2 and 3 (Chapters 5 and 6, respectively), fluid replacement strategies were implemented to either rehydrate or maintain euhydration. Details of fluid consumption/rehydration methods are provided below. When fluid was consumed orally, the volume of fluid ingested (in L) was matched to the loss of body mass (in kg) for each individual.

3.7.3.1 *Systemic (oral) rehydration*

In study 2, participants underwent a 1 h rehydration period (see Chapter 5 for detailed experimental protocol). Participants consumed a bolus of water at room temperature, mixed with 3g $NaCl{\cdot}LH_2O^{-1}$ to improve fluid retention (Maughan and Leiper, 1995). Fluid was divided equally into three bottles, and participants were required to finish each bottle within each of the three 15 min rehydration blocks.

3.7.3.2 *Nebulized (local) rehydration*

In a second condition of study 2 (Chapter 5), local rehydration of the airways was performed using an ultrasonic nebuliser (UltraNeb, DeVilbiss Healthcare Ltd., UK) with isotonic saline solution (0.9 %). The nebuliser was set to the maximum flow rate and participants breathed tidally through a two-way non-rebreathing valve (Series 1410, Hans Rudolph Inc, Kansas, USA) with a nose-clip in place. Each bout of nebulisation lasted 15 min, and participants performed a total of three bouts over the 1 h rehydration period.

3.7.3.3 *Exercise fluid replacement*

In study 3, fluid replacement was delivered during exercise in the control trial (see Chapter 6 for detailed experimental design). Fluid replacement was matched to target body mass loss and was delivered as four equal boluses of room temperature water mixed with 40 g electrolyte powder (36 g carbohydrate, 20 $mmol{\cdot}L^{-1}$ sodium; SiS GO Electrolyte Powder, Science in Sport®, UK) *per* 500 mL of water to maintain fluid balance (Evans et al., 2017). Participants were required to finish each bolus within 30 min.

3.8 Pulmonary function

In all experimental studies, pulmonary function tests (PFT) were performed in the same order: impulse oscillometry (IOS, Chapter 6 only), spirometry and then whole body plethysmography. All pulmonary function equipment was calibrated prior to each data collection time point using a 3 L calibration pump (3 L calibration syringe, Carefusion, Hoechberg, Germany). The pump piston was pulled back and forth slowly and at a consistent speed, ensuring the pump was completely emptied and completely filled with each repetition. The correction factors were logged by the computerised system; an error message was produced if the volume deviated from the acceptable range (i.e., ± 10%), and calibration was then repeated until requirements were met. For IOS, the screen flap at the back of the pneumotach was open throughout the volume calibration, and was closed prior to and throughout measurement, as per the manufacturer's instructions (Carefusion, 2017).

3.8.1 Impulse oscillometry

Impulse oscillometry was performed as the first lung function test at all data collection points, as the forced manoeuvres of spirometry and whole body plethysmography can impact the resistance and reactance values measured with IOS (Brashier and Salvi, 2015). The main principle of IOS is that impulses of multiple frequency sound waves are pushed into the lungs as pressure waves to allow the measurement of respiratory resistance and reactance (Brashier and Salvi, 2015).

In accordance with the guidelines of Brashier and Salvi, (2015), participants were asked to assume a seated position on a chair (legs un-crossed). The mouthpiece was adjusted to a comfortable height for the participant, so the neck was slightly extended, and a nose-clip was worn throughout the test. An add-on mouth clip was worn to keep the tongue at the bottom of the mouth. Participants were asked to press their hands firmly against their cheeks for the duration of the test to reduce impedance of extra-thoracic airway walls as a result of pressure oscillations applied at the mouth (Brashier and Salvi, 2015). Participants were then asked to perform normal tidal breathing in a relaxed state for 30-45 s. A minimum of three tests were performed until three technically acceptable manoeuvres were completed. In line with the ATS/ERS guidelines (Oostveen et al., 2003), tests were discarded and repeated if any breathing segments contained the following artefacts: poor cheek support, tongue position causing interference, air leaks, swallowing, breath holding,

and/or vocalisation. Respiratory resistance at 5 and 20 Hz (R_5 and R_{20}, respectively), frequency-dependence of resistance ($R_5 – R_{20}$), respiratory reactance at 5 Hz (X_5), area of reactance (A_x), and resonant frequency (F_{res}) were recorded in accordance with the ATS/ERS guidelines (Oostveen et al., 2003) and are detailed in Table 3.3. The mean values over three acceptable tests were retained for analysis.

Table 3.3. Definitions and application of IOS parameters reported within this thesis.

Parameter	Definition	Units	Relevance
R_5	Respiratory resistance at 5 Hz	$kPa \cdot L^{-1} \cdot s^{-1}$	Total airway resistance
R_{20}	Respiratory resistance at 20 Hz	$kPa \cdot L^{-1} \cdot s^{-1}$	Resistance at the large airways
R_5-R_{20}	Difference between R_5 and R_{20}	$kPa \cdot L^{-1} \cdot s^{-1}$	Resistance at the small airways
X_5	Respiratory reactance	$kPa \cdot L^{-1} \cdot s^{-1}$	Provides information about the distensible airways and reflects elastic recoil at peripheral airways
A_x	Integrated low frequency respiratory reactance magnitude between 5 Hz and F_{res}	$kPa \cdot L^{-1}$	Respiratory compliance and small airway patency. Reflects changes in the degree of peripheral airway obstruction
F_{res}	Resonant frequency	Hz	Reflects the frequency that the lung tissue moves from passive distension to active stretch.

kPa, kilopascals; L, litres; s, seconds; Hz, hertz.

3.8.2 Spirometry

Spirometry measurements of FEV_1 (L), FVC (L), FEF_{25-75} ($L \cdot s^{-1}$), FEV_1/FVC (%) ratio and PEF ($L \cdot s^{-1}$) were recorded using a spirometer (studies 1 and 2 used a MasterScreen PFT, Carefusion, Hoechberg, Germany; study 3: MicroLoop, Micromedical Limited, Kent, England). The alternative spirometer was used for study 3 due to the requirement for a portable device. Figure 3.2 provides a graphical representation of the lung volumes and flow rates measured using spirometry. In accordance with the ATS/ERS guidelines (Miller et al., 2005b), participants were required to perform the test in a seated, upright position, with a nose-clip affixed to occlude and prevent air leakage from the nostrils. Prior to the commencement of the test, the procedure was explained in full. Participants were instructed to breathe tidally into the spirometer via a mouthpiece for ~ 5-10 s and were then asked to inhale rapidly and forcefully to TLC. This was immediately followed by a forced expiration

(the duration being a minimum of 6 s). Participants were encouraged by the experimenter throughout the test. The manoeuvres were compared to the published acceptability criteria (within- and between-manoeuvre) (Miller et al., 2005b). Three technically acceptable manoeuvres were required, with two manoeuvres within 150 mL of each other (for FEV_1 and FVC). The highest FEV_1 and FVC of two repeatable manoeuvres, and the highest peak expiratory flow (PEF) of three acceptable manoeuvres were recorded. The forced expiratory flow at 25-75 % (FEF_{25-75}) was selected from the manoeuvre with the highest sum FEV_1 + FVC. A maximum of eight manoeuvres were conducted *per* time point.

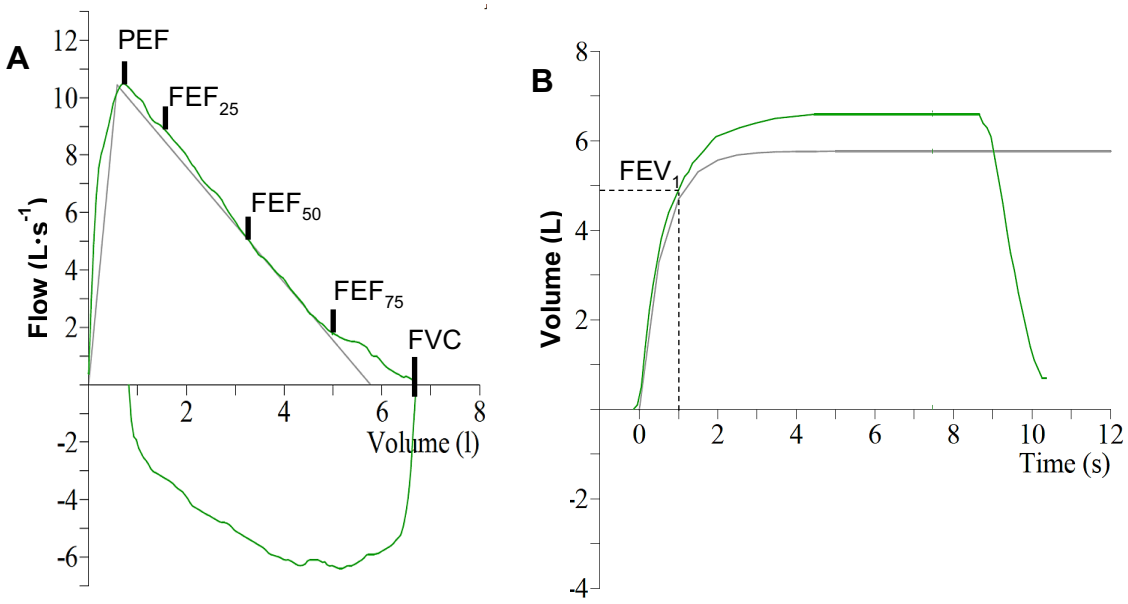

Figure 3.2. A) Spirometry flow-volume loop and B) volume-time graph showing lung volumes and flow rates from a forced expiratory manoeuvre. PEF, peak expiratory flow; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; FEF_{25}, forced expiratory flow at 25 % of FVC; FEF_{50}, forced expiratory flow at 50 % of FVC; FEF_{75}, forced expiratory flow at 75 % of FVC.

3.8.3 Whole body plethysmography

Whole body plethysmography was performed in all studies following ATS/ERS guidelines (Wanger et al., 2005). Participants were required to enter the MasterScreen plethysmograph box (Carefusion, Hoechberg, Germany) and sit for 1-2 min to allow stabilisation of pressure and temperature. Participants were instructed to breathe at a rate of 25 breaths·min^{-1} into the mouthpiece (with nose-clip on). At the end of a normal expiration, a shutter was closed

for ~2-3 s. Participants were required to continue breathing normally against the closed shutter without increasing their effort. Upon the shutter re-opening, participants were asked to perform a full exhalation to RV (L), and then a full inspiration to TLC (L), followed by a final forced exhalation. Participants were verbally encouraged throughout each manoeuvre. FRC (L) was calculated (JLab software, Carefusion, Germany) and the mean FRC was selected for analysis. RV was derived from the mean FRC minus mean expiratory reserve volume (ERV), and TLC was calculated as the maximum vital capacity (VC) *plus* RV. A minimum of three manoeuvres were performed to ensure reproducibility (FRC was required to be within 5% across the three trials) A graphical representation of the static lung volumes recorded via whole body plethysmography is presented in Figure 3.3.

The basic principle of whole body plethysmography applies Boyle's law and works on the detection of changes in mouth pressure or with flow rates under defined breathing conditions (Criée et al., 2011). Boyle's Law states that *'the pressure of a given mass of a given gas is inversely proportional to its volume at a constant temperature'.* Thus, relative volume changes can be inferred from pressure changes. The key principle in body plethysmography is that changes in alveolar pressure may be inferred from changes in plethysmograph pressure (Goldman et al., 2005). The small volume changes detected due to compression and decompression of the lungs allows for the measurement of gas flow into and out of the lungs.

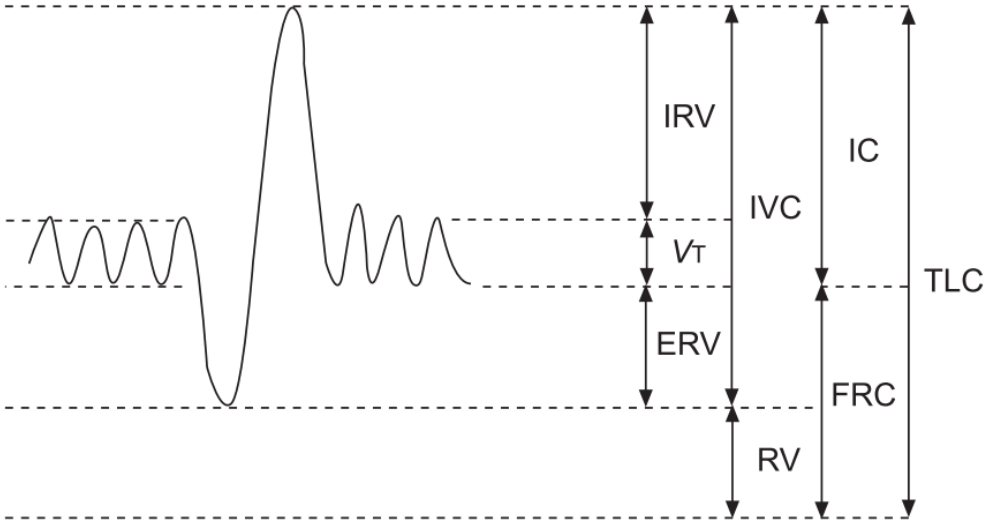

Figure 3.3. Static lung volumes and capacities based on a volume–time graph of an inspiratory vital capacity (IVC) manoeuvre. IC: Inspiratory capacity; IRV: inspiratory reserve volume; V_T: tidal volume; ERV: expiratory reserve volume; RV: residual volume; IC: inspiratory capacity; FRC: functional residual capacity; TLC: total lung capacity, from Wanger et al., (2005).

The volume calibration of the pneumotachograph was performed using the same procedures as described above (see section 3.8) prior to each test. The plethysmograph pressure transducer was calibrated following the manufacturer's instructions (Carefusion, 2017) and performed prior to each test using the 'box calibration' function. The door of the box was closed to allow box pressure and volume to stabilise. The automatic calibration was internally performed and controlled by a motor-driven syringe. Following 2 min of volume and pressure stabilisation, 30–50 mL of air was repeatedly inserted and removed from the box. Changes in pressure following calibration reflected the changes in thoracic gas volume resulting from compression/decompression of thoracic gas. When the participant entered the box to perform a manoeuvre, a one min rest period was first observed to allow the calibration to correct for the participant's body volume. The final calibration coefficient was calculated using the body mass of the participant.

3.8.3.1 *Airway resistance*

Airway resistance is defined as the pressure difference between the mouth and the alveoli, divided by the airflow (Sidebotham and Le Grice, 2007). In this thesis, airway resistance was measured using the interrupter (i.e., airway occlusion) technique, as described by Goldman et al., (2005). This technique relies on short interruptions of airflow (i.e., when the shutter closes – as described above), based on the assumption that during periods of no airflow, pressures through the respiratory system will equilibrate rapidly. Specific airway resistance (sRaw) was calculated by taking the difference between the recorded alveolar pressure (box pressure) and mouth pressure and dividing this value by airflow.

3.9 Other physiological measurements

3.9.1 Heart rate

Heart rate (beats·min^{-1}) was measured via a telemetric heart rate monitor (Polar, FS1, Birmingham, UK). Participants wore a telemetric belt around their chest at all times during the exercise trials. Heart rate was recorded from a paired watch at regular intervals as necessitated by the specific experimental trial and was continuously monitored to ensure participant safety. Maximum heart rate (HR_{max}) was calculated using the following equation (Fox et al., 1971):

$$HR_{max} = 220 - age$$

3.9.2 Core temperature

Measurement of T_{core} was performed using tympanic temperature (T_{tymp}) in study 2 and using rectal temperature (T_{rec}) in study 3. Tympanic temperature was measured at the tympanic membrane using ear canal placement (ThermoscanExactemp 6022, Braun, Germany). The temperature monitor was placed into the ear canal on three occasions for each measurement point, and the mean recorded value was taken as the tympanic temperature at each point. Whilst T_{tymp} typically underestimates T_{rec} or other deep body sites [by ~0.5-1 ˚C (Roth et al., 1996)], it is still recognised as an appropriate method for monitoring changes in T_{core} (Taylor et al., 2014).

Due to the increased exercise intensity and severity of dehydration induced in study 3, T_{rec} was used as the measure of T_{core}. Oesophageal temperature (an alternative internal

measurement site) was considered for use, as this measurement site may respond faster to changes in body temperature (Taylor et al., 2014). However, T_{rec} is regarded as the gold standard measure for T_{core} due to its precision (Ganio et al., 2009a). Further, due to i) the primary experimental outcomes (i.e. to avoid interference with pulmonary and ventilatory parameters) and ii) to improve comfort of participants during the experimental trials (>6 h in duration), T_{rec} was considered the most appropriate method of temperature measurement.

Participants were provided with a rectal thermistor (RET-1 Rectal probe, Physitemp, New Jersey, USA) and were instructed to self-insert the thermistor in the privacy of a bathroom. The thermistor was inserted to a marked depth of 10 cm into the anal sphincter for measurement of T_{rec} (Taylor et al., 2014). Recordings of T_{rec} were taken every 15 min and were monitored continuously to 0.01˚C throughout exercise using a temperature logger (TC-2000 Thermocouple Meter, Sable Systems International, North Las Vegas, USA) to ensure participant safety. In accordance with our ethics application, participants were to be removed from the chamber if T_{rec} increased above 40˚C (no incidence of this occurred in the studies presented within this thesis).

3.9.3 Oxygen saturation

In study 2, peripheral oxygen saturation (SpO_2) was measured throughout the nebulised rehydration trials via a pulse oximeter (9590 Onyx Vantage Finger Pulse Oximeter, Nonin Medical, Plymouth, USA) placed on the tip of the participants' index finger. In study 3, SpO_2 was measured throughout the $\dot{V}O_{2max}$ test using a pulse oximeter (Xpod® 3012LP External OEM Pulse Oximeter, Nonin Medical, Plymouth, USA) attached to the earlobe of the participant. Resting SpO_2 was recorded after ~1 min of rest to allow stability of readings. Subsequent values were recorded at the end of each 2 min stage throughout the incremental test.

3.10 Perceptual scales

3.10.1 Breathing discomfort

The breathing discomfort scale was used to determine subjective feelings of breathing difficulty. A modified Borg Scale (Appendix D) was used to rate breathing discomfort on a scale of 0 = 'no discomfort at all' to 10 = 'very, very severe discomfort' (Mahler and Horowitz, 1994). Participants were shown the breathing discomfort scale at rest (prior to

each trial), when the rating system was explained in detail. During study 2, participants were asked to rate breathing discomfort at the end of each stage (i.e., during the last 5 min of each cycling and stepping bout) by pointing to the appropriate rating on the scale. In study 3, breathing discomfort was recorded every 2 min throughout the $\dot{V}O_{2max}$ test, and every 15 min throughout the exercise-induced dehydration trials.

3.10.2 Rating of perceived exertion

Participants were asked to rate their perceived exertion (RPE) on a Borg Scale (Borg, 1982) from 6 = 'very very light' to 20 = 'maximal' (see Appendix E). Participants were shown the RPE scale at rest prior to each trial, when the rating system was explained in detail. In study 2, participants were asked to rate RPE at the end of each stage (i.e. during the last 5 min of each cycling and stepping bout). In study 3, participants were asked to rate RPE every 2 min during the $\dot{V}O_{2max}$ test, and every 15 min during the exercise-induced dehydration trial.

3.11 Statistical analysis

All statistical analyses were performed using statistical software (SPSS 20, SPSS, Chicago, IL). Data were tested for normality using the Shapiro-Wilk test. Statistical significance was set at $p \leq 0.05$. Data are presented as mean ± SD, unless otherwise stated. Specific statistical tests used for each study are reported in the relevant experimental chapters.

3.11.1 Sample size determination

Sample size was determined based on previous work (see specific study chapters for details) and was conducted using G*Power Version 3.9.1.2 (Faul et al., 2007). Mean changes of key dependent variables presented in previous studies with similar methods were used to determine the sample size required to detect a meaningful change at a power of 80% and an alpha level of significance of 0.05. In study 2, eighteen participants were recruited and ten completed all experimental trials. In study 3, seventeen participants were recruited and eleven completed all experimental trials.

3.11.2 Statistical significance

Results within this thesis have been classed as statistically significant if the probability (p) value was ≤ 0.05. This means that the probability that the test statistic would be greater than or equal to the observed results (when the null hypothesis is true) is beyond the 0.05 level (i.e., there is 95% confidence that the outcome has not been reached by chance). The statistical test used to determine statistical significance varies between experimental chapters, with the most appropriate test for each individual research question implemented in each case. The statistical test used for each study is detailed in the relevant experimental chapters.

3.11.3 Tests of normality

Tests of normality were performed on all data sets, to ensure samples were normally distributed. The Shapiro-Wilk test was used to test for normality, and thus helped determine the correct statistical test to be used for analysis. The Shapiro-Wilk test compares the sample to a normally distributed set of scores with the same mean and standard deviation (Field, 2017). If the Shapiro-Wilk test is non-significant ($p > 0.05$), it can be assumed that the there is no difference in the distribution of the sample, and thus the data is classed as normally distributed. All data collected within this thesis was found to be normally distributed, and thus alternative tests for non-normally distributed data were not required.

3.11.4 Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA) is a set of univariate statistical techniques used to compare means from experiments with three or more treatment conditions or groups (Gray and Kinnear, 2012). Within this thesis, ANOVA was determined to be the most appropriate test to assess whether a significant difference existed between means; this test compares the variances within and/or between groups and whether or not they are equal. Within and/or between conditions analysis have been conducted in this thesis using either: one-way, two-way, or three-way ANOVA (detail of the specific statistical test implemented is provided within the relevant experimental chapters). In studies 2 and 3, three-way ANOVA were implemented to include further comparison of an additional independent variable. If the ANOVA demonstrated statistical significance, a post hoc analysis was conducted – these are detailed in section 3.11.5 below.

3.11.5 Post hoc analysis

Post hoc tests are pairwise comparisons that are used to compare all different combinations of the analysis, and thus locate the specific differences (Field, 2017). Where statistical significance was demonstrated using ANOVA, the Bonferroni correction was implemented as the post hoc test to determine differences between multiple comparisons. The Bonferroni correction divides the overall type I error rate across all conditions by the number of comparisons, to ensure that the type I error rate is below 0.05 (Field, 2017); this is the most robust univariate technique if sphericity is violated (Field, 2017).

Chapter 4 Inter-day repeatability and intra-day reproducibility in pulmonary function in healthy adults

4.1 Abstract

Pulmonary function testing is used on a regular basis for diagnosis of pulmonary illness and for research purposes. Pulmonary function research often seeks to detect relatively small changes in lung volumes and capacities, and therefore it is essential that the tests performed are not impacted by confounding factors, and that measurements are reliable and reproducible . The present study aimed to investigate in healthy adults *i)* the inter-day repeatability of pulmonary function measured by spirometry and whole body plethysmography and *ii)* the intra-day reproducibility in spirometry and whole body plethysmography. Participants performed repeated pulmonary function tests on *i)* four separate occasions (at the same time of day) to assess inter-day repeatability, and *ii)* multiple times within the same day (1 morning and 2 afternoon visits) to assess intra-day reproducibility. Pulmonary function tests (FVC, FEV_1, PEF, RV, FRC) displayed 'excellent' repeatability (ICC > 0.9 and CV < 5%) when performed on multiple days, and were shown to display 'very good' reproducibility when performed on multiple times within the same day. These findings provide support for the use of spirometry and whole body plethysmography in experimental research aiming to detect small, but physiologically relevant alterations in pulmonary function of healthy adults across multiple days, and at different times within the same day.

4.2 Introduction

Pulmonary function tests (PFT) are used daily in hospitals and research settings for the diagnosis and management of disease, and to evaluate the effectiveness of experimental interventions (Douglas et al., 2008; Strivens, 2017). Spirometry, the most commonly used PFT (Dancer and Thickett, 2012), assesses the volume of inhaled and exhaled airflow from an individual as a function of time. The two key parameters of spirometry tests are FVC – which provides an index of lung size, and FEV_1 – which provides an index of airway calibre. The FEV_1/FVC ratio represents the portion of the vital capacity that an individual can expire in the first second of expiration, and is > 70% in healthy adults (Vogelmeier et al., 2017). A reduced FEV_1/FVC ratio indicates an obstructive lung defect (such as asthma or COPD), whereas reduced FEV_1 and FVC, with a normal ratio (>70%), implies presence of a restrictive lung defect (Pellegrino et al., 2005). Spirometry is considered 'gold standard' in pulmonary function; however, it only allows partial pulmonary function assessment, as lung volumes (i.e., RV, FRC, and TLC) are not captured.

Whole body plethysmography is often performed in conjunction with spirometry for the measurement of lung volumes and capacities. Plethysmography provides measures of the lung volumes and capacities not captured by spirometry, including TLC, RV, FRC, ERV, VC, and IRV, as well as measurement of airway resistance (sRaw). Minor increases in static lung volumes, such as RV and FRC, can indicate possible air trapping and/or pulmonary dysfunction (Ruppel, 2012). When associated with reductions in vital capacity (Dykstm et al., 1999; Lutfi, 2017), reduced RV and FRC suggest that the dysfunction is localised at the level of the small airway.

Precision of PFT is critical in clinical settings to inform diagnosis and, in research setting, to confidently detect pulmonary function changes (that may be small, but meaningful) in response to interventions. Compared to spirometry, the variability of whole body plethysmography is greater which limits the sole use of whole body plethysmography in clinical trials (Houghton et al., 2005; Tattersfield and Keeping, 1979), and supports the use of both techniques in combination. Knowing the reproducibility [i.e., variation in measurements made on a subject under changing conditions (Bartlett and Frost, 2008)] and the repeatability [i.e., variation in repeat measurements made on the same subject under identical conditions (Bartlett and Frost, 2008)] of PFT is important for interpretation of the

results. Variation in PFT results may arise from: faulty equipment; observer influences; biological factors [including inter (within)- and intra (between)-day variations] (Miller et al., 2005b). Intra-day variations in pulmonary functions are expected in some disease states, such as asthma [which is characterised by variability of airway calibre (GINA, 2020)]. Spengler and Shea, (2000) also presented increases in FEV_1 of up to 18% when testing was performed in healthy adults in the afternoon compared to the evening, which was thought to be associated with an endogenous circadian rhythm. The intra-day variation in whole body plethysmography has not been reported as widely as that of spirometry; however Hruby and Butler (1975) presented early evidence of increased airway resistance in the afternoon compared to the morning. Estimating intra- and inter-day reproducibility and repeatability in PFT will ensure that variation can be recorded and accounted for in following experimental interventions, thereby reducing the risk of over- or under-estimation of the effect of the intervention.

The first aim of this experimental chapter was to investigate the inter-day repeatability of pulmonary function measured by spirometry and whole body plethysmography in healthy adults. The second aim was to test, in a similarly healthy population, intra-day reproducibility in spirometry and whole body plethysmography. It was hypothesised that, in healthy young adults, *i)* a high level of agreement would be noticed between PFT data when tests are repeated on separate days, and *ii)* variations in spirometry and whole body plethysmography data may be present when PFT are repeated at multiple times on a single day.

4.3 Method

The study was split into two parts: Part A focused on inter-day repeatability, and part B on intra-day reproducibility.

4.3.1 Participants

For Part A, ten participants (two females) aged between 18 and 50 years were recruited. Six of these participants (two females) took part in Part B, with an additional four participants recruited (one female). For descriptive data the reader is referred to section 4.4.1. All participants were healthy, non-smokers, with no history of chronic respiratory

disease. All participants provided written informed consent prior to taking part and adhered to the pre-trial controls described in Chapter 3.4.1.

4.3.2 Experimental design

In both protocols, participants were asked to abstain from exercise, caffeine and alcohol for 24 h prior to the experimental visits.

4.3.2.1 *Part A: inter-day repeatability*

Participants attended the laboratory on four separate days, with a minimum of 48 h between visits. Each visit started with a hydration status assessment (via U_{osm}) and a nude body mass measurement (section 4.3.3). Pulmonary function testing was then performed. Participants arrived between 08:00 h – 10:00 h over the four experimental visits (participants arrived at the same time of day for each visit).

4.3.2.2 *Part B: Intra-day reproducibility*

Participants attended the laboratory at three separate times on the same day. Between 08:15 - 10:15 h (AM), 12:15 - 14:15 h (PM1, early afternoon) and 13:30 - 15:30 h (PM2, mid-afternoon), hydration status (via U_{osm}; section 4.3.3) and pulmonary function (via spirometry, then whole body plethysmography; see section 4.3.4) were assessed (*Note*. Times were matched with data collection times used in Chapter 5). Participants were free to leave the laboratory between data collection points but were asked to consume a minimum of 500 mL water between data points to ensure euhydration was maintained.

4.3.3 Hydration status

Participants were required to arrive at the laboratory in a euhydrated state. Hydration status was checked via U_{osm}, with a threshold of < 700 $mOsm.kg^{-1}$ used for inclusion (details of the technique can be found in Chapter 3.7.2). This was followed by a nude body mass measurement.

4.3.4 Pulmonary function

Pulmonary function was assessed in a seated position using spirometry and whole body plethysmography (described in detail in Chapter 3.8). ATS/ERS guidelines were strictly followed (Miller et al., 2005b; Wanger et al., 2005), with participants reminded of the

procedures for each manoeuvre before the commencement of the test. Tests were always performed in the same order [i.e., spirometry first, in line with ERS/ATS guidelines (Miller et al., 2005b)].

4.3.5 Statistical analysis

All data were analysed using SPSS Statistical Software (Version 25, SPSS, Chicago, IL), as described in Chapter 3.11. Data were analysed for normality of distribution using the Shapiro-Wilk test. Data are presented as mean ± SD, with 95% confidence intervals (CI), whenever appropriate. Statistical significance was accepted at $p < 0.05$.

4.3.5.1 *Part A: inter-day repeatability*

A repeated measures ANOVA was conducted to assess changes in U_{osm} between visits. Bonferroni post hoc adjusted pairwise comparisons were used where significant main effects occurred. Coefficient of variation (CV) and intraclass correlation coefficients (ICC) were calculated to test inter-day repeatability of pulmonary function. Intra-subject CV (in %) was calculated and defined as the ratio of the SD to the mean (where SD represents the standard deviation and the mean calculated from values recorded on four separate days). A CV < 5% was deemed to indicate 'excellent' repeatability. ICC is a measure of agreement to determine how closely data within the same group agree with each other and was calculated from PFT outputs collected in the morning on four separate experimental days. An ICC > 0.800 or > 0.900 indicates 'good' or 'excellent' agreement between measurements, respectively (Liljequist et al., 2019). Technical error of measurement (TEM; the variability encountered when the same specimen is measured at multiple sessions, where error due to both biological and technical factors is considered (Harris and Smith, 2009)) was calculated for all PFT outputs (detail of this measurement is provided in Chapter 3.7.1.5). Average inter-day variation in pulmonary function was calculated using the minimum and maximum values recorded over the four visits.

4.3.5.2 *Part B: intra-day reproducibility*

To assess intra-day reproducibility, repeated measures ANOVA were conducted on hydration status (body mass and U_{osm}), spirometry and whole body plethysmography parameters recorded at three different times on the same day (AM, PM1, PM2). Bonferroni post hoc adjusted pairwise comparisons were used where significant main effects occurred.

4.4 Results

4.4.1 Participants

4.4.1.1 *Part A: inter-day repeatability*

Participants were aged 29 ± 8 y, had a body mass of 62.8 ± 8.5 kg and a stature of 173 ± 10 cm at visit 1.

4.4.1.2 *Part B: intra-day reproducibility*

Participants were aged 29 ± 7 y, had a body mass of 68.0 ± 11.7 kg and a stature of 175 ± 11 cm at visit 1.

4.4.2 Hydration status

4.4.2.1 *Part A: inter-day repeatability*

All participants were euhydrated upon arrival, and their U_{osm} did not significantly differ between visits (visit #1: 200 ± 137 mOsm·kg^{-1}; visit #2: 255 ± 200 mOsm·kg^{-1}; visit #3: 170 ± 140 mOsm·kg^{-1}; visit #4: 130 ± 130 mOsm·kg^{-1}).

4.4.2.2 *Part B: intra-day reproducibility*

All participants remained euhydrated throughout the day, with a U_{osm} of 150 ± 95 mOsm·kg^{-1} at AM, 180 ± 100 mOsm·kg^{-1} at PM1 and 250 ± 160 mOsm·kg^{-1} at PM2; no difference was noted between time points for U_{osm}. Body mass did not significantly change during the experimental day (AM: 68.0 ± 11.7 kg; PM1: 68.0 ± 11.8 kg; PM2: 68.0 ± 11.6 kg).

4.4.3 Pulmonary function

4.4.3.1 *Part A: inter-day repeatability*

CV, ICC and TEM for each day, as well as mean values for all spirometry and whole body plethysmography parameters are presented in Table 4.1. The inter-day ICC exceeded 0.900 for both spirometry (range: 0.966 – 0.994) and whole body plethysmography (range: 0.944 – 0.998). CV below 5% (suggesting excellent repeatability) were noted for all data, except for FEF_{25-75} (6.9%), ERV (9.1%) and sRaw (8.7%). The average inter-day variation for

FEV_1 and FVC was 188 ± 66 mL and 182 ± 71 mL, respectively. The average inter-day variation in RV, FRC, and TLC was 202 ± 103 mL, 364 ± 247 mL, and 255 ± 195 mL, respectively.

Table 4.1. Inter-day variability of resting pulmonary function measurements in ten healthy adults (two females).

Variable	Visit 1	Visit 2	Visit 3	Visit 4	ICC (95% CI)	CV (%)	Variance (%)	Absolute TEM
Spirometry								
FEV_1 (L)	3.94 ± 0.75	4.01 ± 0.73	4.02 ± 0.77	4.00 ± 0.77	0.994	2.6	5.4 ± 2.0	0.08
(% predicted)	(100 ± 12%)	(102 ± 11%)	(102 ± 12%)	(101 ± 11%)	(0.985 – 0.998)			
FVC (L)	5.03 ± 1.08	5.10 ± 1.06	5.04 ± 1.07	5.04 ± 1.08	0.998	1.8	3.3 ± 2.7	0.06
(% predicted)	(107 ± 13%)	(109 ± 13%)	(107 ± 12%)	(107 ± 12%)	(0.995 – 0.999)			
FEV_1/FVC (%)	79.1 ± 6.8	79.3 ± 6.8	80.4 ± 7.0	80.0 ± 6.7	0.987 (0.967 – 0.966)	1.8	3.5 ± 2.5	1.2
PEF ($L \cdot s^{-1}$)	9.57 ± 1.96	9.78 ± 1.98	9.49 ± 2.03	9.59 ± 1.96	0.983	4.6	9.2 ± 5.1	0.33
(% predicted)	(105 ± 10%)	(107 ± 13%)	(104 ± 14%)	(105 ± 12%)	(0.955 – 0.995)			
FEF_{25-75} ($L \cdot s^{-1}$)	3.52 ± 0.82	3.60 ± 0.80	3.71 ± 0.93	3.62 ± 0.84	0.966	6.9	13.9 ± 6.6	0.20
(% predicted)	(76 ± 16%)	(78 ± 15%)	(80 ± 19%)	(78 ± 16%)	(0.912 – 0.990)			
Whole body plethysmography								
TLC (L)	7.07 ± 1.49	7.06 ± 1.49	7.12 ±1.55	7.10 ± 1.45	0.998 (0.994 – 0.999)	1.6	3.5 ± 2.6	0.09
FRC (L)	4.03 ± 1.00	3.91 ± 0.99	4.06 ± 1.12	4.07 ± 1.03	0.990 (0.974 – 0.997)	4.3	8.1 ± 5.4	0.13
RV (L)	2.14 ± 0.52	2.19 ± 0.53	2.22 ± 0.55	2.23 ± 0.53	0.991 (0.977 – 0.997)	4.1	8.6 ± 4.2	0.07
RV/TLC (%)	30 ± 5	31 ± 4	31 ± 5	31 ± 4	0.974 (0.932 – 0.993)	3.9	8.1 ± 4.9	1
ERV (L)	1.89 ± 0.55	1.72 ± 0.48	1.85 ± 0.66	1.83 ± 0.53	0.968 (0.919 – 0.991)	9.1	16.0 ± 8.8	0.13
IC (L)	3.12 ± 0.70	3.22 ± 0.80	3.10 ± 0.66	3.09 ± 0.63	0.985 (0.960 – 0.996)	4.5	9.1 ± 5.1	0.11
IVC (L)	4.94 ± 1.15	4.78 ± 1.24	4.90 ± 1.15	4.90 ± 1.04	0.990 (0.975 – 0.997)	3.6	7.4 ± 9.0	0.13
sRaw ($kPa \cdot s^{-1}$)	0.99 ± 0.19	1.05 ± 0.21	1.01 ± 0.22	1.00 ± 0.25	0.944 (0.854 – 0.984)	8.7	16.9 ± 6.1	0.07

Data are means ± SD. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow; $FEF_{25-75,}$ forced expiratory flow at 25–75% of forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; ERV, expiratory reserve volume; IC, inspiratory capacity; IVC, inspiratory vital capacity; sRaw, specific airway resistance; ICC, intraclass correlation; CV, coefficient of variation. TEM, technical error of measurement.

4.4.3.2 *Part B: intra-day reproducibility*

Throughout the experimental day, a significant time effect was detected for FEV_1 (p = 0.003), with an increase of 152 ± 98 mL (3.7 ± 2.3%) from morning to early afternoon (AM to PM1; Figure 4.1). This increase in FEV_1 was not sustained, as the 96 ± 137 mL (2.3 ± 3.3%) difference between AM and PM2 did not reach statistical significance (p = 0.162). Four participants (40%) presented a significant (≥ 200 mL) increase in FEV_1 at PM1, with two of those four presenting a sustained increase at PM2 (Figure 4.1).

A significant time effect was observed for FVC (p = 0.010). However, post hoc analysis did not reveal any significant difference between individual time points: AM to PM1 (118 ± 130 mL; p = 0.055), AM to PM2 (61 ± 106 mL; p = 0.305), and PM1 to PM2 (-57 ± 84 mL; p = 0.184). Four participants presented a ≥ 200 mL increase in FVC at PM1, with none reaching this threshold at PM2 (Figure 4.1). Neither FEV_1/FVC, nor PEF and FEF_{25-75} changed significantly during the day (Table 4.2).

For whole body plethysmography, a significant time effect was detected for RV (p = 0.046), with an increase in RV from AM to PM2 (103 ± 84 mL; p = 0.011), but no difference between AM and PM1 (3 ± 162 mL), or PM1 and PM2 (106 ± 162 mL). FRC and TLC remained stable from AM to PM1 (FRC: -31 ± 217 mL; TLC: 62 ± 201 mL) and AM to PM2 (FRC: 40 ± 128 mL; TLC: 58 ± 128 mL), as did all the other parameters (Table 4.2).

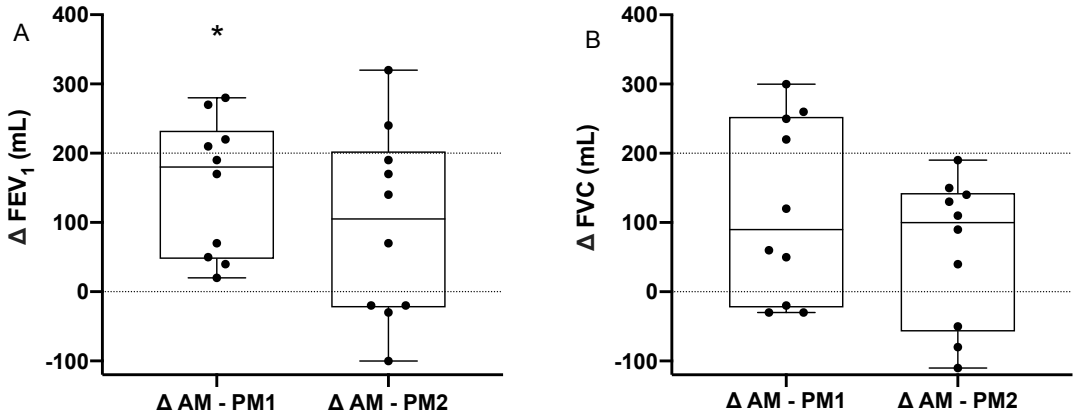

Figure 4.1. Changes in A) FEV_1 (mL) and B) FVC (mL) between 09:15 ± 1:00 h and 13:15 h ± 1:00 h (Δ AM-PM1), and between 09:15 ± 1:00 h and 14:30 ± 1:00 h (Δ AM-PM2) in ten healthy adults (two females). Boxplots represent the median and interquartile range, with whiskers representing the minimum and maximum values. *$p < 0.05$, significant difference between AM and PM1.

Table 4.2. Spirometry and whole body plethysmography measurements recorded at 09:15 ± 1:00 h (AM), 13:15 ± 1:00 h (PM1) and 14:30 ± 1:00 h (PM2) in ten healthy participants (two females).

	AM	PM1	PM2	Maximum difference
Spirometry				
FEV_1 (L)	4.09 ± 0.63	4.24 ± 0.66 *	4.19 ± 0.67	0.18 ± 0.08
FVC (L)	5.20 ± 0.93	5.32 ± 0.93	5.27 ± 0.97	0.16 ± 0.09
FEV_1/FVC (%)	79.3 ± 7.6	80.2 ± 6.6	80.1 ± 7.5	2.1 ± 1.0
PEF ($L \cdot s^{-1}$)	9.59 ± 1.53	9.71 ± 1.55	9.71 ± 1.60	0.56 ± 0.52
FEF_{25-75} ($L \cdot s^{-1}$)	3.87 ± 0.86	3.96 ± 0.90	3.96 ± 0.90	0.45 ± 0.25
Whole body plethysmography				
TLC (L)	7.12 ± 1.18	7.19 ± 1.29	7.18 ± 1.24	0.18 ± 0.11
FRC (L)	3.96 ± 0.74	3.93 ± 0.77	4.00 ± 0.75	0.22 ± 0.11
RV (L)	1.97 ± 0.52	1.96 ± 0.50	2.07 ± 0.47 *	0.18 ± 0.11
RV/TLC (%)	28 ± 7	28 ± 7	29 ± 7	2 ± 2
ERV (L)	1.99 ± 0.44	1.96 ± 0.48	1.93 ± 0.47	0.13 ± 0.11
IC (L)	3.24 ± 0.68	3.27 ± 0.72	3.25 ± 0.67	0.18 ± 0.11
IVC (L)	5.16 ± 1.05	5.17 ± 1.05	5.11 ± 1.11	0.13 ± 0.07
sRaw ($kPa \cdot s^{-1}$)	1.12 ± 0.23	1.05 ± 0.22	1.06 ± 0.21	0.12 ± 0.11

Data are means ± SD. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow; FEF_{25-75}, forced expiratory flow at 25–75% of forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; ERV, expiratory reserve volume; IC, inspiratory capacity; IVC, inspiratory vital capacity; sRaw, specific airway resistance. * Significant difference vs. AM ($p<0.05$).

4.5 Discussion

The aim of the present study was to investigate the inter-day repeatability (Part A) and intra-day reproducibility (Part B) of pulmonary function, assessed via spirometry and whole body plethysmography, in healthy young adults. It was hypothesised that a high level of agreement would be apparent between PFT parameters when repeated on separate days, but slight variations may be present when PFT are repeated at multiple times on a single day.

This study showed that, in agreement with the first hypothesis, the repeatability of pulmonary volumes and capacities measured via spirometry and whole body plethysmography was 'excellent' (i.e., ICC >0.9 and CV <5%) when the tests were performed on multiple days. Intra-day reproducibility was also 'very good' for both PFT, in partial agreement with hypothesis two, whereby the majority of assessed parameters remained stable throughout the day. Small, but statistically significant differences between morning and early afternoon measurements were observed for a limited number of pulmonary function measurements (i.e., FEV_1 and RV). These alterations were not sustained between the two afternoon time points. Findings from the present study suggest that, in healthy young participants, spirometry and whole body plethysmography parameters are stable when recorded on multiple days. Whilst small, but statistically significant intra-day variation occurs in selected spirometry and plethysmography parameters (i.e. FEV_1 and RV), the majority of pulmonary function parameters remain stable throughout the day. These findings support the widespread use of these two PFT, both in clinical and research settings, and provide confidence for implementing these methods to detect changes in pulmonary function of healthy adults under future experimental conditions.

4.5.1 Inter-day repeatability

4.5.1.1 *Spirometry*

The small CV reported in this study for FEV_1 (5.6%) and FVC (3.3%) are aligned with data from Rozas and Goldman (1982), who reported CV of 2.8% for both FEV_1 and FVC in 15 healthy adults. The absolute variations recorded in participants from the present study (188 ± 66 mL for FEV1 and 182 ± 71 mL for FVC) remain, on average, below the 200 mL threshold which is considered of clinical significance (Pellegrino et al., 2005). Variation in

FEV_1 measured on recurrent visits has previously been associated with asthma and instability of airway calibre. According to the Global Initiative for Asthma (GINA, 2020), a decrease in FEV_1 >200 mL (or a >12% change) between visits may be indicative of airflow limitation and can therefore be used in the diagnosis of asthma. Dean et al., (2018) presented a mean inter-visit variability in FEV_1 of 7.3% in 584 adults with asthma, and 4.8% in 380 adults without asthma. Data from the present study shows an average inter-day variability in FEV_1 of 5.4 ± 2.0% in healthy adults, which is comparable to the variation presented in healthy adults by Dean et al., (2018). Further, in the present study inter-day variations in FEV_1 and FVC were maintained below 150 mL in 84% of tests, which conforms to the normal spirometry intra-test repeatability criteria (Graham et al., 2019).

PEF and FEF_{25-75} were the most variable of the recorded spirometry parameters in the present study, with an average variance of 9.2 ± 5.1% and 13.9 ± 6.6%, respectively. However, repeatability remained 'excellent', with ICC of 0.983 and 0.966 for PEF and FEF_{25-75}, respectively. PEF principally represents large airway flow and is well known to be dependent upon effort from the participant; hence, as recommended by ATS/ERS (Graham et al., 2019), encouragements were provided by the experimenter during completion of all FVC manoeuvres. In regard to FEF_{25-75} variability, Hansen, Sun and Wasserman (2006) examined spirometry tests from 5,938 non-smokers and 3,570 current smokers (both male and female) and highlighted that the use of FEF_{25-75} to confirm airway obstruction led to false-negative results in 42% of patients. This study presented high variability of FEF_{25-75} and warned against potentially misleading results when using this parameter for diagnosing airway obstruction. In addition, Quanjer et al., (2014) examined spirometry measurements from 22,767 patients (aged 2-94 years), and presented evidence that, in 3% of cases, FEF_{25-75} left airway obstruction undetected. Finally, as FEF_{25-75} represents the mean expiratory flow between 25 and 75% of FVC, changes in FVC directly impact on FEF_{25-75} readings. The interpretation of FEF_{25-75} data should therefore be made with caution.

On the whole, the present study indicates excellent repeatability of spirometry parameters, particularly for the most physiologically and clinically meaningful indexes (i.e. FVC, FEV_1, and FEV_1/FVC). These findings therefore justify the wide use of spirometry for assessing pulmonary function changes in experimental trials.

4.5.1.2 *Whole body plethysmography*

The findings from the present study also indicate 'excellent' repeatability for whole body plethysmography measurements taken on multiple days. Whilst whole body plethysmography parameters are, in general, more variable than those recorded during spirometry testing, findings from the present study show excellent repeatability. The healthy young adults assessed here displayed CV of 4.3%, 4.1% and 1.6% for FRC, RV and TLC, respectively. Previously, Hankinson et al., (1998) reported the following CV for plethysmography parameters in adults: FRC 3.9 to 10%, RV 2.4 to 14.0%, and TLC 1.5 to 4%, however, these data should be interpreted with caution since some of the studies analysed did not include descriptions of their participants' respiratory health status. In fact, data reported in the present study show slightly lower CV, highlighting the quality of data recorded here, which is supported by the 'excellent' ICC values (i.e., FRC: 0.990, RV: 0.991, TLC: 0.998). Further, in fourteen healthy adults performing five consecutive measurements (within 24 h), Teculescu *et al.*, (1982) reported CV of 7.5% and 4.2% for FRC and TLC, respectively (variation in RV was not reported). These data support the findings from the present study and confirm the very good repeatability of whole body plethysmography.

The data from the healthy young adults assessed within this study suggest that the most variable whole body plethysmography parameters are ERV (with a CV of 9.1%) and sRaw (CV of 8.7%); however, the 16.9% variance in sRaw remains lower than previously reported. Teculescu *et al.*, (1982) reported a variation of 28% (ranging from 17.8 to 43.4%) in four healthy young adults (aged 21 – 43 years), although these findings should be interpreted with care due to the small sample size used. Whilst sRaw is an important parameter for the assessment of airway resistance, the variability of this parameter may be elevated due to the complexity/discomfort involved in the test manoeuvre. As described in Chapter 3.8.3, the manoeuvre requires participants to continue to breathe 'normally' whilst a shutter occludes their airways. This element of the test is the most prone to artefacts, especially due to the lack of participant cooperation (Gosselink and Stam, 2005). Findings from the present study, alongside previous work presented above, suggest that sRaw and ERV are not best suited for detecting small changes in airway resistance and that their interpretation should account for inter-day variability. Overall however, these findings indicate excellent repeatability of whole body plethysmography, and render the use of this

technique highly appropriate in respiratory-focused experiments involving healthy young adults.

4.5.2 Intra-day reproducibility

4.5.2.1 *Spirometry*

Healthy young adults performing spirometry at multiple times within the same day produced highly reproducible results for FVC, FEV_1/FVC, PEF, and FEF_{25-75}. Aside from FEV_1, no significant changes were noted in spirometry performance undertaken in the morning *vs* the afternoon.

Contradictory results have previously been reported regarding the intra-day variation of FVC. The finding that no significant variation in FVC was noted participants of the present study is well aligned with Spengler and Shea's results (2000). In that study, no significant variation in FVC was noticed in ten healthy males who performed spirometry every 2 h over a 41 h time period. An absolute range of variation of 0.2–8.9% was reported by Spengler and collaborators (2000), which was statistically non-significant. These findings are similar to the present study, in which the range of variation was 0.7–7.6% from morning to early afternoon. Goel *et al.*, (2015) also compared intra-day variation of pulmonary function and found little variation in FVC in 161 healthy young adults (average morning FVC of 4.02 ± 0.57 L compared to an afternoon average of 4.02 ± 0.56 L). In contrast, Borsboom *et al.*, (1999) displayed average increases in FVC of 200 mL from 09:00h to 12:00h in 876 healthy adults. They reported that the increased FVC occurred more commonly in their older participants compared to the younger adults and suggested that changes to airway calibre with age may contribute to intra-day variation in pulmonary function. The range of participants recruited within the study by Borsboom *et al.*, (1999) highlight the importance of interpreting their presented data with caution.

In the present study, minimal variance in FVC across a single day was displayed. These findings, supported by the little variation reported in previous work (Goel et al., 2015; Spengler and Shea, 2000), provide confidence that spirometry can be used in healthy young adults to compare small changes in pulmonary function.

Although no change in FVC occurred throughout the day in participants of the present study, FEV_1 transiently increased by 152 ± 98 mL (3.7%) between morning and early

afternoon. Spengler and Shea (2000) reported similar findings, with mean diurnal change in FEV_1 of 3.5% (range 1.9–18.2%) in ten healthy young adult males (aged 24 ± 4 years). Further, Troyanov et al., (1994) presented average maximum elevation in FEV_1 of 9.6 ± 3.3% (range 3.5–18.4%) in ten healthy adults when spirometry was performed every 2 h between 08:00 h and 22:00 h; with the peak FEV_1 being reported at 13:12 ± 1:24 h. These findings are comparable to the 0.5–6.8% range reported in the present study. Troyanov et al., (1994) also reported that asthmatic individuals recorded a greater change in FEV_1 (average 16.5 ± 6.3%, range 7.0–26.9%) compared to healthy participants (*Note*. this study did not report changes in FVC). Given that airflow limitation and variable airway tone are characteristics of asthma (Barnes, 2008), it is to be expected that variation of FEV_1 (an index of airway calibre) would be higher in those patients compared to healthy participants.

4.5.2.2 *Whole body plethysmography*

Static lung volumes and capacities in the present study displayed very good reproducibility, with only RV displaying a significant increase between morning and afternoon. Experiments investigating the intra-day variability of whole body plethysmography indexes are currently sparse. In an early study, Hruby and Butler (1975) compared pulmonary function and airway resistance in fifteen adults [healthy: $n = 6$, obstructive lung disease patients (Note. No patients with asthma were included): $n = 5$, restrictive lung disease : $n = 4$] at multiple time points across a single day (08:00 h, 10:00 h, 12:00 h, 15:00 h, and 18:00 h) using an iso-pressure body plethysmograph. In line with the findings of the present study, no significant diurnal change was noted for FRC or VC. Increased sRaw was however noted in the afternoon, which is in contrast to the data collected in the present study (with no change in sRaw detected). This discrepancy in sRaw is likely due to the inclusion of clinical patients within the analysis from Hruby and Butler (1975). Further, a significant intra-day reduction in RV of ~144 mL (7%) has previously been noted in the aforementioned study by Borsboom et al., (1999), when measurements were taken at 09:00 h and 12:00. This compares to a 103 ± 84 mL increase in RV (+5.6 ± 4.4%) in our study participants, though it is important to consider that the large sample size (and therefore statistical power) of the study by Borsboom et al., (1999), which increases the reliability of those findings. These observed changes in RV may indicate a role for air trapping and/or airflow limitation in intra-day variation of pulmonary function (Ruppel, 2012). Since TLC remained stable throughout the day, it is unlikely that the observed changes in RV are attributable to intra-day changes in lung elastic recoil.

4.5.3 Study limitations

Based on guidelines from an ERS/ATS task force (Miller et al., 2005a), dynamic lung volume assessments (i.e., spirometry) were performed prior to static lung volume assessments (i.e., whole body plethysmography). Whilst in individuals with asthma, the deep inspiration of the spirometry manoeuvre may influence the airway resistance readings obtained through plethysmography [due to deep inspiration-induced bronchoconstriction (Burns and Gibson, 2002)], this is unlikely to have occurred in the healthy young participants assessed in the present work (Chapman et al., 2009; Kapsali et al., 2000). As a precaution, the order of PFT will remain consistent throughout future experiments presented within this thesis.

It is also worth noting that the intra-day evaluation of pulmonary function performed in this study does not provide a direct assessment of circadian rhythms/diurnal variation. Whilst this was not the aim of the present study, it is important to consider when making comparisons with previous work [i.e., work from Troyanov et al., (1994); Borsboom et al., (1999); Goel et al., (2015); Spengler & Shea, (2000)]. Refinetti et al., (2007) suggested that ANOVA should not be used for assessment of circadian rhythms, as this test does not evaluate the existence of a rhythmic pattern. However, the purpose of the present study was not to assess circadian rhythms per se, but instead to estimate variation (if any) of key pulmonary function parameters across a single day. The use of the ANOVA to assess variation between time points therefore allows understanding of variation within a single day and ensures that this can be accounted for in the interpretation of findings in the subsequent experimental chapters of this thesis.

4.5.4 Significance of findings

The findings from this study have substantial relevance to future/subsequent research presented both, within and beyond this thesis. Reproducibility and repeatability of data are integral for conducting robust scientific experiments. Ensuring PFT are repeatable and reproducible between and within days allows for trusted interpretation of results, particularly when assessing small variations in specific parameters. Furthermore, acknowledgment of inherent variations in pulmonary function parameters can contribute to the design of robust scientific experimental studies and acquisition of high-quality scientific data.

4.5.5 Conclusion

Spirometry and whole body plethysmography in healthy young adults displayed ‘excellent’ repeatability when performed on multiple days, and ‘very good’ reproducibility when performed at multiple time points within the same day. These findings therefore support the use of both PFT in experimental studies aiming to detect small but physiologically meaningful changes in pulmonary function of healthy young adults.

Chapter 5 Systemic but not local rehydration restores dehydration-induced changes in pulmonary function in healthy adults

5.1 Abstract

Water transport and local (airway) hydration are critical for the normal functioning of lungs and airways. Currently, there is uncertainty regarding the effects of systemic dehydration on pulmonary function. The aims of this study were: *i)* to clarify the impact of exercise- or fluid restriction-induced dehydration on pulmonary function in healthy adults; and *ii)* to establish whether systemic or local rehydration can reverse dehydration-induced alterations in pulmonary function. Ten healthy participants performed four experimental trials in a randomized order (2 h exercise in the heat twice, and 28 h fluid restriction twice). Pulmonary function was assessed using spirometry and whole-body plethysmography in the euhydrated, dehydrated, and rehydrated states. Oral fluid consumption was used for systemic rehydration, and nebulized isotonic saline inhalation for local rehydration. Both exercise and fluid restriction induced mild dehydration (2.7 ± 0.7% and 2.5 ± 0.4% body mass loss, respectively; $p < 0.001$) and elevated plasma osmolality ($p < 0.001$). Dehydration across all four trials was accompanied by a reduction in forced vital capacity (152 ± 143 mL, $p < 0.01$) and concomitant increases in residual volume (216 ± 177 mL, $p < 0.01$) and functional residual capacity (130 ± 144 mL, $p < 0.01$), with no statistical differences between modes of dehydration. These changes were normalized by fluid consumption, but not nebulization. These results suggest that, in healthy adults: *i)* mild systemic dehydration induced by exercise or fluid restriction leads to pulmonary function impairment, primarily localized to small airways; and *ii)* systemic, but not local, rehydration reverses these potentially deleterious alterations.

5.2 Introduction

Systemic dehydration commonly occurs in individuals who perform sustained physical activity in hot environments (Sawka et al., 2007), as well as in patients and older adults (Allison and Lobo, 2004). Even at a mild level [i.e., ~2-3% body mass loss (Cheuvront and Kenefick, 2014)], systemic dehydration can have unfavourable effects on multiple organ systems, including the cardiovascular, renal and nervous systems (Popkin et al., 2010), and can compromise physical and cognitive performance (Cheuvront and Kenefick, 2014). Limited and contradictory data currently exist regarding the effects of systemic dehydration on the respiratory system.

Two previous studies have showed deleterious alterations in expiratory flow or lung volumes in healthy adults (Govindaraj, 1972) and in athletes with mild asthma (Simpson et al., 2017) following mild systemic dehydration (up to 2.5% body mass loss). A third study (Javaheri et al., 1987) however showed improvements in selected measures of pulmonary function (i.e., FEV_1 and FEF) in healthy adults following moderate dehydration (4.5% body mass loss). The different population groups and severities of dehydration, as well as the various modes of dehydration [i.e., fluid restriction (Govindaraj, 1972) *vs* exercise (Simpson et al., 2017) *vs* diuretic drug (Javaheri et al., 1987)], have generated uncertainty regarding the impact of systemic dehydration on pulmonary function.

Fluid supply to the airways stems primarily from the bronchial circulation, which itself arises from the systemic circulation (Baile, 1996). Optimal lung fluid balance is a critical component of normal pulmonary functioning (Boucher, 2003), with ASL dehydration implicated in several respiratory disease states, such as cystic fibrosis (Haq et al., 2016) and EIB (Anderson and Daviskas, 1997). Water flows across the airway epithelia in response to an osmotic gradient. Thus, when individuals become dehydrated and bronchial blood flow and/or composition changes, water movement to the airways is modified and airway hydration may become compromised. Alterations in ASL thickness (‘depth’), composition, and/or rheology can promote peripheral or small airway instability and provoke premature airway closure (Macklem et al., 1970), potentially increasing occurrence of respiratory symptoms (in particular, breathlessness) and worsening respiratory reserve in susceptible individuals.

Exercise in the heat and/or insufficient fluid intake are two common causes of systemic dehydration. Both modes of dehydration lead to hypertonic-hypovolemia and are associated with an increase in P_{osm} (Cheuvront et al., 2013). Fluid losses and the associated fluid shifts occur at different rates during acute exercise compared to prolonged periods of insufficient fluid intake. Exercise- and low fluid intake-induced dehydration may therefore affect bronchial blood flow and/or composition. Whether a fast rate of fluid loss (as occurs during acute exercise) leads to more severe alterations in pulmonary function than a slow rate (with prolonged fluid restriction) is currently unknown. Further, whether changes in pulmonary function induced via fluid loss can be reversed by replacing that fluid also remains undetermined. Since it is proposed that direct water lost from the airways (as a result of systemic dehydration) could be responsible for the change in pulmonary function, it is also of interest to compare different types of fluid replacement (i.e., systemic rehydration *vs* direct local airway rehydration) and their subsequent effects upon pulmonary function.

The aim of this study was to clarify the impact of mild systemic dehydration on pulmonary function in healthy adults. In line with previous findings following research in athletes with asthma (Simpson et al., 2017), it was hypothesised that pulmonary function would be compromised in dehydrated healthy adults, as evidenced by changes in spirometry and whole body plethysmography variables. To test whether the mode of dehydration influences the pulmonary response, the effects of 2 h of exercise in the heat (fast rate of fluid loss) were compared to 28 h of fluid restriction (slow rate of fluid loss). Additionally, this study aimed to establish: *i)* whether dehydration-induced changes in pulmonary function are reversible with immediate rehydration, and *ii)* whether local rehydration delivered directly at the site of the airways (via nebulised isotonic saline) is superior to systemic rehydration (via oral fluid intake) in restoring pulmonary function.

5.3 Methods

5.3.1 Ethical approval

All protocols and procedures were approved by the Brunel University London Research Ethics Committee (6639-TISS-Jul/2017- 7774-2; Appendix A) and conformed to the guidelines of the Declaration of Helsinki, 7th version (World Medical Association, 2013).

5.3.2 Participants

Seventeen (five female) participants, aged 19 to 42 years, were initially recruited to take part in this study. Seven participants failed to complete all four experimental visits and were subsequently excluded from the analysis (six participants voluntarily withdrew from the study, and one participant developed pneumonia between experimental visits and was excluded from further involvement). All participants were healthy and had no history of respiratory illness (incl. asthma and EIB). Pulmonary function was checked for normality at study entry via spirometry (with all FEV_1 >80% predicted values and all FEV_1/FVC ratio >70% predicted), using GLI-2012 equations as reference (Quanjer et al., 2012). Participants provided written informed consent prior to taking part, and adhered to the pre-trial control measures described in Chapter 3.4.1 (including avoiding caffeine, alcohol, and strenuous exercise in the 24 h prior to the test).

5.3.3 Experimental overview

A repeated-measures, randomized crossover design was utilized, with all participants completing four experimental trials on separate days (Figure 5.1). Alcohol, caffeine and strenuous exercise were prohibited in the 24 h prior to testing. Before the first trial, participants completed a 24 h food diary, which they subsequently replicated before each trial (as described in Chapter 3.4.1)

Participants arrived at the laboratory at between 08:00h – 10:00h in a euhydrated state. Basic anthropometrics, hydration status, and pulmonary function were assessed. Resting $\dot{V}_E$ was then recorded to allow for estimation of ASL loss over the 28 h fluid restriction trials. Following baseline measurements, participants underwent one of two dehydration trials: 28 h fluid restriction (FR) or exercise-induced (EX) dehydration. Upon completion of the dehydration trials, hydration status and pulmonary function were re-assessed; this was followed by a period of rehydration with oral fluid or isotonic saline nebulisation. Spirometry was performed 15 and 35 min within the rehydration periods. Hydration status and pulmonary function were recorded 60 min after commencing rehydration.

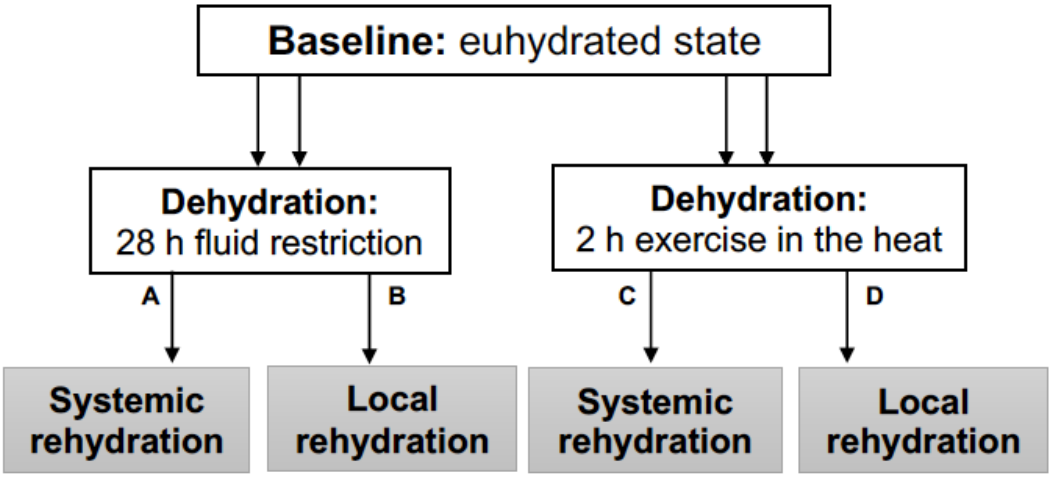

Figure 5.1. Schematic representation of the four experimental trials: A) fluid restriction with systemic rehydration (oral fluid intake); B) fluid restriction with local rehydration (nebulised isotonic saline); C) exercise with systemic rehydration; D) exercise with local rehydration. The order of trials was randomized.

5.3.4 Hydration status

Hydration status was assessed via changes in nude body mass, $P_{osm,}$ and U_{osm}. A U_{osm} of < 700 mOsm·kg^{-1} was used as inclusion criteria for euhydration at the start of each visit (Sawka et al., 2007). Details of techniques are provided in Chapter 3.7.

5.3.5 Pulmonary function

Pulmonary function was assessed at each time point via spirometry and whole body plethysmography (in that order) at baseline and 60 min of rehydration. At 15 min and 35 min rehydration, forced manoeuvres were performed in duplicate only (as long as FEV_1 and FVC were reproducible). sRaw was measured using the interrupter (i.e., airway occlusion) technique (Goldman et al., 2005). All tests were performed in accordance with ATS/ERS guidelines (Miller et al., 2005b; Wanger et al., 2005) and are described in detail in Chapter 3.8.

5.3.6 Dehydration protocols

5.3.6.1 *Fluid Restriction*

As done previously (Szinnai et al., 2005), participants were prohibited from consuming any fluid and were restricted to consuming foods with low water content (< 30%) from a list of acceptable/prohibited foods (Popkin et al., 2010) for 28 h. Participants kept a diary of all food consumed, which they replicated during the second fluid restriction trial. Participants were fitted with an activity monitor (ActivPal, PAL Technologies Ltd., Glasgow, UK) and asked to limit physical activity for the entire duration of the fluid restriction. Local environmental temperature and humidity were recorded throughout 28 h using a portable logger (Hygrochron, iButton, Maxim Integrated, California, USA) and were later used to estimate absolute water content of inspired air and ASL loss (Anderson and Daviskas, 1997; Daviskas et al., 1991). Expired water content was assumed to equal 33 mg $H_2O \cdot L^{-1}$ of air (Anderson and Daviskas, 1997). The difference between inspired and expired absolute water content was calculated and multiplied by resting $\dot{V}_E$. Total water loss over the duration of the fluid restriction trial was estimated assuming negligible variations in $\dot{V}_E$ over the 28 h period.

5.3.6.2 *Exercise-induced dehydration*

Participants completed 2 h of low intensity exercise in hot conditions (37°C, 50% RH) with total fluid restriction. The exercise protocol was identical to that used in previous work (Simpson et al., 2017), alternating cycling on an Excalibur Sport stationary bike (Lode BV, Groningen, The Netherlands) at 25% of estimated peak power output (Section 3.5.2) and stepping (on a 20 cm step, at a rate of 45 steps per minute regulated by a metronome). At the end of each bout of cycling (20 min) and stepping (10 min), the following measurements were taken: heart rate (via telemetry: FT1, Polar Electro Oy, Kempele, Finland), tympanic temperature (Thermoscan Exactemp 6022, Braun, Germany), and ratings of perceived exertion (RPE) and breathing discomfort [via modified Borg scales (Borg, 1982)]. During the final 5 min of each bout of cycling/stepping, $\dot{V}_E$ and $\dot{V}O_2$ were recorded breath-by-breath (Vyntus CPX, Carefusion, Germany), with mean $\dot{V}_E$ over the final minute kept for analysis (details of measurement techniques are provided in Chapter 3.6 and 3.9). Mean $\dot{V}O_2$ over 2 h of exercise was used for estimation of ASL loss, based on calculations provided by Mitchell *et al*. (Mitchell et al., 1972):

$$\dot{m}_e = 0.019\ \dot{V}O_2\ (44 - Pa)$$

Where $\dot{m}_e$ is the rate of evaporative water loss in the expired air ($g \cdot min^{-1}$), Pa is the ambient water vapour pressure (mmHg), and $\dot{V}O_2$ is the oxygen uptake ($L \cdot min^{-1}$ STPD).

5.3.7 Rehydration protocols

5.3.7.1 *Systemic Rehydration*

Participants rehydrated by ingesting water at room temperature, mixed with 3 g NaCl·L H_2O^{-1} to improve fluid retention (Evans et al., 2017). The volume of fluid ingested (L) matched the loss of body mass (kg). Participants ingested water in three equal boluses (550 ± 176 mL) over 15 min, with a 5 min break between boluses to perform spirometry.

5.3.7.2 *Local Rehydration*

An ultrasonic nebuliser (UltraNeb, DeVilbiss Healthcare Ltd., UK) and isotonic saline (0.9%) were used to locally rehydrate the airways at a measured output of 1.4 ± 0.2 mL·min^{-1}. Participants were required to breathe tidally through a two-way non-rebreathing valve (Series 1410, Hans Rudolph Inc, Kansas, USA) with a nose clip in place. Participants were exposed to 3 × 15 min bouts of nebulisation, with 5 min breaks in-between.

5.3.8 Statistical power

Power analyses were conducted using G*Power Software (see Chapter 3.11.1 for detail). Utilising FVC data from a previous experiment which investigated the effects of dehydration via exercise in the heat upon pulmonary function of asthmatic individuals (Simpson et al., 2017), it was calculated that a minimum of eight participants will be required to reach a statistical power of 80%.

5.3.9 Statistical analysis

Statistical analyses were performed using dedicated software (SPSS version 26, SPSS, IBM Corp., Armonk, USA). Data were tested for normality using the Shapiro-Wilk test. To assess changes in spirometry, plethysmography and hydration within and between trials, 3-way repeated measures ANOVA were used (with mode of dehydration, mode of rehydration and time as main factors). Post hoc Bonferroni-adjusted pairwise comparisons were used where significant main or interaction effects were detected. A within-subjects repeated measures correlation (Bland and Altman, 1995) was used to determine the

relationship between changes in hydration and pulmonary function. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SD, unless otherwise stated.

5.4 Results

5.4.1 Participants

Ten healthy, non-smoking individuals (2 females; age 29 ± 8 y, body mass 62.8 ± 8.5 kg, stature 173 ± 10 cm) completed the study. At study entry, baseline spirometry data were within normal range for all participants, with group mean data of 105 ± 7 % predicted for FEV_1, 111 ± 6% predicted for FVC and FEV_1/FVC 80 ± 5%.

5.4.2 Dehydration protocols

5.4.2.1 *Fluid Restriction*

Over the 28 h period, participants spent 21 ± 3 h sedentary, 5 ± 2 h standing, and 2 ± 0.2 h stepping/walking. Based on the recorded environmental conditions (23.8 ± 2.5°C, 52 ± 9% RH) and resting $\dot{V}_E$ (9 ± 3 $L \cdot min^{-1}$), estimated total water loss from the airways was 357 ± 110 mL (13 ± 4 $mL \cdot h^{-1}$), with no difference between the two trials (systemic rehydration trial: 344 ± 130 mL, 12 ± 5 $mL \cdot h^{-1}$; local rehydration trial: 370 ± 93 mL, 13 ± 3 $mL \cdot h^{-1}$).

5.4.2.2 *Exercise*

Estimated peak power output was 273 ± 52 W, therefore participants cycled at 25% peak power output (71 ± 9 W) in both exercise trials. No difference was reported between the two exercise trials for heart rate, tympanic temperature, $\dot{V}_E$, RPE, breathing discomfort or estimated water loss from the airways (all $p > 0.05$, data are provided in Table 5.1). Compared to fluid restriction, the estimated total water loss from the airways during exercise was smaller ($p < 0.001$), whilst the rate of water loss was larger ($p < 0.001$).

Table 5.1. Average physiological responses throughout 2 h of exercise in the heat with no fluid replacement *(n=10)*.

	EX-Systemic	**EX-Local**
Heart Rate (beats·min^{-1})	131 ± 23	130 ± 27
Tympanic temperature (°C)	37.1 ± 0.4	37.0 ± 0.4
$\dot{V}_E$ (L·min^{-1})	37 ± 7	39 ± 7
RPE	12 ± 2	11 ± 3
Breathing discomfort	3 ± 2	2 ± 2
Airway water loss (mL)	79 ± 16	80 ± 19
Airway water loss (mL·h^{-1})	39 ± 8	40 ± 10

Data are means ± SD. EX-Systemic, exercise with systemic rehydration; EX-Local, fluid restriction with local rehydration (nebulised isotonic saline); $\dot{V}_E$: minute ventilation; RPE: rating of perceived exertion.

5.4.3 Hydration status

Data for hydration status are shown in Table 5.2.

5.4.3.1 *Effect of dehydration*

Both modes of dehydration induced a mild level of dehydration, with a reduction in body mass of 2.5 ± 0.4% after exercise and 2.7 ± 0.7% after fluid restriction [main effect of time: $F_{2,18} = 183.18$, $p < 0.001$, interaction effect (i.e. dehydration method x time): $F_{2, 18} = 23.89$, $p < 0.001$]. The reduction in body mass was associated with an increase in P_{osm} in all trials (main effect of time: $F_{2, 18} = 55.32$, $p < 0.001$). U_{osm} increased following exercise and fluid restriction (main effect of time: $F_{2,18} = 124.48$ $p < 0.001$), but the increase was greater following fluid restriction (interaction effect: $F_{2, 18} = 23.89$, $p = 0.001$). Plasma volume, haemoglobin and haematocrit did not change after exercise or fluid restriction.

5.4.3.2 *Effect of rehydration*

Following systemic, but not local rehydration, body mass increased (interaction effect rehydration method x time: $F_{2, 18} = 126.62$; $p < 0.001$ *vs* dehydration). Post-systemic rehydration, body mass remained slightly lower compared to baseline (interaction effect: $F_{2,18} = 126.62$, $p < 0.001$). Post-hoc tests revealed that P_{osm} was restored following systemic rehydration but remained elevated following local rehydration ($p < 0.001$ *vs* baseline).

Neither systemic nor local rehydration normalized U_{osm} ($p < 0.001$ *vs* baseline). In fact, following local rehydration, U_{osm} increased ($p = 0.001$ *vs* dehydration). Consequently, U_{osm} was higher after local compared to systemic rehydration (interaction effect: $F_{2,18} = 23.89$, $p = 0.001$). Systemic rehydration increased plasma volume ($p = 0.013$ *vs* dehydrated, $p = 0.012$ *vs* baseline), alongside reductions in haemoglobin ($p = 0.017$). Haematocrit was lower following systemic *vs* local rehydration ($p = 0.004$). No change was noted following local rehydration for plasma volume, haemoglobin concentration, or haematocrit. Detailed statistical analysis is presented in Table 9.1 (Appendix F).

Table 5.2. Hydration status at baseline, immediately after fluid restriction- and exercise-induced dehydration and following systemic and local (airway) rehydration (n = 10).

	FR-Systemic	EX-Systemic	FR-Local	EX-Local
Body mass (kg)				
Baseline	62.9 ± 8.7	62.5 ± 8.6	63.1 ± 8.8	63.0 ± 8.5
Dehydration	61.2 ± 8.4*	61.0 ± 8.5*	61.4 ± 8.5*	61.4 ± 8.3*
Rehydration	62.5 ± 8.7$^{\dagger\,\ddagger}$	62.2 ± 8.6$^{\dagger\,\ddagger}$	61.3 ± 8.5*	61.3 ± 8.3*
P_{osm} (mOsm·kg^{-1})				
Baseline	294 ± 5	294 ± 6	291 ± 5	293 ± 7
Dehydration	300 ± 4*	300 ± 4*	300 ± 5*	300 ± 4*
Rehydration	293 ± 4§‡	292 ± 5§‡	301 ± 5*	300 ± 7*
U_{osm} (mOsm·kg^{-1})				
Baseline	201 ± 137	166 ± 137	255 ± 199	132 ± 134
Dehydration	897 ± 103^{*a}	477 ± 188*	966 ± 135^{*a}	442 ± 232*
Rehydration	544 ± 261*‡	694 ± 224*‡	1024 ± 99†	851 ± 171†
Haemoglobin (g·L^{-1})				
Baseline	147 ± 15	150 ± 16	145 ± 14	148 ± 18
Dehydration	151 ± 14	148 ± 11	150 ± 18	150 ± 15
Rehydration	143 ± 13	142 ± 20	147 ± 13	148 ± 18
Haematocrit (%)				
Baseline	45 ± 4	45 ± 6	45 ± 5	45 ± 5
Dehydration	45 ± 5	45 ± 3	45 ± 5	45 ± 5
Rehydration	44 ± 4‡	44 ± 5‡	46 ± 5	46 ± 5
Plasma Volume (%)				
Baseline	55.4 ± 4.2	55.3 ± 5.8	54.8 ± 4.6	55.1 ± 5.4
Dehydration	53.5 ± 6.1	55.7 ± 2.7	53.6 ± 6.6	54.0 ± 5.1
Rehydration	60.1 ± 6.3†‡	59.5 ± 5.4†‡	55.4 ± 4.4	54.7 ± 6.3

Data are means ± SD. FR-Systemic, fluid restriction with systemic rehydration (oral fluid intake); EX-Systemic, exercise with systemic rehydration; FR-Local, fluid restriction with local rehydration (nebulised isotonic saline); EX- Local, exercise with local rehydration; P_{osm}, plasma osmolality; U_{osm}, urine osmolality. $^{*}p < 0.05$ *vs* baseline; $^{\dagger}p < 0.05$ *vs* baseline and dehydration; $^{\ddagger}p < 0.05$ *vs* FR-Local and EX-Local at corresponding time point; $^{\S}p < 0.05$ *vs* dehydrated; $^{a}p < 0.05$ *vs* FR-Systemic and FR-Systemic at corresponding time point. *Note. For detailed statistical output see Appendix F.*

5.4.4 Spirometry

Spirometry data are presented in Table 5.3. Due to a technical issue, data from one participant had to be excluded from the analysis ($n = 9$).

5.4.4.1 *Effect of dehydration*

At baseline, spirometry values did not differ between trials. Mild dehydration induced by exercise and fluid restriction was associated with a reduction in FVC (main effect for time: $F_{4,36} = 17.52$, $p < 0.001$), but not FEV_1. The magnitude of change in FVC was not different between modes of dehydration (exercise: -173 ± 168 mL; fluid restriction: -131 ± 70 mL; Figure 5.2). As FEV_1 did not change, the ratio FEV_1/FVC increased post- dehydration (main effect for time: $F_{4, 36} = 22.58$, $p < 0.001$), with the increase slightly greater following exercise (interaction effect: $F_{4,36} = 2.94$, $p = 0.032$). No differences were noted between or within trials for PEF.

5.4.4.2 *Effect of rehydration*

After only 15 min of systemic rehydration, FVC was restored (main effect for time: $F_{4,36} = 17.52$, $p < 0.001$, interaction effect: $F_{4,36} = 4.537$, $p = 0.005$; $p > 0.05$ at 15 min of rehydration *vs* baseline). With local rehydration, however, FVC remained below baseline over the entire 60 min period of rehydration (Figure 5.2). FEV_1/FVC was restored by systemic rehydration (interaction effect rehydration method x time: $F_{4, 36} = 6.69$, $p > 0.05$ *vs* baseline at all recovery time points), while only transient improvements in FEV_1/FVC were noted at 15 min and 35 min during local rehydration ($p > 0.05$ *vs* baseline; Figure 5.2). FEV_1 and PEF remained unaltered following rehydration.

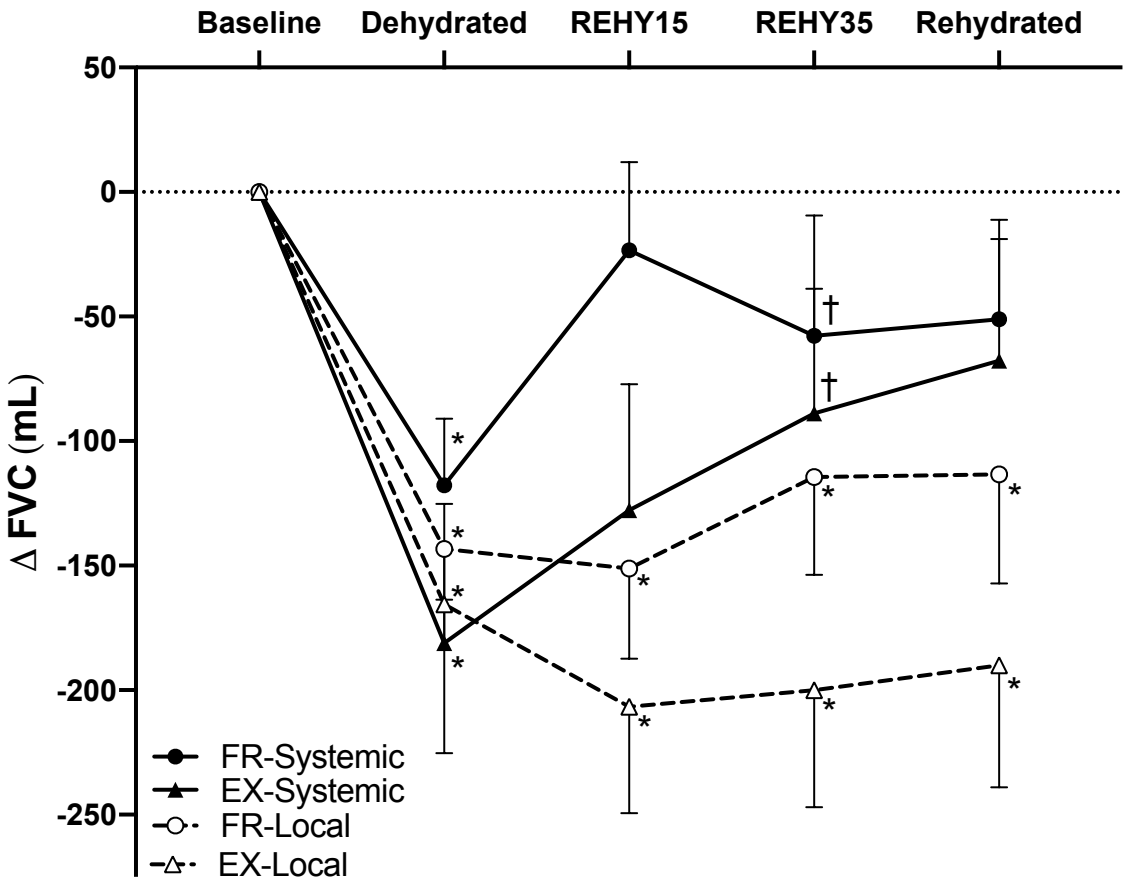

Figure 5.2. Changes in forced vital capacity (FVC) following fluid restriction- and exercise- induced dehydration, and at 15, 35 and 60 min of systemic (oral fluid intake) and local (nebulised isotonic saline) rehydration. Values are mean ± SEM for nine healthy adults (2 females). *$p < 0.05$ *vs* baseline, † $p < 0.05$ *vs* dehydration.

Table 5.3. Spirometry data at baseline, after fluid restriction- and exercise- induced dehydration, and at 15, 35 and 60 min of systemic and local (airway) rehydration *(n* = 9*)*.

	FR-Systemic	EX-Systemic	FR-Local	EX-Local
FEV_1 (L)				
Baseline	3.95 ± 0.79	4.01 ± 0.81	4.00 ± 0.78	4.01 ± 0.81
Dehydration	3.94 ± 0.86	4.04 ± 0.87	3.94 ± 0.80	4.03 ± 0.79
15 min rehydration	3.94 ± 0.80	3.98 ± 0.89	3.95 ± 0.78	3.98 ± 0.84
35 min rehydration	3.92 ± 0.83	3.97 ± 0.86	3.96 ± 0.79	3.99 ± 0.80
60 min rehydration	3.93 ± 0.80	3.97 ± 0.86	3.98 ± 0.82	3.99 ± 0.80
FVC (L)				
Baseline	5.03 ± 1.15	5.04 ± 1.14	5.11 ± 1.12	5.06 ± 1.14
Dehydration	4.92 ± 1.19*	4.86 ± 1.22*	4.96 ± 1.13*	4.89 ± 1.06*
15 min rehydration	5.01 ± 1.17	4.91 ± 1.22	4.96 ± 1.11*	4.85 ± 1.11*
35 min rehydration	4.98 ± 1.20†	4.95 ± 1.20†	4.99 ± 1.14*	4.86 ± 1.07*
60 min rehydration	4.98 ± 1.16	4.97 ± 1.19	4.99 ± 1.19*	4.87 ± 1.07*
FEV_1/FVC (%)				
Baseline	79.1 ± 7.2	80.3 ± 7.4	79.0 ± 7.1	79.9 ± 7.1
Dehydration	81.1 ± 7.8*‡	84.1 ± 8.0*	79.9 ± 6.7*‡	83.0 ± 5.8*
15 min rehydration	79.4 ± 7.5†	81.8 ± 6.9†	80.3 ± 6.7	82.8 ± 5.6
35 min rehydration	79.7 ± 7.1†	81.0 ± 7.2†	79.9 ± 6.2	82.6 ± 5.8
60 min rehydration	79.7 ± 7.4†§	80.5 ± 6.9†§	80.5 ± 6.8*	82.4 ± 6.0*
PEF ($L \cdot s^{-1}$)				
Baseline	9.41 ± 2.01	9.22 ± 1.96	9.63 ± 2.05	9.42 ± 2.00
Dehydration	9.39 ± 2.06	9.21 ± 2.03	9.55 ± 1.90	9.41 ± 1.85
15 min rehydration	9.54 ± 2.04	9.04 ± 1.94	9.22 ± 1.76	9.08 ± 2.08
35 min rehydration	9.40 ± 2.01	9.05 ± 1.95	9.51 ± 2.02	8.98 ± 2.03
60 min rehydration	9.53 ± 2.01	9.19 ± 1.96	9.43 ± 2.01	9.21 ± 1.96

Data are mean ± SD. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow; FR-Systemic, fluid restriction with systemic rehydration (oral fluid intake); EX-Systemic, exercise with systemic rehydration; FR-Local, fluid restriction with local rehydration (nebulised isotonic saline); EX- Local , exercise with local rehydration. $^{*}p < 0.05$ *vs* baseline, $^{\dagger}p < 0.05$ *vs* dehydration, $^{\ddagger}p < 0.05$ *vs* FR-Systemic and FR-Local at corresponding time point, $^{\S}p < 0.05$ *vs* FR-Local and EX-Local at corresponding time point. *Note. For detailed statistical output see Appendix F.*

5.4.5 Whole body plethysmography

Whole body plethysmography data are presented in Table 5.4.

5.4.5.1 *Effect of dehydration*

Static lung volumes and capacities were not different at baseline between trials. Following dehydration with both fluid restriction and exercise, increases in FRC (main effect for time: $F_{2,18} = 12.38$, $p < 0.001$) and RV (main effect for time: $F_{2,18} = 25.39$, $p < 0.001$) were noted. The magnitude of change in FRC (fluid restriction: 134 ± 152 mL; exercise: 131 ± 151 mL) and RV (fluid restriction: 151 ± 131 mL; exercise: 282 ± 194 ml) was not different between modes of dehydration (Figure 5.3). As TLC remained unchanged following dehydration, the RV/TLC ratio increased (main effect for time: $F_{2,18} = 28.44$, $p < 0.001$), with exercise inducing a larger change (interaction effect: $F_{2,18} = 4.44$, $p = 0.023$). ERV and sRaw were unaffected by dehydration.

5.4.5.2 *Effect of rehydration*

FRC and RV were both restored following systemic rehydration (both p>0.05 *vs* baseline) but did not change following local rehydration (FRC: interaction effect: $F_{2,18} = 4.41$, $p = 0.028$; RV: main effect for time $F_{2,18} = 25.39$, $p < 0.001$, main effect for rehydration method: $F_{1,9} = 11.06$, $p = 0.008$; Figure 5.3). TLC was not impacted by rehydration. Consequently, RV/TLC returned to baseline after systemic rehydration (main effect for time: $F_{2,18} = 28.44$, $p < 0.001$, main effect for rehydration method: $F_{1,19} = 8.311$, $p = 0.015$), but not after local rehydration ($p = 0.007$ *vs* baseline; Figure 5.3). ERV was slightly reduced after 60 min of systemic and local rehydration (main effect for time: $F_{2,18} = 5.33$ $p = 0.015$). sRaw was unaltered by rehydration.

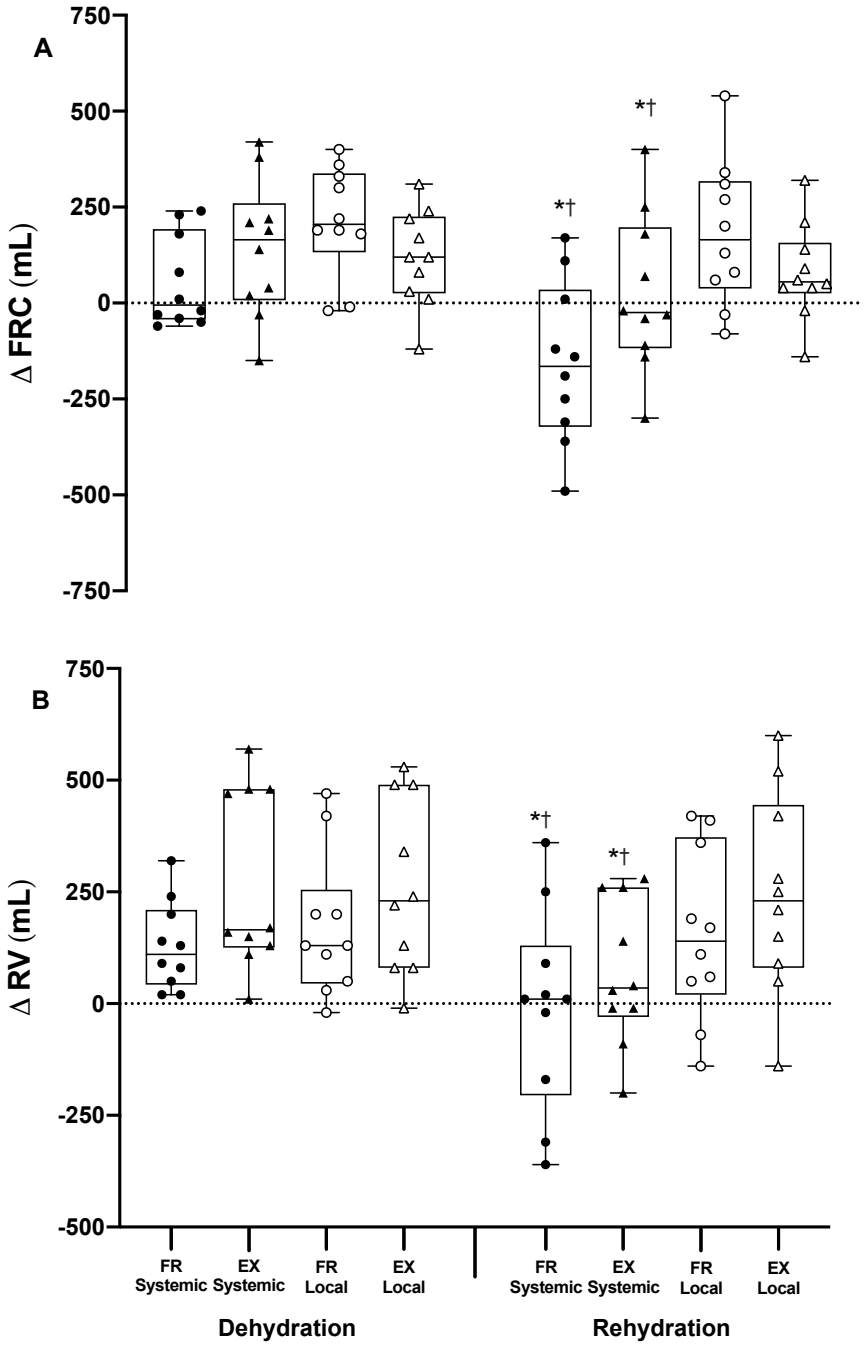

Figure 5.3. Changes in A) functional residual capacity (FRC) and B) residual volume (RV) after fluid restriction- and exercise- induced dehydration and following systemic (oral fluid intake) and local (nebulised isotonic saline) rehydration in ten healthy adults (2 females). Boxplots represent the median and interquartile range, with whiskers representing the minimum and maximum values. FR-Systemic, fluid restriction with systemic rehydration; EX-Systemic, exercise with systemic rehydration; FR-Local, fluid restriction with local rehydration; EX-Local, exercise with local rehydration. *$p < 0.05$ *vs* dehydration, †$p < 0.05$ *vs* FR-Local and EX-Local at corresponding time point.

Table 5.4. Whole body plethysmography data at baseline, after fluid-restriction- and exercise- induced dehydration, and after 60 min of systemic and local (airway) rehydration *(n =10).*

	FR-Systemic	EX-Systemic	FR-Local	EX-Local
TLC (L)				
Baseline	7.05 ± 1.48	7.06 ± 1.54	7.06 ± 1.49	7.10 ± 1.45
Dehydration	7.09 ± 1.46	7.10 ± 1.55	7.12 ± 1.40	7.14 ± 1.56
Rehydration	7.07 ± 1.46	7.19 ± 1.48	7.15 ± 1.50	7.17 ± 1.60
FRC (L)				
Baseline	4.03 ± 1.00	4.01 ± 1.07	3.91 ± 0.99	4.07 ± 1.03
Dehydration	4.08 ± 1.08*	4.15 ± 1.01*	4.12 ± 0.93*	4.19 ± 1.02*
Rehydration	3.90 ± 0.97†‡	4.02 ± 1.00†‡	4.09 ± 1.02*	4.15 ± 1.11*
RV (L)				
Baseline	2.14 ± 0.52	2.19 ± 0.55	2.19 ± 0.53	2.23 ± 0.53
Dehydration	2.27 ± 0.54*	2.49 ± 0.62*	2.36 ± 0.51*	2.49 ± 0.69*
Rehydration	2.13 ± 0.47†‡	2.29 ± 0.55†‡	2.35 ± 0.60*	2.47 ± 0.72*
RV/TLC (%)				
Baseline	30.3 ± 4.9	31.0 ± 5.0	31.0 ± 4.4	31.4 ± 4.3
Dehydration	32.1 ± 5.2*§	35.3 ± 6.3*	33.5 ± 5.4*§	34.9 ± 6.5*
Rehydration	30.4 ± 3.3†‡	31.9 ± 4.1†‡	32.9 ± 5.2*	34.4 ± 6.7*
ERV (L)				
Baseline	1.89 ± 0.55	1.83 ± 0.63	1.72 ± 0.48	1.83 ± 0.53
Dehydration	1.81 ± 0.59	1.69 ± 0.54	1.76 ± 0.46	1.70 ± 0.49
Rehydration	1.75 ± 0.55*	1.73 ± 0.50*	1.74 ± 0.48*	1.68 ± 0.52*
sRaw (kPa·s^{-1})				
Baseline	0.99 ± 0.19	1.01 ± 0.22	1.05 ± 0.21	1.00 ± 0.25
Dehydration	1.03 ± 0.22	1.06 ± 0.22	1.08 ± 0.27	1.03 ± 0.24
Rehydration	0.97 ± 0.23	1.04 ± 0.29	1.06 ± 0.26	1.05 ± 0.26

Data are mean ± SD. TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; ERV, expiratory reserve volume; sRaw, specific airway resistance; FR-Systemic, fluid restriction with systemic rehydration (oral fluid intake); EX-Systemic, exercise with systemic rehydration; FR-Local, fluid restriction with local rehydration (nebulised isotonic saline); EX-Local, exercise with local rehydration. $^{*}p < 0.05$ *vs* baseline; $^{\dagger}p < 0.05$ *vs* dehydration; $^{\ddagger}p < 0.05$ *vs* FR-Local and EX- Local at corresponding time point, $^{\S}p < 0.05$ vs. FR- Systemic and FR-Local at corresponding time point. *Note. For detailed statistical output see Appendix F.*

5.4.6 Correlation analysis

The percent change in body mass at dehydration and rehydration (60 min) showed a moderate positive correlation with the change in FVC (r = 0.643, p < 0.001; Figure 5.4A), and moderate negative correlations with FRC (r = -0.644, p < 0.001; Figure 5.4C) and RV (r = -0.693, p < 0.001; Figure 5.4E). The change in P_{osm} following the dehydration and rehydration interventions showed a moderate negative correlation with the change in FVC (r = -0.653, p < 0.001; Figure 5.4B), and moderate positive correlations with FRC (r = 0.524, p < 0.001; Figure 5.4D) and RV (r = 0.587, p < 0.001; Figure 5.4F). No other significant relationships were noted.

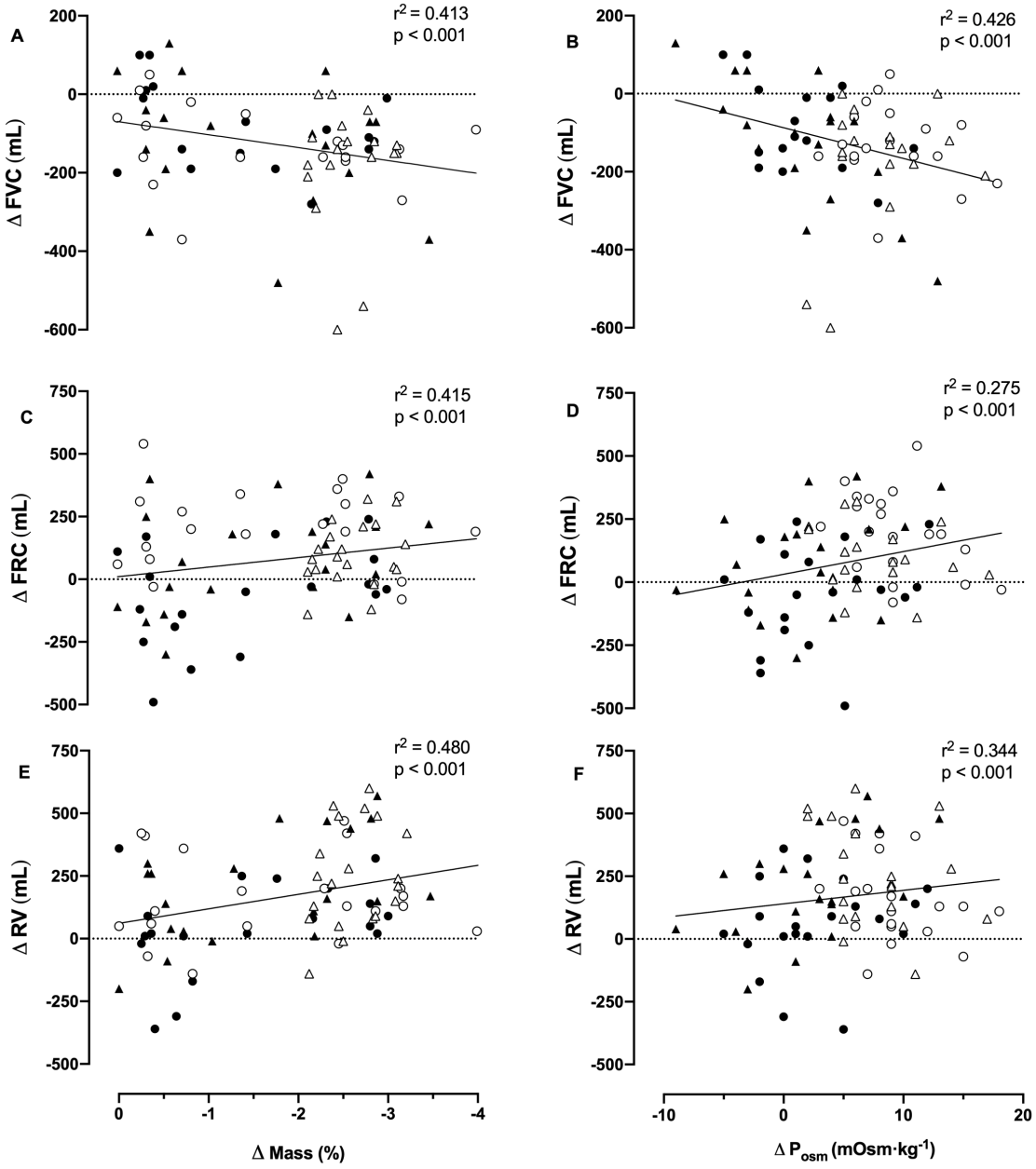

Figure 5.4. Relationship between changes in body mass and changes in A) FVC, C) FRC, and E) RV; and relationship between changes in plasma osmolality (P_{osm}) and changes in B) FVC, D) FRC, and F) RV over the course of four trials (at dehydration and rehydration) in healthy adults (*n = 9* for FVC and *n* = 10 for FRC and RV; 2 females). Closed circles, fluid restriction with systemic rehydration; closed triangles, exercise with systemic rehydration; open circles, fluid restriction with local rehydration; open triangles, exercise with local rehydration.

5.5 Discussion

The findings from this study show negative alterations in pulmonary function in mildly dehydrated healthy young adults at rest. The observed reduction in FVC combined with an elevated RV and FRC suggest that the dehydration-induced pulmonary alteration is primarily localised to the small airways. That the mode of dehydration (i.e., 28 h of fluid restriction and 2 h of exercise in the heat) did not influence the airway response indicates that the severity, rather than the rate, of dehydration is likely responsible for the observed alterations. In this study, dehydration-induced alterations in pulmonary function were reversed following acute systemic rehydration (via oral fluid intake), but not following local rehydration of the airways (via nebulised isotonic saline). Systemic hydration via restoration of P_{osm} may therefore play a regulatory role in the maintenance of small airway patency.

5.5.1 Comparison to previous research

5.5.1.1 *Effects of dehydration*

This study shows that mild systemic dehydration, induced by both exercise and fluid restriction, leads to a reduction in FVC (~150 mL) and elevations in RV (~220 mL) and FRC (~130 mL) in healthy adults. These findings are in line with previous work that demonstrated negative alterations in FVC, RV and FRC in athletes with mild asthma following 2 h of exercise in the heat (Simpson et al., 2017). The present study was able to replicate the findings from Simpson et al., (2017) in a healthy population, which suggests that dehydration-induced pulmonary alteration is a general phenomenon that is present irrespective of the presence of pulmonary or airway disease. The reduced severity of the pulmonary function alterations [mean reduction in FVC following exercise ~170 mL *vs* ~300 mL in asthmatic individuals (Simpson et al., 2017)], with only 30% of healthy participants in the present study reaching the 'clinical threshold' of 200 mL (Pellegrino et al., 2005) suggest that, whilst still affected, the airways of healthy individuals have a higher tolerance to systemic dehydration in comparison to individuals with pre-existing lung conditions.

The spirometry results in the present study are in contrast to those previously obtained in healthy individuals (Govindaraj, 1972; Javaheri et al., 1987). Govindaraj (1972) reported

that mild dehydration (2.0 ± 0.9% loss of body mass) induced by 16 h of fluid restriction caused negligible changes in FVC, but was associated with a significant reduction in FEV_1 (~180 mL). While the differences observed in FEV_1 are difficult to explain, the absence of a detectable change in FVC may be explained by the fact that in the study by Govindaraj (1972) only five out of the twenty participants lost >2% body mass, whereas all participants in the present study reached this threshold. According to Cheuvront & Kenefick (2014), a day-to-day change in body mass >2% provides 95% probabilistic certainty that dehydration has occurred. A further study conducted by Javaheri *et al.* (1987) showed improvements in pulmonary function (incl. FEV_1, FEV_1/FVC, and flow rates at all lung volumes) following moderate dehydration (i.e., 4.0 to 4.5% loss of body mass) induced by diuretics in a small sample (n = 6) of healthy men. The use of chlorthalidone could have contributed to the divergent findings, as diuretics cause iso-osmotic hypovolemia, when exercise and fluid restriction lead to hyper-osmotic hypovolemia (Cheuvront et al., 2013).

The comparison of two modes of dehydration (i.e., exercise *vs* fluid restriction) is a novel aspect of the present research that supports the idea that hyper-osmotic hypovolemia can compromise pulmonary function in humans. Indeed, with matched changes in body mass loss and in P_{osm}, comparable alterations in pulmonary function (i.e., reduced FVC, and elevated RV and FRC) were noted across trials. As the magnitude of changes in lung volumes were not different when the dehydration rate was fast (i.e., 2 h exercise in the heat) and slow (i.e., 28 h fluid restriction), it is inferred that the rate of dehydration is unlikely the key determinant of dehydration-induced pulmonary alterations.

5.5.1.2 *Effects of rehydration*

A further novel aspect of the current study is the fact that systemic rehydration was effective at restoring selected lung volumes and capacities (i.e., FVC, RV and FRC). The positive effect of oral fluid intake on FVC was noted after only 15 min, which suggests a rapid reversal of the pulmonary alterations. Previously, dehydration-induced alterations in FVC, RV, and FRC were not restored following 40 min *ad libitum* water intake in individuals with asthma (Simpson et al., 2017). The use of a matched-volume rehydration strategy [with 100% of fluid replaced *vs* 61 ± 19% in previous work (Simpson et al., 2017)], together with administration of a hypertonic solution known to improve fluid retention (Evans et al., 2017), enabled body mass to return close to baseline within an hour. In contrast, following nebulised isotonic saline rehydration, body mass remained below (-1.7 ± 0.5 kg) and P_{osm}

above (9 ± 4 $mOsm \cdot kg^{-1}$) baseline, and FVC, RV, and FRC were not restored. These findings therefore suggest that oral hypertonic fluid intake, but not nebulised isotonic saline solution, is an effective strategy to reverse dehydration-induced pulmonary alterations.

5.5.2 Interpretation of findings

A decrease in FVC alongside concomitant increases in RV, RV/TLC and FRC is usually indicative of airway closure and air trapping (Macklem et al., 1970). These findings therefore suggest that systemic dehydration may selectively impair small airway function. Similar alterations in spirometry and plethysmography were observed following both modes of dehydration (i.e., exercise and fluid restriction), at a time when P_{osm} was elevated. The reversal of FVC, RV, FRC and RV/TLC under systemic rehydration only (i.e., when P_{osm} was normalized), alongside the significant association between P_{osm} and lung volumes, points toward P_{osm} as a key determinant of the small airway impairment.

Pogson et al. (2008) reported an inverse correlation between increased serum osmolality and decreased FVC and FEV_1 in a large population (>10,000) of patients suffering from chronic obstructive pulmonary disease. The authors suggested a causal relationship, mediated by airway epithelial cells, between increased serum plasma osmolality and reduced pulmonary function. Airway epithelial cells are ‘osmotic transducers’ (Willumsen et al., 1994) that respond to changes in osmolarity of both their extracellular and intracellular environments. Through controlled secretion and/or absorption of salt and water, airway epithelial cells preserve hydration of the airways and maintain water and osmolyte homeostasis in human lungs (Bartoszewski et al., 2017). In the dehydration trials of the present study, it can be postulated that airway epithelia "detected" the increase in P_{osm} in bronchial vasculature, which, in turn, would have influenced water supply to the airways and altered the composition and/or content of the ASL. The common functional implication of perturbations to the ASL is peripheral airway instability and premature airway closure (Macklem et al., 1970), which aligns with the lung volume changes observed in the participants of the present research. During the systemic rehydration trials, the rapid normalization of P_{osm} would have returned ASL to its normal state, thus decreasing surface tension and re-opening the collapsed airways, thereby explaining the rapid restoration of lung volumes to baseline. That previous studies (Maughan and Leiper, 1995; Nose et al., 1988a) have evidenced restorations of plasma volume and P_{osm} within the timeframe used for our rehydration trials (i.e., 60 min) supports the idea that

extracellular hypervolemia following fluid ingestion could rapidly restore small airway patency.

5.5.3 Methodological considerations

The ~2.6% body mass loss of the present study was well matched *i)* with the ~2.3% body mass loss in athletes with mild asthma (Simpson et al., 2017) and *ii)* between the four experimental trials conducted. Whilst the 28 h duration was required to induce the target degree of dehydration (Szinnai et al., 2005), it did not allow the recording of pulmonary function at the same time of day within trials. Based on findings presented in Chapter 4, however, a confounding influence of diurnal variation on the observed alterations in pulmonary function can be excluded, as the direction of changes reported in Chapter 4 are opposite to the changes recorded post-dehydration in the present study (i.e., gain of 85 ± 92 mL for FVC, and reductions of 76 ± 200 mL for RV and of 54 ± 198 mL for FRC between morning and afternoon readings in Chapter 4 compared to reductions in FVC of 152 ± 143 mL and elevations in RV and FRC of 216 ± 177 mL and 130 ± 144 mL, respectively).

To account for the possible effect of evaporative water loss – through pulmonary ventilation – on ASL osmolarity (Boucher, 1999), airway water losses were estimated during the dehydration trials. Airway water loss was greater during fluid restriction (~340 mL) compared to exercise (~80 mL) and accounted for 23% of total body water loss during the 28 h fluid restriction period *vs* only 5% during the 2 h exercise. As non-significant differences in pulmonary alterations were noted between the two modes of dehydration, it seems unlikely that evaporative water loss contributed significantly to the observed changes.

In the absence of a 'gold standard' method for assessment of small airway function (Konstantinos Katsoulis et al., 2016), a combination of highly reproducible functional tests were implemented (i.e., spirometry and whole body plethysmography which displayed very good intra-day reproducibility and excellent inter-day repeatability as reported in Chapter 4). Alongside these functional tests, imaging techniques such as high-resolution computed tomography or magnetic resonance imaging could have helped to quantify small airway dysfunctions (Konstantinos Katsoulis et al., 2016). Although an ultrasonic nebuliser was used to ensure the highest flow rate and even distribution of water vapor delivered to the

airways, the rate of delivery ranged from 1.0 to 1.8 mL·min^{-1}. This is lower than expected [e.g., (Katial et al., 2000)] and may have influenced the ability of the implemented local rehydration method to restore lung volumes and capacities. Further, the nebulised isotonic solution may also have become hypotonic upon delivery (as the solution was only isotonic when delivered in a euhydrated state). In individuals with asthma, both hyper- and hypo-osmotic nebulised saline can compromise pulmonary function (Schoeffel et al., 1981). It therefore cannot be excluded that the local rehydration strategy of the present study modified ASL ion concentration, ultimately preventing restoration of pulmonary function.

Finally, it is possible that oral fluid consumption might have led to psychological benefits and, thereby, contributed to improved effort during volitional respiratory manoeuvres. Cognitive task performance and mood have indeed been shown to improve following rehydration with oral fluid in healthy men (Zhang et al., 2019). However, PEF, an effort dependent variable, did not significantly change at any time in the present study. It can therefore be confidently stated that the effort produced by the participants remained consistent throughout the trials and that any psychological effects on the research outcomes were likely minimal.

5.5.4 Clinical and functional significance

These findings have potential relevance to both healthy and clinical populations. In particular, endurance athletes are at increased risk for exercise-induced dehydration and commonly report respiratory symptoms (including breathlessness and cough) whilst exercising (Couto and Moreira, 2016). Older adults, especially those with chronic obstructive pulmonary disease, often experience exertional breathlessness (O'Donnell et al., 2014) and are particularly prone and vulnerable to dehydration (Allison and Lobo, 2004). Further work is now needed to determine the impact of the reported alterations on susceptibility to respiratory symptoms, and to understand the risk of pulmonary function deterioration in dehydrated states. Whether dehydration, by increasing gas trapping, triggers or exacerbates dynamic lung hyperinflation and, thereby, promotes breathlessness during physically demanding tasks, remains to be determined.

5.5.5 Conclusion

Mild systemic dehydration was associated with a reduction in pulmonary function, primarily localised to the small airways. These changes occurred in healthy young adults

after both acute exercise in the heat and following prolonged periods of fluid deprivation. Oral fluid ingestion, but not nebulised isotonic saline, quickly and successfully reversed these alterations in pulmonary function. Therefore, oral rehydration appears to be the most effective strategy for reversing dehydration-induced pulmonary alterations. More work is now needed to understand whether a greater severity of dehydration might lead to clinically significant changes in pulmonary function in healthy adults, and to determine the functional implications of the observed changes.

Chapter 6 Effect of moderate dehydration on resting pulmonary function and ventilatory responses to exercise in physically active adults

6.1 Abstract

Mild dehydration in healthy adults can lead to negative alterations in resting pulmonary function. However, it is not yet known whether these alterations are exacerbated at higher severities of dehydration, and whether these alterations may impact the ventilatory response to exercise. The aims of this study were *i)* to investigate the impact of moderate dehydration upon resting pulmonary function of healthy physically active individuals, and *ii)* to establish the functional implications of dehydration-induced airway dysfunction upon ventilatory and perceptual responses to exercise in the same population. Eleven healthy individuals performed an incremental exercise test before and after 2 h of exercise in the heat either with (euhydration) or without (dehydration) fluid replacement. Pulmonary function was assessed via spirometry, whole body plethysmography, and impulse oscillometry. Moderate dehydration (-3.5 ± 0.4% body mass; $p < 0.001$) was associated with significant elevations in P_{osm} (13 ± 5 mOsm·kg^{-1}). Once dehydrated, participants showed reduction in resting FVC (-210 ± 270 mL, $p = 0.029$) and elevations in RV and FRC (401 ± 348 mL; $p = 0.003$ and 255 ± 183 mL; $p = 0.001$, respectively). Ventilatory parameters (i.e., $\dot{V}_E$, and V_T f_b), operating lung volumes (i.e. IC, EELV and EILV), and perceptual responses (respiratory discomfort) recorded during exercise were however not significantly impacted by fluid loss. This study demonstrated that significant alterations in resting pulmonary function are present in a state of moderate dehydration; however these changes do not seem to translate into significant modifications in the ventilatory response to exercise, at least not in healthy individuals.

6.2 Introduction

In Chapter 5, young adults with no pre-existing respiratory condition displayed negative pulmonary responses to mild dehydration (as demonstrated by a reduction in FVC and

elevations in RV and FRC). This study extended previous work in individuals with asthma, showing negative alterations in small airway function following mild dehydration (Simpson et al., 2017). Findings presented in Chapter 5 therefore confirmed that dehydration-induced pulmonary alteration is a reproducible phenomenon. Further, it was established that this phenomenon exists following various dehydration methods, including prolonged fluid restriction and exercise in the heat. Whilst mild dehydration can commonly occur in trained and untrained populations, athletes (Sawka et al., 2007) and individuals undertaking physical activity in hot environments (Hendrie et al., 1997; Wagoner et al., 2020) are susceptible to greater magnitudes/severities of body water loss.

Overall understanding of the effect of moderate dehydration [i.e., a reduction in body mass of 3 to 5% (Cheuvront and Kenefick, 2014)] on the pulmonary system is currently limited. Only one study to-date (Javaheri et al., 1987) has investigated the impact of moderate dehydration (4.0-4.5% body mass loss via diuretics) on resting pulmonary function. Contrary to the mild dehydration studies (Govindaraj, 1972; Simpson et al., 2017), improvements in expiratory flow rates (FEV_1 and PEF) and in maximal voluntary ventilation were observed in six healthy male participants following diuretic (i.e. 200 mg chlorthalidone) administration. However, this contradictory result may be attributable to the different modes of dehydration method employed; the hypotonic-hypovolemia (induced by Javaheri et al., (1987) would have led to extracellular dehydration, whereas hypertonic-hypovolemia following exercise in the heat (employed in Chapter 5) directly alters P_{osm}.

Whilst negative alterations in pulmonary function have been previously observed with exercise and fluid restriction (i.e. hypertonic-hypovolemia) in healthy [Chapter 5; (Govindaraj, 1972)] and asthmatic individuals (Simpson et al., 2017), the magnitude of the reported alterations, though statistically significant, have been relatively small, ranging from: ~150–300 mL for FVC, ~220–260 mL for RV and ~130–260 mL for FRC. It is currently unknown whether *i)* a more severe state of dehydration leads to more pronounced pulmonary alterations, and *ii)* dehydration-induced negative changes on the pulmonary system observed at rest have functional implications during exercise; if present, it is likely that any functional implications would be most prominent when ventilatory demand is high, such as during maximal exercise.

Endurance-trained athletes are known to develop very high ventilatory rates during exercise, with peak $\dot{V}_E$ >200 L·min^{-1} reported in elite groups (Wells and Norris, 2009). Endurance athletes are also prone to respiratory symptoms, such as breathlessness and breathing discomfort (Smoliga et al., 2016) during exercise. Further, several reports have highlighted that dynamic hyperinflation, characterised by an elevation in EELV above resting levels and premature termination of expiration, is experienced by some endurance athletes during exercise (Guenette et al., 2007; Johnson et al., 1992; Taylor et al., 2013). Air trapping at the level of the small airways is thought to contribute to the onset of dynamic hyperinflation (O'Donnell and Laveneziana, 2006). The small airway dysfunctions observed during hypertonic-hypovolemia [Chapter 5; Simpson et al., (2017)] may therefore contribute to the onset of dynamic hyperinflation in athletes. As dynamic hyperinflation has been shown to strongly correlate with breathlessness (O'Donnell, 1994), dehydration may also lead to an increased sensation of respiratory discomfort.

The aims of this study were: first, to investigate the impact of moderate dehydration on resting pulmonary function in physically-active individuals; and second, to establish the functional implications of dehydration-induced airway dysfunctions on ventilatory and perceptual responses to exercise. In line with Chapter 5, it was hypothesised that moderate dehydration would lead to deleterious alterations in resting pulmonary function, characterised by a reduction in FVC and elevations in RV and FRC. Further, it was hypothesised that dehydration would increase EELV and breathing discomfort toward the end of an incremental test to exhaustion.

6.3 Method

6.3.1 Ethical approval

All protocols and procedures were approved by the Brunel University London Research Ethics Committee (11730-TISS-Jun/2018-13047-1) and conformed to the guidelines of the Declaration of Helsinki, 7th version (World Medical Association, 2013).

6.3.2 Participants

Eighteen participants (six female) were initially recruited to take part in this study. Seven participants however failed to complete all three experimental visits and were subsequently excluded from the analysis (two participants were unable to complete the 2 h exercise bout

in the heat; two participants did not meet the required dehydration threshold of >3% body mass loss; one participant was excluded based on performance in the incremental test during the familiarisation visit; and two further participants self-withdrew from the study after the familiarisation visit). All participants were apparently healthy, non-smokers, with no history of respiratory illness (in particular, no asthma or EIB). Participants regularly took part in cycling/triathlon endurance training (training a minimum of three times *per* week). All participants provided written informed consent prior to taking part and adhered to the pre-trial control measures described in Chapter 3.4.1.

6.3.3 Experimental design

A repeated-measures crossover design was employed (Figure 6.1). Eligible participants were required to attend the Centre for Human Performance, Exercise and Rehabilitation at Brunel University London on three separate occasions. The first visit was used to determine eligibility for the study, to introduce participants to the experimental set up, and to familiarise participants with the experimental protocols and techniques. Visits 2 and 3 consisted of the experimental trials (i.e., dehydration and euhydration trial, in a randomised order). For each visit to the laboratory, participants arrived in a euhydrated state (as quantified by urine osmolality – see Chapter 3.7.2).

6.3.3.1 *Familiarisation visit*

Basic anthropometrics, hydration status and pulmonary function (IOS, spirometry, and whole body plethysmography) were assessed upon arrival to the laboratory. Following recording of baseline measurements in temperate laboratory conditions (20 ± 2°C, 46 ± 8% RH), participants performed an incremental cardiopulmonary exercise test (CPET) to exhaustion. Following completion of the CPET, pulmonary function was assessed (spirometry only), and participants then entered an environmental chamber (Procema Ltd., Twickenham, UK) to perform 2 h of cycling in hot conditions (36°C, 50% RH) at 40% maximum power output (PO_{max}) with no fluid intake. Following completion of the 2 h exercise bout in the heat, participants exited the environmental chamber and recorded nude body mass to calculate sweat loss. The familiarisation visit concluded following this measurement.

6.3.3.2 *Experimental visits*

The experimental visits commenced at the same time of day for all participants (between 08:00 h – 10:00 h), with a minimum of 48 h and maximum of 14 days between visits. Participants followed an identical protocol to the familiarisation visit; however, following completion of the 2 h exercise in the heat and post-exercise nude body mass assessment, participants then performed spirometry followed by a second incremental CPET (CPET-2) to exhaustion [in temperate conditions and following the exact same procedures as during the first CPET (CPET-1)]. A final hydration and pulmonary function assessment (including the full series of tests, as performed at baseline) was then completed to conclude the trial. Participants performed one experimental visit with (euhydration), and one without (dehydration) fluid replacement during the 2 h exercise bout in the heat. The dehydration trial involved 2 h of cycling exercise in the heat (36°C and 50% RH) with fluid restriction. In the euhydration trial, participants followed an identical protocol, but were provided with fluid throughout the exercise. Fluid intake mass was calculated as 3.5% of the participants body mass (i.e., the target fluid loss for the dehydration trials), and was provided in four equal boluses to be consumed within 30 min (see Chapter 3.7.3.3 for details). Water was mixed with 80 $g \cdot L^{-1}$ electrolyte powder (SiS GO Electrolyte Powder, Science in Sport®, UK) to maintain fluid balance (Evans et al., 2017).

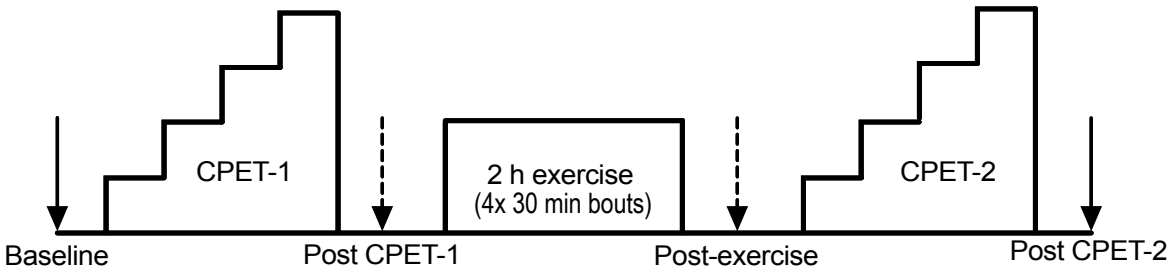

Figure 6.1. Schematic representation of the experimental trials (visits 2 and 3). The 2 h exercise bout was broken down in four 30 min blocks of cycling, interspersed with 5 min of rest, and took place in hot conditions (36°C, 50% relative humidity) at 40% maximal power output. Solid arrows represent full pulmonary function and hydration assessment; dashed arrows represent spirometry and body mass only. CPET: cardiopulmonary exercise test.

6.3.4 Hydration status

Hydration status was assessed using changes in nude body mass and measures of P_{osm} and U_{osm}. A U_{osm} of < 700 mOsm·kg^{-1} was used as inclusion criteria for each visit (Sawka et al., 2007). Details of techniques are provided in Chapter 3.7.

6.3.5 Pulmonary function

Pulmonary function was assessed via IOS, spirometry, and whole body plethysmography (in this order) upon arrival and at the end of each experimental visit. Additional spirometry measurements were performed immediately before and after 2 h of exercise in the heat (Figure 6.1). All tests were performed in line with the ATS/ERS guidelines (Miller et al., 2005b; Wanger et al., 2005), as described in Chapter 3.8.

6.3.6 Cardiopulmonary exercise test (CPET)

Participants cycled on an electronically braked cycle ergometer (Lode Excalibur, Lode, B.V, Groningen, The Netherlands) to establish $PO_{max,}$ $\dot{V}O_{2peak,}$ the ventilatory response to exercise and operating lung volumes during exercise. Participants cycled at 50 W for 3 min prior to each incremental test to ensure a standardised warm up was performed; this was followed by increments of 50 W every 2 min. To elicit similarity in test duration between sexes, males began the first test stage at 100 W, whilst females began at 50 W. Cadence was maintained between 70 and 90 rpm. Measurements of $\dot{V}_E$, $\dot{V}_T$, $\dot{V}O_2$, $\dot{V}CO_2$, f_b, T_i and T_{tot} were recorded continuously via breath-by-breath analysis (Vyntus CPX, Carefusion, Hoechberg, Germany) and reported at the end of each 2 min stage. Recordings of HR via telemetry (Polar, FS1, Birmingham, UK), T_{core} via rectal temperature (RET-1 Rectal probe, Physitemp, New Jersey, USA), and SpO_2 via earlobe pulse oximetry (Xpod® 3012LP External OEM Pulse Oximeter, Nonin Medical, Plymouth, USA) were taken at the end of each 2 min stage, with ventilatory parameters averaged over 20 s. Participants were asked to rate their RPE and breathing discomfort using a modified Borg scale (Borg, 1982) at the end of each 2 min stage (see Chapter 3.10). Participants performed an IC manoeuvre at the end of each 2 min stage to allow for assessment of operating lung volumes (described in detail below). The test was terminated when cadence dropped below 60 rpm for >5 seconds and HR reached within 10 beats·min^{-1} of maximum and/or RER was >1.15. Participants were encouraged throughout the test by the researcher (see Chapter 3.5.4 for a detailed description of the criteria for CPET termination).

6.3.7 Operating lung volumes

Operating lung volumes were assessed using duplicate IC manoeuvres performed at rest, at the end of each 2 min stage, and immediately before termination of exercise (at exhaustion). The manoeuvre involved the participant taking a complete maximal inspiratory breath at the end of a normal expiration, and then returning to normal breathing (Guenette et al., 2013). For a detailed description of the manoeuvre, see Chapter 3.6.3. Verbal encouragement was given to ensure a maximal inspiratory effort was performed. Participants were provided with clear instructions regarding the manoeuvre prior to each test, and performed practice manoeuvres at rest and during the warm up to ensure full understanding and compliance with the technique. Following completion of the incremental exercise test, IC manoeuvres were analysed by the experimenter and dynamic changes in operating lung volumes (i.e., EELV and EILV) were recorded.

6.3.8 Exercise in the heat

The exercise involved four bouts of 30 min cycling, interspersed with 5 min passive rest. The workload for the cycling bout was set at 40% of the PO_{max} achieved in CPET-1 based on pilot work performed to determine the workload required to reach moderate dehydration within 2 h of exercise. Participants maintained a cadence of 70-90 rpm throughout the exercise protocol. At 15 min intervals, ventilatory parameters ($\dot{V}_E$, V_T, $\dot{V}O_2$, $\dot{V}CO_2$, f_b, T_i, and T_{tot}) were recorded for 5 min using online breath-by-breath analysis (described in Chapter 3.6.2). HR, T_{core}, breathing discomfort and RPE were also recorded at 15 min intervals during exercise (details of measurement techniques are provided in Chapter 3.9 and 3.10).

6.3.9 Statistical power

Power analyses were conducted using G*Power Software (see Chapter 3.11.1 for detail). Sample size was based on previous work that investigated the impact of exercise-induced dehydration upon pulmonary function in healthy adults (Chapter 5) and individuals with asthma (Simpson et al., 2017). It was predicted that the reduction in FVC following moderate dehydration would be more severe compared to the mild dehydration previously induced (Chapter 5). To detect a 200 ml difference in FVC between pre- and post-

dehydration, it was calculated that a sample size of ten participants would give approximately 80% power, for an alpha level at 5%.

6.3.10 Statistical analysis

All data were analysed using SPSS Statistical Software (Version 26, SPSS, Chicago, IL). Data were checked for normality using the Shapiro-Wilk test and are displayed as mean ± SD, unless otherwise stated. A two-way repeated measures ANOVA was used to assess differences in resting pulmonary function parameters (incl. IOS, spirometry and whole body plethysmography parameters) and hydration status (body mass, $P_{osm,}$ U_{osm}) between conditions (euhydration *vs* dehydration) and time (pre *vs* post-2h of cycling in the heat), and to compare peak power output between tests (CPET-1 *vs* CPET-2) and conditions. For data collected during CPET, three-way repeated measures ANOVA was used to assess differences in ventilatory ($\dot{V}_E$, V_T, $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$, f_b, T_i, $T_{tot,}$ and T_i/T_{tot}), physiological ($\dot{V}O_2$, $\dot{V}CO_2$, HR, T_{core}, and SpO_2) and perceptual parameters (breathing discomfort and RPE) between experimental conditions (euhydration *vs* dehydration), tests (CPET-1 *vs* CPET-2) and times (0 , 2 , 4 , 6 min, and at peak exercise). Five time points were analysed as the duration of test varied between participants. For data collected during the 2 h exercise bout, a two-way repeated measures ANOVA was performed to assess changes in physiological and perceptual responses over time (rest, 30, 60, 90, and 120 min) and between conditions (dehydration *vs* euhydration). For all ANOVA, post hoc Bonferroni's adjusted pairwise comparisons were used where significant main effects occurred. Statistical significance was set at $p < 0.05$.

6.4 Results

6.4.1 Participants

Eleven participants (four female), aged 33 ± 12 years, with a body mass of 73.5 ± 10.8 kg, and a stature of 173 ± 8 cm completed the study. All participants were physically active ($\dot{V}O_{2peak}$: 47.7 ± 6.9 ml·kg·min^{-1}, peak power output: 291 ± 102 W, peak $\dot{V}_E$: 112 ± 25 L·min^{-1}, HR_{max}: 164 ± 15 b·min^{-1}). Baseline spirometry data were within normal range for all participants (group average of 105 ± 9% predicted for FEV_1, 103 ± 8% predicted for FVC, and FEV_1/FVC of 84 ± 5%).

6.4.2 Hydration status

Hydration status data are shown in Table 6.1. There was no difference in body mass at baseline between conditions. Moderate dehydration was successfully induced via exercise, with a reduction in body mass of 3.5 ± 0.4% in the dehydration condition compared to 0.3 ± 0.5% in the euhydration condition (main effect of time: $F_{1,10} = 185.60$, $p < 0.001$; main effect of condition: $F_{1,10} = 159.30$, $p = 0.003$; interaction effect: $F_{1,10} = 149.50$, $p < 0.001$). There was an increase in P_{osm} of 13 ± 5 mOsm·kg^{-1} from baseline to post CPET-2 in the dehydration condition ($p < 0.001$), whereas no change (0 ± 5 mOsm·kg^{-1}) occurred in the euhydration condition (main effect of time: $F_{1,10} = 29.16$, $p < 0.001$; main effect of condition: $F_{1,10} = 62.05$, $p < 0.001$; interaction effect: $F_{1,10} = 40.24$, $p < 0.001$). Urine osmolality increased from baseline to post CPET-2 in both the dehydration ($p = 0.003$) and the euhydration ($p = 0.045$) conditions, with no difference between experimental conditions (main effect for time: $F_{1,10} = 13.55$, $p = 0.004$). Haemoglobin mass increased ($p = 0.009$), and plasma volume decreased ($p = 0.002$), from baseline to post CPET-2 in the dehydration condition, with no change noted for either parameter in the euhydration condition. There was no effect of time or hydration status upon haematocrit.

Table 6.1. Markers of hydration status before (baseline) and after (Post CPET-2) 2 h of exercise in the heat with (euhydration) and without (dehydration) fluid intake in eleven healthy physically-active individuals (four females).

	Dehydration		**Euhydration**	
	Baseline	*Post CPET-2*	*Baseline*	*Post CPET-2*
Body mass (kg)	72.1 ± 9.8	69.6 ± 9.3 [a #]	71.9 ± 9.7	71.6 ± 9.6 [a]
P_{osm} (mOsm·kg^{-1})	291 ± 4	304 ± 4 [a #]	293 ± 2	292 ± 4
U_{osm} (mOsm·kg^{-1})	271 ± 193	566 ± 225 [a]	256 ± 152	409 ± 195 [a]
Haemoglobin (g·L^{-1})	138 ± 9	144 ± 10 [a]	141 ± 10	139 ± 12
Haematocrit (%)	43 ± 3	44 ± 3	43 ± 4	44 ± 3
Plasma volume (%)	57 ± 3	54 ± 4 [a]	57 ± 4	57 ± 4

Data are mean ± SD; P_{osm}, plasma osmolality; U_{osm}, urine osmolality; [a] $p < 0.05$ *vs* baseline, [#] $p < 0.05$ *vs* euhydration at corresponding time point.

6.4.3 Ventilatory, physiological and perceptual responses to exercise in the heat

Table 6.2 displays the ventilatory, physiological and perceptual responses at rest, 30, 60, 90 and 120 min of exercise in the heat. At the start of exercise, there was no difference between conditions for any recorded variables.

6.4.3.1 *Ventilatory response*

During the 2 h bout of exercise in the heat, ventilatory variables as well as $\dot{V}O_2$, and $\dot{V}CO_2$ displayed an increase over the first 30 min, and then remained stable (main effects of time: $\dot{V}_E$: $F_{4, 32} = 188.58$; V_T: $F_{4, 40} = 26.18$; f_b: $F_{4, 40} = 25.66$; $\dot{V}O_2$: $F_{4, 40} = 125.85$; $\dot{V}CO_2$: $F_{4, 40}$: 121.50; all $p < 0.001$). Conversely, T_i and $T_{tot,}$ decreased from rest to 30 min (main effects of time: T_i: $F_{4, 40} = 20.79$; T_{tot}: $F_{4,40} = 38.56$; both $p < 0.001$) and remained stable thereafter No difference was noted between the dehydration and euhydration conditions.

6.4.3.2 *Other physiological responses*

There was no difference in HR or T_{core} between experimental conditions at rest. From rest to 30 min, HR increased in both conditions (main effect of time: $F_{4, 40} = 91.37$; $p < 0.001$); and from 30 min onward, HR was higher in the dehydration compared to the euhydration

condition (interaction effect condition x time: $F_{4, 40} = 12.86$; $p < 0.03$). In the dehydration condition, HR showed a continuous increase from 30 min to 120 min (all $p < 0.001$). In the euhydration condition, HR remained stable from 30 to 90 min, but was elevated at 120 min ($p = 0.031$ *vs* 30 min). For $T_{core,}$ a gradual increase occurred in the dehydration condition over the entire duration of the exercise test (main effect of time: $F_{4, 40} = 24.00$; $p < 0.005$). In the euhydration condition, T_{core} was elevated at all time points compared to rest (interaction effect condition x time: $F_{4, 40} = 4.28$; $p < 0.01$), but remained stable from 30 min to 60 min and from 60 min to 90 min. T_{core} was higher in dehydration *vs* euhydration at 60 min ($p = 0.034$) and 90 min ($p = 0.034$), but not at 120 min ($p = 0.054$).

6.4.3.3 *Perceptual responses*

Breathing discomfort was increased at all time points during exercise in comparison to rest (main effect of time: $F_{4, 40} = 25.78$; $p < 0.01$). In both conditions, breathing discomfort increased from rest to 60 min (both $p < 0.01$), and remained stable thereafter. A steady increase in RPE was shown in both conditions from rest to 90 min (main effect of time: $F_{4, 40} = 181.94$; $p < 0.02$; interaction effect condition x time: $F_{4.40} = 4.28$, $p = 0.005$). RPE then continued to rise in the dehydration condition ($p = 0.014$, 90 min *vs* 120 min), while it remained stable in the euhydration condition ($p = 0.341$ from 90 min to 120 min). In the dehydration condition, RPE was higher than euhydration from 60 min onward ($p < 0.05$ *vs* euhydration). On average, breathing discomfort was 0.5 higher in dehydration compared to euhydration ($p = 0.005$).

Table 6.2. Ventilatory, physiological and perceptual responses to 2 h exercise in the heat (cycling at 40% PO_{max}), with (euhydration) or without (dehydration) fluid replacement in eleven healthy physically-active individuals (four females).

		2 h cycling exercise (36°C, 50% RH)				
		Rest	**30 min**	**60 min**	**90 min**	**120 min**
$\dot{V}_E$ **(L)**	*Euhydration*	14 ± 5 ^{bcde}	47 ± 8 ^{a}	46 ± 5 ^{a}	47 ± 7 ^{a}	46 ± 8 ^{a}
	Dehydration	17 ± 8 ^{bcde}	48 ± 7 ^{ad}	49 ± 8 ^{a}	51 ± 10 $^{ab\#}$	52 ± 11 ^{a}
V_T **(L·min^{-1})**	*Euhydration*	0.96 ± 0.52 ^{bcde}	1.64 ± 0.62 ^{a}	1.46 ± 0.35 ^{a}	1.58 ± 0.42 ^{a}	1.53 ± 0.37 ^{a}
	Dehydration	0.88 ± 0.44 ^{bcde}	1.63 ± 0.42 ^{a}	1.55 ± 0.55 ^{a}	1.65 ± 0.59 ^{a}	1.82 ± 0.71 ^{a}
f_b **(breaths·min^{-1})**	*Euhydration*	17 ± 4 ^{bcde}	28 ± 7 ^{a}	30 ± 8 ^{a}	30 ± 7 ^{a}	29 ± 9 ^{a}
	Dehydration	18 ± 4 ^{bcde}	28 ± 5 ^{a}	30 ± 7 ^{a}	31 ± 8 ^{a}	33 ± 11 ^{a}
$\dot{V}O_2$ **(L·min^{-1})**	*Euhydration*	0.40 ± 0.09 ^{bcde}	1.96 ± 0.27 ^{ae}	2.01 ± 0.41 ^{a}	2.16 ± 0.36 ^{a}	2.23 ± 0.32 ^{ab}
	Dehydration	0.60 ± 0.53 ^{bcde}	2.10 ± 0.36 ^{ae}	2.23 ± 035 ^{a}	2.15 ± 0.47 ^{a}	2.10 ± 0.33 ^{ab}
$\dot{V}CO_2$ **(L·min^{-1})**	*Euhydration*	0.34 ± 0.10 ^{bcde}	1.53 ± 0.23 ^{a}	1.51 ± 0.28 ^{a}	1.58 ± 0.21 ^{a}	1.60 ± 0.18 ^{a}
	Dehydration	0.51 ± 0.44 ^{bcde}	1.62 ± 0.26 ^{a}	1.68 ± 0.27 ^{a}	1.61 ± 0.38 ^{a}	1.57 ± 0.23 ^{a}
T_i **(s)**	*Euhydration*	1.56 ± 0.55 ^{bcde}	1.05 ± 0.31 ^{a}	0.93 ± 0.42 ^{a}	0.92 ± 0.28 ^{a}	0.91 ± 0.25 ^{a}
	Dehydration	1.54 ± 0.38 ^{bcde}	1.01 ± 0.25 ^{a}	1.01 ± 0.23 ^{a}	1.14 ± 0.33 ^{a}	1.10 ± 0.38 ^{a}
T_{tot} **(s)**	*Euhydration*	3.45 ± 0.84 ^{bcde}	2.33 ± 0.77 ^{a}	2.31 ± 0.65 ^{a}	2.11 ± 0.59 ^{a}	2.05 ± 0.64 ^{a}
	Dehydration	3.70 ± 0.40 ^{bcde}	2.38 ± 0.55 ^{a}	2.32 ± 0.50 ^{a}	2.52 ± 0.61 ^{a}	2.34 ± 0.69 ^{a}
T_i/T_{tot}	*Euhydration*	0.46 ± 0.08	0.46 ± 0.05	0.40 ± 0.13	0.44 ± 0.04	0.45 ± 0.03
	Dehydration	0.42 ± 0.08	0.43 ± 0.08	0.44 ± 0.05	0.46 ± 0.07	0.47 ± 0.06

Cardiac and thermoregulatory responses						
HR	*Euhydration*	88 ± 20^{bcde}	123 ± 22^{ae}	125 ± 22^{ade}	128 ± 22^{ac}	130 ± 22^{abc}
(beats·min^{-1})	*Dehydration*	82 ± 12^{bcde}	$129 \pm 22^{acde\#}$	$136 \pm 22^{ae\#}$	$139 \pm 23^{ae\#}$	$143 \pm 23^{abcd\#}$
T_{core} (°C)	*Euhydration*	37.2 ± 0.4^{bcde}	37.6 ± 0.3^{ade}	37.8 ± 0.4^{ae}	37.8 ± 0.4^{ab}	37.9 ± 0.6^{abc}
	Dehydration	37.3 ± 0.4^{bcde}	37.6 ± 0.2^{acde}	$38.0 \pm 0.3^{abde\#}$	$38.2 \pm 0.5^{abce\#}$	38.4 ± 0.5^{abcd}
Perceptual responses						
RPE	*Euhydration*	6 ± 0^{bcde}	12 ± 1^{acde}	14 ± 1^{abde}	15 ± 2^{abc}	15 ± 3^{abc}
	Dehydration	6 ± 0^{bcde}	13 ± 2^{acde}	$14 \pm 2^{abde\#}$	$16 \pm 2^{abce\#}$	$17 \pm 2^{abcd\#}$
Breathing	*Euhydration*	0 ± 0^{bcde}	3 ± 2^{ade}	4 ± 3^{a}	4 ± 3^{ab}	5 ± 3^{ab}
discomfort	*Dehydration*	0 ± 0^{bcde}	4 ± 3^{ade}	5 ± 3^{a}	5 ± 3^{ab}	5 ± 3^{ab}

Mean ± SD. $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_b, breathing frequency; $\dot{V}O_2$, volume of oxygen uptake; $\dot{V}CO_2$, volume of expired carbon dioxide; T_i, inspiratory time; T_{tot}, total time of breath; T_i/T_{tot}, inspiratory duty cycle; HR, heart rate; T_{core}, core temperature; RPE, rating of perceived exertion. $^{a}p < 0.05$ *vs* rest, $^{b}p < 0.05$ *vs* 30 min, $^{c}p < 0.05$ *vs* 60 min, $^{d}p < 0.05$ *vs* 90 min, $^{e}p < 0.05$ *vs* 120 min, $^{\#}p < 0.05$ *vs* euhydration.

6.4.4 Pulmonary function

6.4.4.1 *Spirometry*

All spirometry data are presented in Table 6.3. At baseline, spirometry values did not differ between conditions. For FVC, main time and condition effects were noted, as well as a two-way interaction (Table 6.3); i.e. moderate dehydration was associated with a reduction in FVC after: 2 h of exercise in the heat (-210 ± 270 mL, $p = 0.029$) and post CPET-2 (-170 ± 200 mL *vs* baseline; $p = 0.018$). Six out of the eleven (55%) participants experienced a reduction in FVC of >200 mL. There was no change in FVC at any time in the euhydration condition (Figure 6.2). A significant main effect of time only was present for FEV_1 ($p = 0.001$; Table 6.3), whereby an increase occurred from baseline to post CPET-2 in both the dehydration (120 ± 130 mL) and the euhydration conditions (150 ± 120 mL) ($p = 0.017$). As FEV_1 and FVC were both altered, FEV_1/FVC showed a main effect of time (Table 6.3), whereby an increase was shown: post CPET-1, post 2 h of exercise in the heat, and post CPET-2 (all $p < 0.01$ *vs* baseline). A significant main effect for condition, and a significant interaction effect were also shown, whereby FEV_1/FVC was higher in the dehydration condition at post 2 h of exercise in the heat and post CPET-2 *vs* euhydration ($p < 0.002$). PEF remained unchanged throughout the experimental trials.

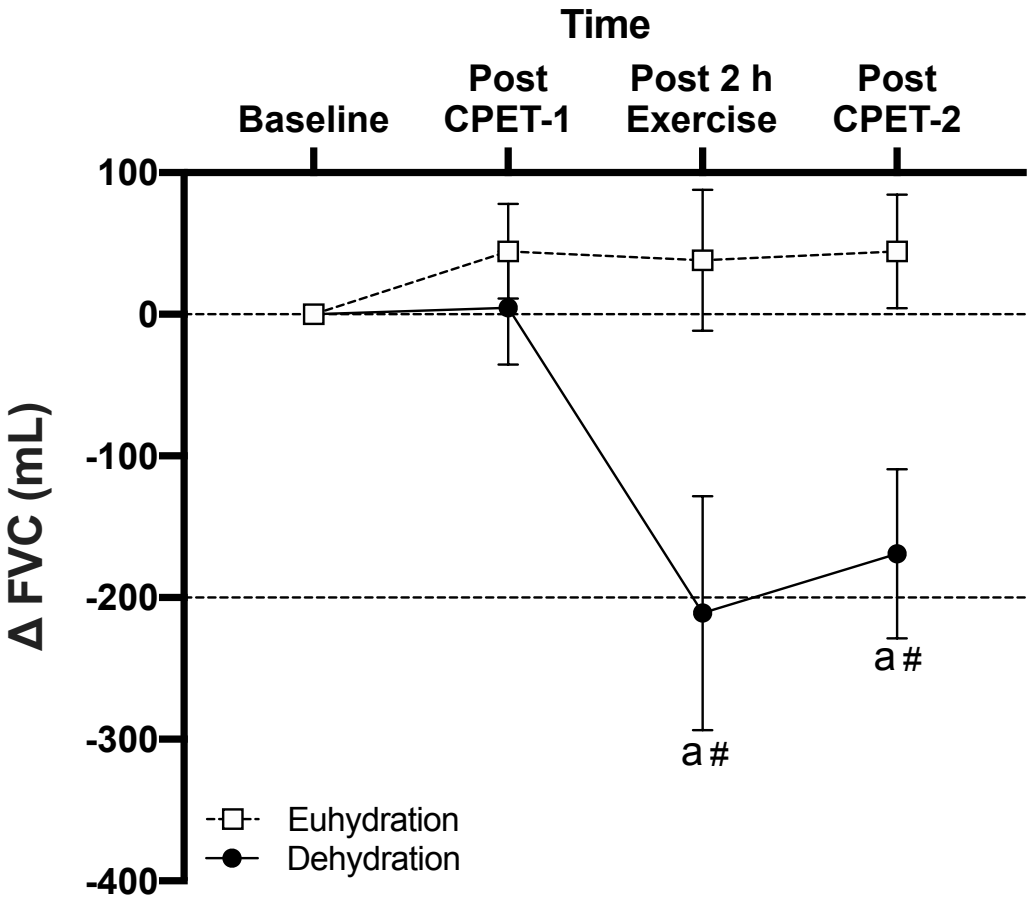

Figure 6.2. Change in forced vital capacity (FVC) from baseline, following the first incremental exercise test (post CPET-1), following 2 h of exercise in the heat (post 2 h exercise), and following the second incremental exercise test (post CPET-2), with (euhydration) or without (dehydration) fluid replacement. Values are mean ± SEM for eleven participants (four females). [a] $p < 0.05$ *vs* baseline, # $p < 0.05$ *vs* euhydration at corresponding time point.

Table 6.3. Spirometry parameters (mean ± SD) at baseline, after the first incremental exercise test (CPET-1), after 2 h exercise in the heat, and after the second incremental exercise test (CPET-2), with (euhydration) or without (dehydration) fluid replacement *(n=11)*.

		Time				ANOVA output		
		Baseline	**Post CPET-1**	**Post 2 h exercise**	**Post CPET-2**		***F***	***p***
FVC	*Euhydration*	4.85 ± 0.77	4.90 ± 0.90	4.89 ± 0.75	4.90 ± 0.72	**Condition**	14.072	0.027
(L)	*Dehydration*	4.85 ± 0.83	4.86 ± 0.79	4.64 ± 0.75 [a #]	4.68 ± 0.77 [a #]	**Time**	3.531	0.004
						Condition x Time	15.873	0.001
FEV_1	*Euhydration*	4.01 ± 0.61	4.13 ± 0.66	4.15 ± 0.52	4.17 ± 0.54 [a]	**Condition**	0.528	0.484
(L)	*Dehydration*	4.02 ± 0.59	4.10 ± 0.61	4.13 ± 0.69	4.13 ± 0.66 [a]	**Time**	6.631	0.001
						Condition x Time	0.408	0.748
FEV_1/FVC	*Euhydration*	83 ± 5	85 ± 6 [a]	85 ± 6 [a]	85 ± 6 [a]	**Condition**	23.673	0.001
(%)	*Dehydration*	83 ± 5	85 ± 6 [a]	89 ± 6 [a #]	88 ± 5 [a #]	**Time**	16.943	0.000
						Condition x Time	16.945	0.000
PEF	*Euhydration*	8.69 ± 1.79	8.96 ± 2.56	9.28 ± 2.76	9.13 ± 2.47	**Condition**	2.562	0.322
($L \cdot s^{-1}$)	*Dehydration*	9.07 ± 2.72	8.68 ± 2.13	9.08 ± 2.75	8.94 ± 2.22	**Time**	1.116	0.079
						Condition x Time	0.214	0.463

FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; PEF, peak expiratory flow. [a] $p < 0.05$ *vs* baseline, [#] $p < 0.05$ *vs* euhydration.

6.4.4.2 *Whole body plethysmography*

All whole body plethysmography data are presented in Table 6.4. At baseline, whole body plethysmography values did not differ between conditions. For RV, there was a main effect of time, and a two-way interaction effect, for FRC there were main effects of time and condition, and a significant two-way interaction effect (Table 6.4). Post-hoc pairwise comparisons showed that moderate dehydration was associated with significant increases in RV (401 ± 348 mL; $p = 0.003$ *vs.* baseline; $p = 0.010$ *vs* euhydration) and FRC (255 ± 183 mL; $p = 0.001$ *vs.* baseline), whilst these variables remained unchanged in the euhydration condition (Figure 6.3). TLC displayed no significant main or interaction effects. As a result, RV/TLC displayed main effects of time and condition, and a two-way interaction effect (Table 6.4).Post-hoc tests demonstrated that RV/TLC increased by 5 ± 4% in the dehydration condition ($p = 0.003$ *vs* baseline) but did not change in the euhydration condition. There were no main effects of time or condition for sRaw (Table 6.4); however, an interaction effect was noted, whereby an increase in sRaw was observed in the dehydration condition (0.11 ± 0.12 $kPa \cdot s^{-1}$; $p = 0.012$ *vs.* baseline), whereas a decrease was noted in the euhydration condition (-0.10 ± 0.14 $kPa \cdot s^{-1}$; $p = 0.033$ *vs* baseline). At the end of the trial (post CPET-2), sRaw was therefore higher in the dehydration in comparison to the euhydration condition ($p = 0.007$). ERV, IC, and IVC remained unchanged throughout the trials (Table 6.4).

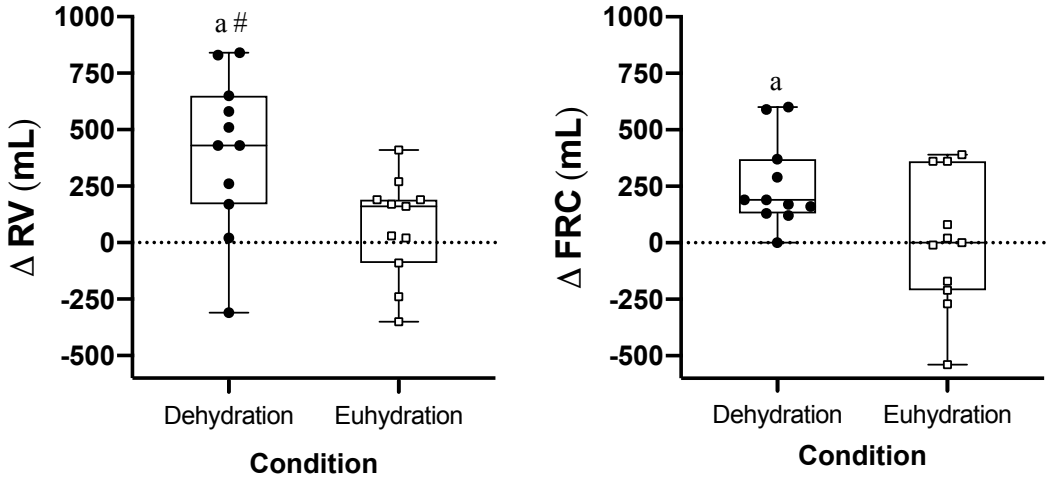

Figure 6.3. Changes in a) residual volume (RV), and b) functional residual capacity (FRC) following the second incremental exercise test (post CPET-2) in the dehydration and euhydration conditions. Boxplots represent the median and interquartile range, with whiskers representing the minimum and maximum values. [a] $p < 0.05$ *vs* baseline; # $p < 0.05$ *vs* euhydration.

Table 6.4. Whole body plethysmography parameters (mean ± SD) at baseline and following the second incremental exercise test (post CPET-2), with (euhydration) or without (dehydration) fluid replacement *(n = 11)*.

		Time		ANOVA output		
		Baseline	**Post CPET-2**		***F***	***p***
TLC (L)	*Euhydration*	6.95 ± 1.31	6.99 ± 0.94	**Condition**	0.318	0.585
	Dehydration	6.95 ± 1.36	7.10 ± 1.51	**Time**	3.723	0.083
				Condition x Time	0.576	0.465
FRC (L)	*Euhydration*	3.93 ± 1.07	3.93 ± 0.87	**Condition**	1.362	0.270
	Dehydration	3.92 ± 1.00	4.17 ± 1.14 [a]	**Time**	5.65	0.039
				Condition x Time	6.09	0.033
RV (L)	*Euhydration*	2.01 ± 0.64	2.08 ± 0.67	**Condition**	4.569	0.058
	Dehydration	2.01 ± 0.69	2.41 ± 0.92 [a #]	**Time**	8.637	0.015
				Condition x Time	19.772	0.001
RV/TLC (%)	*Euhydration*	29 ± 5	29 ± 6	**Condition**	8.94	0.014
	Dehydration	28 ± 5	33 ± 6 [a #]	**Time**	7.112	0.024
				Condition x Time	41.904	<0.001
ERV (L)	*Euhydration*	1.92 ± 0.55	1.85 ± 0.43	**Condition**	0.674	0.431
	Dehydration	1.91 ± 0.44	1.77 ± 0.40	**Time**	2.474	0.147
				Condition x Time	0.689	0.426
IC (L)	*Euhydration*	3.13 ± 0.57	3.14 ± 0.52	**Condition**	0.150	0.222
	Dehydration	3.06 ± 0.54	3.01 ± 0.55	**Time**	0.102	0.756
				Condition x Time	1.694	0.707
IVC (L)	*Euhydration*	4.94 ± 0.79	4.91 ± 0.65	**Condition**	3.846	0.078
	Dehydration	4.95 ± 0.81	4.70 ± 0.72	**Time**	3.658	0.085
				Condition x Time	4.873	0.052
sRaw ($kPa \cdot s^{-1}$)	*Euhydration*	0.97 ± 0.29	0.87 ± 0.23 [a]	**Condition**	1.522	0.246
	Dehydration	0.90 ± 0.19	1.01 ± 0.25 [a #]	**Time**	0.024	0.881
				Condition x Time	12.123	0.006

TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; ERV, expiratory reserve volume; IC, inspiratory capacity; IVC, inspiratory vital capacity; sRaw, specific airway resistance; [a] $p < 0.05$ *vs* baseline, [#] $p < 0.05$ *vs* euhydration at corresponding time point.

6.4.4.3 *Impulse Oscillometry (IOS)*

Data from IOS assessment are presented in Table 6.5. Impulse oscillometry parameters (mean ± SD) at baseline and following the second incremental exercise test (post CPET-2), with (euhydration) or without (dehydration) fluid replacement *(n = 11)*.. No between-conditions difference was reported for IOS values at baseline. Dehydration had no impact on IOS parameters, with no main effects of time or condition, or two-way interactions noted for R_5, R_{20}, R_{5-20}, X_5, A_X and F_{res}.

Table 6.5. Impulse oscillometry parameters (mean ± SD) at baseline and following the second incremental exercise test (post CPET-2), with (euhydration) or without (dehydration) fluid replacement *(n = 11)*.

		Time		**ANOVA output**		
		Baseline	**Post CPET-2**		***F***	***p***
R_5 ($kPa \cdot L^{-1} \cdot s^{-1}$)	*Euhydration*	0.30 ± 0.09	0.29 ± 0.05	**Condition**	0.795	0.521
	Dehydration	0.30 ± 0.09	0.31 ± 0.12	**Time**	0.089	0.772
				Condition x Time	0.433	0.394
R_{20} ($kPa \cdot L^{-1} \cdot s^{-1}$)	*Euhydration*	0.29 ± 0.09	0.28 ± 0.05	**Condition**	1.129	0.313
	Dehydration	0.29 ± 0.10	0.30 ± 0.11	**Time**	0.312	0.588
				Condition x Time	0.643	0.441
R_{5-20} ($kPa \cdot L^{-1} \cdot s^{-1}$)	*Euhydration*	0.00 ± 0.03	0.00 ± 0.02	**Condition**	1.897	0.199
	Dehydration	0.01 ± 0.03	0.01 ± 0.01	**Time**	0.74	0.410
				Condition x Time	0.650	0.439
X_5 ($kPa \cdot L^{-1} \cdot s^{-1}$)	*Euhydration*	-0.08 ± 0.01	-0.08 ± 0.01	**Condition**	0.023	0.882
	Dehydration	-0.08 ± 0.03	-0.08 ± 0.03	**Time**	3.75	0.082
				Condition x Time	2.664	0.134
A_X ($kPa \cdot L^{-1}$)	*Euhydration*	0.18 ± 0.08	0.16 ± 0.06	**Condition**	0.234	0.639
	Dehydration	0.16 ± 0.11	0.16 ± 0.20	**Time**	0.108	0.749
				Condition x Time	0.099	0.759
F_{res} ($kPa \cdot L^{-1} \cdot s^{-1}$)	*Euhydration*	9.65 ± 2.26	9.25 ± 1.85	**Condition**	0.363	0.560
	Dehydration	9.31 ± 1.54	10.02 ± 2.85	**Time**	0.817	0.387
				Condition x Time	1.084	0.322

R_5, respiratory resistance at 5 Hz; R_{20}, respiratory resistance at 20 Hz; X_5, frequency-dependence of resistance respiratory reactance at 5 Hz; A_X area of reactance; F_{res}, resonant frequency; [a] $p < 0.05$ *vs* baseline, [#] $p < 0.05$ *vs* euhydration at corresponding time point.

6.4.5 Cardiopulmonary exercise tests (CPET)

Peak power output during CPET-2 (dehydration: 205 ± 50 W; euhydration 245 ± 50 W) was lower than CPET-1 (dehydration: 285 ± 41 W; euhydration: 270 ± 58 W) in both conditions (dehydration: p = 0.004; euhydration: interaction effect for condition x test x time: $F_{4, 28}$ = 6.24; p = 0.001). Further, during CPET-2, peak power output was lower in dehydration compared to euhydration (interaction effect for condition x test: $F_{4, 28}$ = 6.24, p = 0.041). During CPET-2 in the dehydration condition: two participants completed three stages less than in CPET-1 (one participant experienced muscle cramps); three participants completed two less stages; and three participants completed one less stage than CPET-1 (i.e., 80% of participants completed a minimum of one less stage). In the euhydration condition, only four participants (40%) completed one less stage in CPET-2 compared to CPET-1.

6.4.5.1 *Ventilatory response*

Ventilatory response to CPET is shown in Table 6.6 and Figure 6.4. At submaximal intensities, there was no difference in $\dot{V}_E$ between CPET-1 and CPET-2; however, V_T was lower in CPET-2 at 4 (p = 0.001 vs CPET-1) and 6 min (p = 0.007 vs CPET-1) (significant interaction effect for test x time: $F_{4,32}$ = 14.03, p = 0.014) and f_b was higher at 2, 4, and 6 min (p = 0.018, p = 0.004, and p < 0.001, respectively) compared to CPET-1(interaction effect for test x time: $F_{4,32}$ = 2.78, p = 0.043). At peak exercise, V_T in CPET-2 was higher than in CPET-1, but f_b was not statistically different. Further, dehydration was associated with a reduction in $\dot{V}_E$ at peak exercise of 26 ± 25 L·min^{-1} (interaction effect for condition x test x time: $F_{4, 28}$ = 4.98, p = 0.004 *vs* euhydration, Figure 6.4, Panel A). Whilst V_T in CPET-2 was 0.39 ± 0.60 L lower in dehydration compared to euhydration (Figure 6.4, Panel B), this did not reach statistical significance (p = 0.143), and f_b was unaffected by dehydration (Figure 6.4, Panel C). The submaximal and maximal data for T_i, T_{tot}, and T_i/T_{tot} displayed changes over time (see Table 6.6 and Appendix G), but were not different between experimental conditions or CPET.

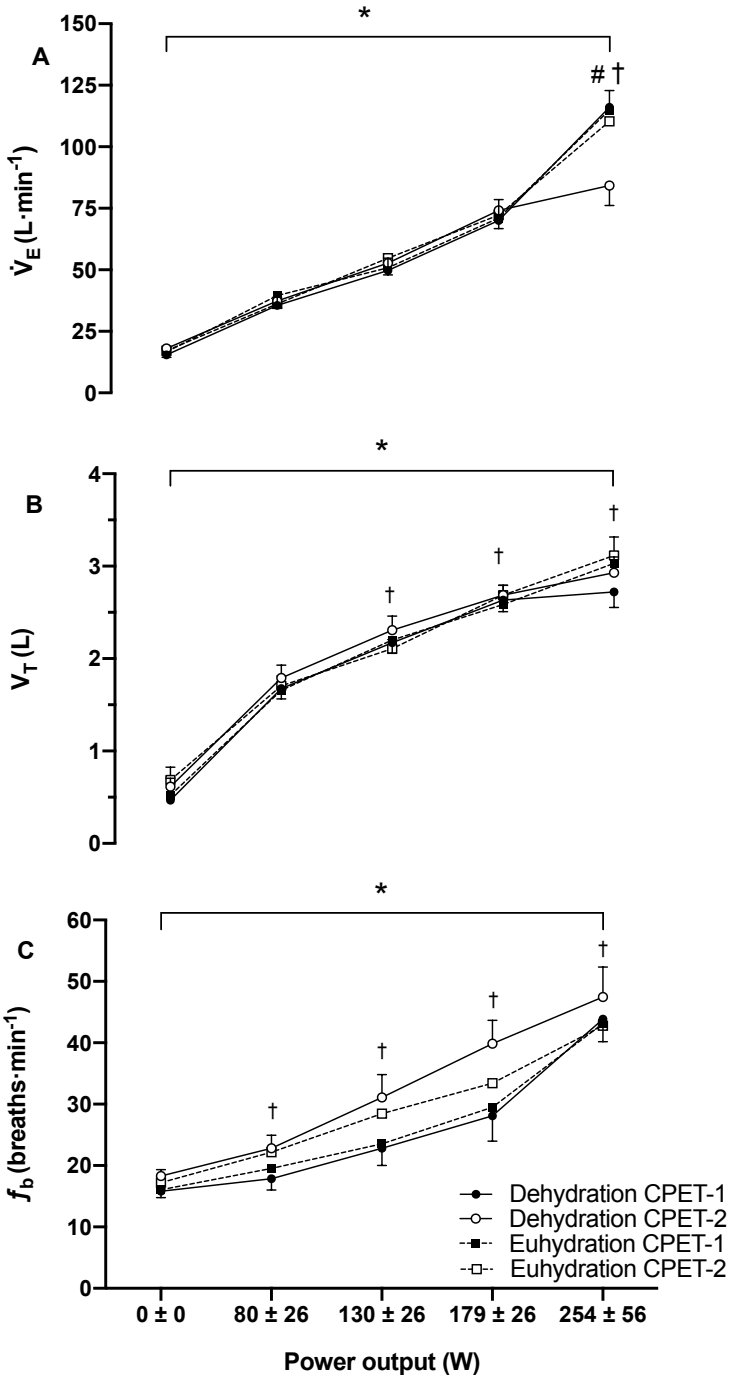

Figure 6.4. Change in a) minute ventilation ($\dot{V}_E$), b) tidal volume (V_T), and c) breathing frequency (f_b) during the incremental exercise tests before (CPET-1) and after (CPET-2) 2 h exercise in the heat, with (euhydration) or without (dehydration) fluid replacement. Power output is displayed as mean ± SD across the four CPET. $^{*}p < 0.05$ main effect of time; # $p < 0.05$ *vs* euhydration at corresponding time point; $^{\dagger}p < 0.05$ *vs* CPET-1 at corresponding time point.

Table 6.6. Ventilatory response to incremental exercise in dehydration and euhydration conditions, before (CPET-1) and after (CPET-2) 2 hours of exercise in the heat, with (euhydration) or without (dehydration) fluid replacement (n=11).

		Incremental exercise test				
Time		**0 min (rest)**	**2 min**	**4 min**	**6 min**	**Peak exercise**
Power Output		**0 ± 0 W**	**80 ± 26 W**	**130 ± 26 W**	**176 ± 26 W**	**254 ± 56 W**
Power output	*Euhydration CPET-1*	0 ± 0 [b c d e]	80 ± 26 [a c d e]	130 ± 26 [a b d e]	180 ± 26 [a b c e]	270 ± 58 [a b c d]
(W)	*Euhydration CPET-2*				180 ± 26 [a b c e]	245 ± 50 [a b c d †]
	Dehydration CPET-1				180 ± 26 [a b c e]	285 ± 41 [a b c d]
	Dehydration CPET-2				175 ± 27 [a b c e]	205 ± 50 [a b c d # †]
Ventilatory variables						
$\dot{V}_E$ (L·min^{-1})	*Euhydration CPET-1*	17 ± 7 [b c d e]	40 ± 12 [a c d e]	51 ± 14 [a b d e]	71 ± 24 [a b c e]	115 ± 24 [a b c d]
	Euhydration CPET-2	17 ± 7 [b c d e]	36 ± 9 [a c d e †]	55 ± 16 [a b d e]	72 ± 26 [a b e †]	110 ± 31 [a b c d]
	Dehydration CPET-1	15 ± 6 [b c d e]	36 ± 11 [a c d e]	50 ± 16 [a b d e]	70 ± 27 [a b c e]	117 ± 21 [a b c d]
	Dehydration CPET-2	18 ± 11 [b c d e]	37 ± 9 [a c d e]	52 ± 15 [a b d e]	74 ± 21 [a b c e †]	84 ± 26 [a b c d # †]
V_T (L)	*Euhydration CPET-1*	1.05 ± 0.60 [b c d e]	2.04 ± 0.86 [a]	2.27 ± 0.81 [a]	2.45 ± 0.77 [a]	2.74 ± 0.90 [a]
	Euhydration CPET-2	0.99 ± 0.51 [b c d e]	1.67 ± 0.44 [a]	1.85 ± 0.65 [a †]	2.21 ± 0.75 [a †]	2.50 ± 0.86 [a †]
	Dehydration CPET-1	1.04 ± 0.70 [b c d e]	2.08 ± 0.78 [a]	2.40 ± 0.64 [a]	2.60 ± 0.73 [a]	2.78 ± 0.79 [a]
	Dehydration CPET-2	0.87 ± 0.39 [b c d e]	1.68 ± 0.58 [a]	1.81 ± 0.49 [a †]	1.89 ± 0.49 [a †]	2.11 ± 0.65 [a †]
f_b	*Euhydration CPET-1*	16 ± 5 [d e]	19 ± 6 [e]	23 ± 8 [e]	28 ± 13 [a e]	43 ± 13 [a b c d]
(breaths·min^{-1})	*Euhydration CPET-2*	17 ± 2 [c d e]	22 ± 7 [c d e]	27 ± 11 [a b d e]	33 ± 13 [a b c e]	41 ± 17 [a b c d]
	Dehydration CPET-1	16 ± 3 [d e]	18 ± 6 [e]	23 ± 9 [e]	28 ± 13 [a e]	43 ± 12 [a b c d]

	Dehydration CPET-2	18 ± 3 ^{cde}	23 ± 7 ^{cde}	31 ± 12 ^{abde}	40 ± 11 ^{abce}	45 ± 16 ^{abcd}
T_i (s)	*Euhydration CPET-1*	1.54 ± 0.45 ^{de}	1.48 ± 0.59 ^{de}	1.24 ± 0.36 ^{e}	1.13 ± 0.43 ^{ab}	0.82 ± 0.35 ^{abc}
	Euhydration CPET-2	1.47 ± 0.36 ^{de}	1.30 ± 0.48 ^{de}	1.09 ± 0.48 ^{e}	0.99 ± 0.42 ^{ab}	0.80 ± 0.30 ^{abc}
	Dehydration CPET-1	1.57 ± 0.46 ^{de}	1.56 ± 0.50 ^{de}	1.36 ± 0.47 ^{e}	1.17 ± 0.48 ^{ab}	0.73 ± 0.24 ^{abc}
	Dehydration CPET-2	1.48 ± 0.55 ^{de}	1.43 ± 0.61 ^{de}	1.02 ± 0.31 ^{e}	0.77 ± 0.21 ^{ab}	0.71 ± 0.30 ^{abc}
T_{tot} (s)	*Euhydration CPET-1*	4.04 ± 1.17 ^{cde}	3.38 ± 1.24 ^{cde}	2.81 ± 0.89 ^{abe}	2.38 ± 0.91 ^{abe}	1.45 ± 0.58 ^{abcd}
	Euhydration CPET-2	3.54 ± 0.65 ^{cde}	2.95 ± 0.92 ^{cde}	2.41 ± 0.99 ^{abe}	2.08 ± 0.88 ^{ab}	1.68 ± 0.67 ^{abc}
	Dehydration CPET-1	3.97 ± 0.94 ^{cde}	3.70 ± 1.17 ^{cde}	3.02 ± 0.96 ^{abe}	2.52 ± 1.02 ^{abe}	1.51 ± 0.50 ^{abcd}
	Dehydration CPET-2	3.40 ± 0.75 ^{cde}	2.68 ± 0.93 ^{cde}	2.14 ± 0.63 ^{abe}	1.62 ± 0.44 ^{ab}	1.53 ± 0.60 ^{abc}
T_i/T_{tot}	*Euhydration CPET-1*	0.39 ± 0.05 ^{de}	0.44 ± 0.02 ^{de}	0.45 ± 0.03 ^{de}	0.48 ± 0.03 ^{abc}	0.49 ± 0.02 ^{abc}
	Euhydration CPET-2	0.38 ± 0.12 ^{de}	0.43 ± 0.04 ^{de}	0.45 ± 0.03 ^{de}	0.47 ± 0.03 ^{abc}	0.48 ± 0.02 ^{abc}
	Dehydration CPET-1	0.40 ± 0.08 ^{de}	0.43 ± 0.04 ^{de}	0.45 ± 0.03 ^{de}	0.47 ± 0.03 ^{abc}	0.48 ± 0.02 ^{abc}
	Dehydration CPET-2	0.42 ± 0.08 ^{de}	0.62 ± 0.54 ^{de}	0.48 ± 0.04 ^{de}	0.47 ± 0.03 ^{abc}	0.50 ± 0.04 ^{abc}

$\dot{V}_{E,}$ minute ventilation; $V_{T,}$ tidal volume; $f_{b,}$ breathing frequency; T_i, inspiratory time; T_{tot}, total time of breath; T_i/T_{tot}, inspiratory duty cycle; ^{a} $p < 0.05$ *vs* 0 min; ^{b} $p < 0.05$ *vs* 2 min; ^{c} $p < 0.05$ *vs* 4 min; ^{d} $p < 0.05$ *vs* 6 min; ^{e} $p < 0.05$ *vs* peak exercise; $^{\#}$ $p < 0.05$ *vs* euhydration at corresponding time point; † $p < 0.05$ *vs* CPET-1 at corresponding time point. *Note. For detailed statistical output see Appendix G.*

6.4.5.2 *Operating lung volumes.*

Operating lung volumes during all CPET are displayed in Figure 6.5. During CPET-1 and CPET-2, EILV increased at 2 min ($p = 0.008$ *vs* 0 min) and remained elevated thereafter in both experimental conditions (main effect of time: $F_{1,8} = 0.86$; all $p < 0.05$ *vs* 0 min). The biggest visual differences in EILV observed at peak exercise in the dehydration condition (i.e. 0.4 ± 0.8 L reduction during CPET-2 compared to CPET-1) did not reached statistical significance ($p = 0.36$). For EELV, a main effect of time was noted ($F_{1, 8} = 3.39$; $p = 0.020$), but post hoc analysis did not reveal significant differences between individual time points. There was no difference in EELV between CPET-1 and CPET-2, or between experimental conditions. No significant changes in IC were noted between CPET-1 and CPET-2 for both conditions.

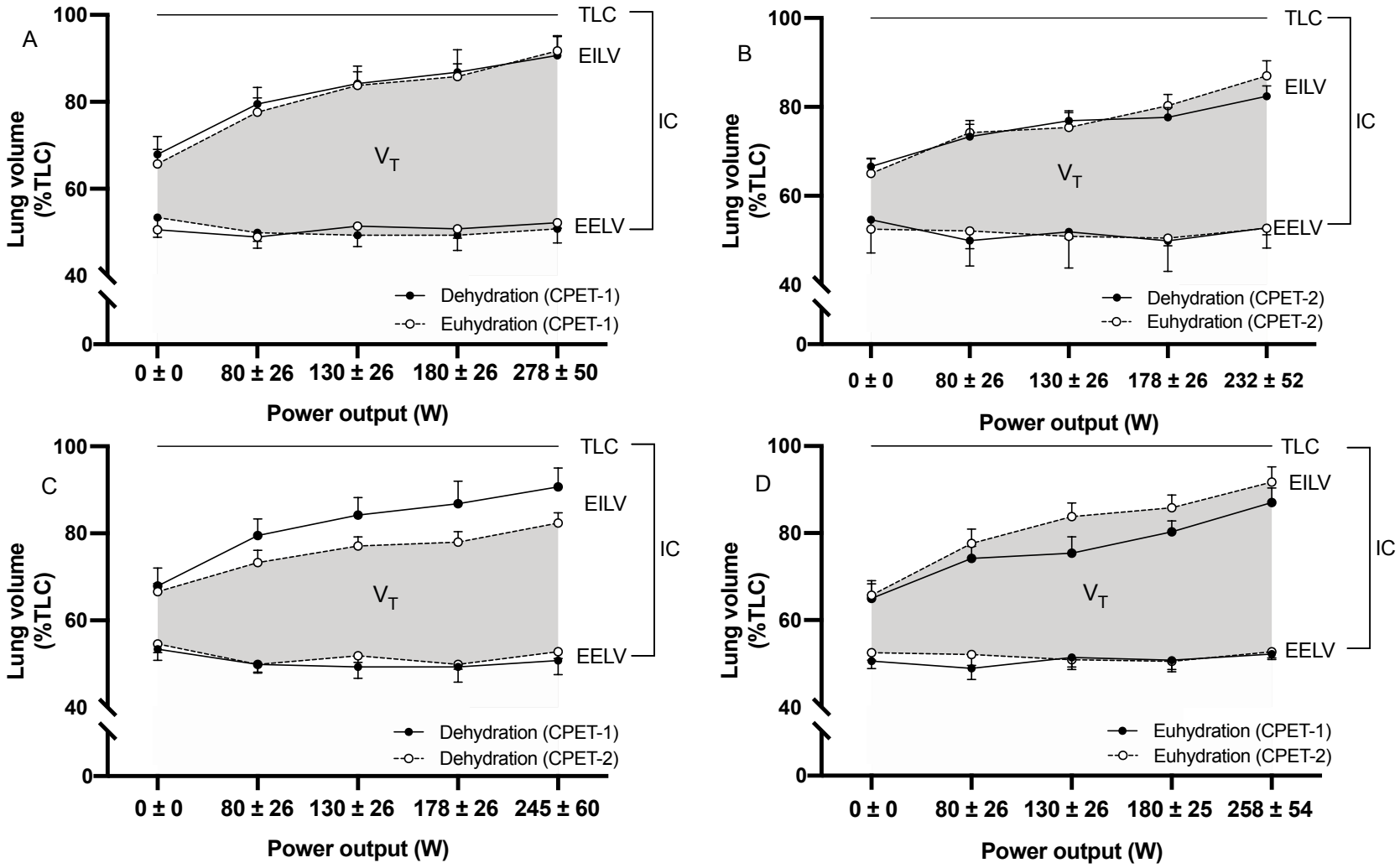

Figure 6.5. Operating lung volumes during incremental tests to exhaustion performed before (CPET-1) and after (CPET-2) 2 h exercise in the heat with (euhydration) or without (dehydration) fluid replacement (n = 11). Inspiratory capacity manoeuvres were performed at rest (0 W), in the last 30 s of each incremental 2 min stage, and at peak exercise. Power output is displayed as mean ± SD across the respective CPET. EELV, end expiratory lung volume; EILV, end inspiratory lung volume; IC, inspiratory capacity; TLC, total lung capacity; V_T, tidal volume. EILV was elevated at 2, 4, 6 min and peak *vs* 0 min in all conditions ($p < 0.010$). Data are mean ± SEM.

6.4.5.3 *Other physiological responses*

Table 6.7 and Figure 6.6 display the physiological responses to CPET. $\dot{V}O_2$ and $\dot{V}CO_2$ progressively increased throughout each CPET($F_{1,9} = 322.01$, $p < 0.001$). At peak exercise, $\dot{V}CO_2$ was higher in CPET-1 compared to CPET-2 (interaction for test x time: $F_{4, 32} = 5.64$, $p = 0.001$), whereas no difference was displayed between CPET-1 and CPET-2 for $\dot{V}O_{2peak}$. There was no impact of dehydration upon $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E/\dot{V}O_2$ or $\dot{V}_E/\dot{V}CO_2$ (all $p > 0.05$ *vs* euhydration). Heart rate progressively increased during both CPET (main effect for time: $F_{1, 8} = 158.98$; $p < 0.001$), with no difference between the dehydration and euhydration conditions. In CPET-2, HR was significantly higher at 0, 2, 4, and 6 min (all $p = 0.001$) compared to CPET-1, but there was no difference at peak exercise between CPET (interaction for test x time: $F_{4,32} = 9.82$, $p < 0.001$). T_{core} was higher during CPET-2 compared to CPET-1 in both conditions (main effect for test: $F_{1, 8} = 36.60$; $p = 0.001$), but was not affected by hydration status. A main effect for time was observed for SpO_2 ($F_{1,8} = 5.23$, $p = 0.002$); however post hoc analysis did not reveal significant difference between individual time points. Dehydration did not affect SpO_2, with no difference observed between CPET-1 and CPET-2 in the euhydration and dehydration conditions.

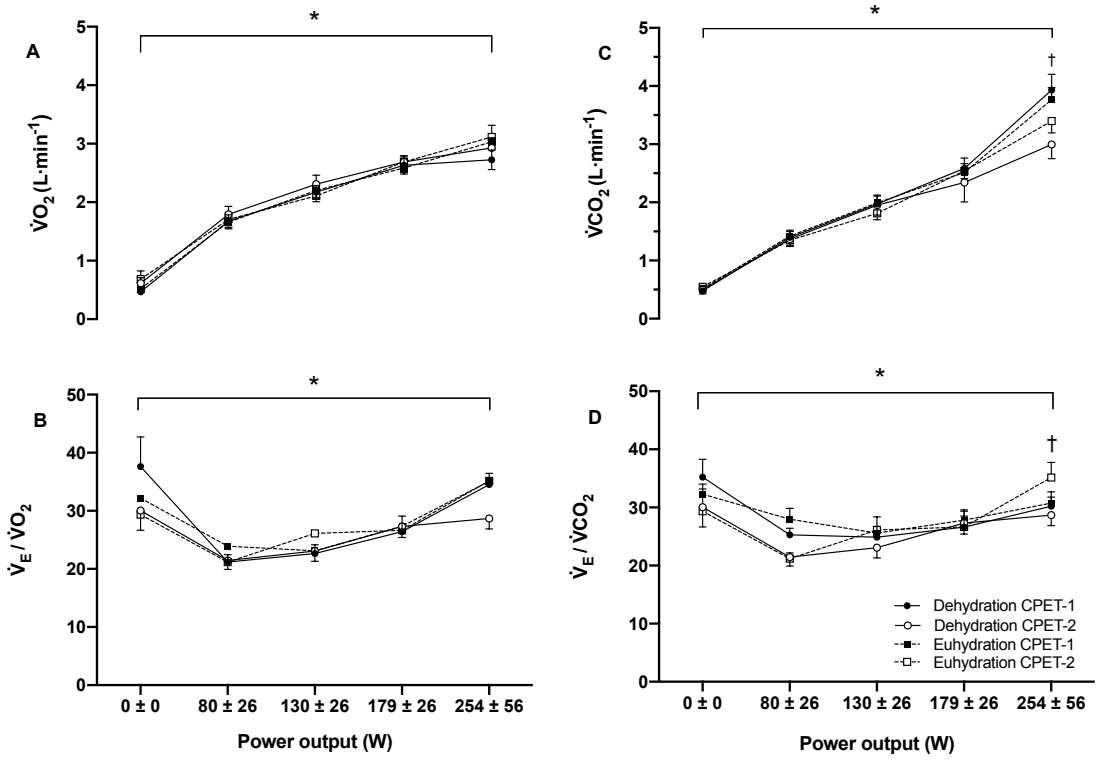

Figure 6.6. Change in A) volume of oxygen uptake ($\dot{V}O_2$) B) ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$), C) volume of exhaled carbon dioxide ($\dot{V}CO_2$) and D) ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}O_2$) during the incremental exercise tests before (CPET-1) and after (CPET-2) 2 h exercise in the heat, with (euhydration) or without (dehydration) fluid replacement (n = 11). Power output is displayed as mean ± SD across the four CPET. $^{*}p < 0.05$ main effect of time; $^{\dagger}p < 0.05$ *vs* CPET-1 at corresponding time point.

Table 6.7. Physiological responses to incremental exercise in dehydration and euhydration conditions, before (CPET-1) and after (CPET-2) 2 hours of exercise in the heat, with (euhydration) or without (dehydration) fluid replacement (n=11).

Time		**0 min (rest)**	**2 min**	**4 min**	**6 min**	**Peak exercise**
Power Output		**0 ± 0 W**	**80 ± 26 W**	**130 ± 26 W**	**176 ± 26 W**	**254 ± 56 W**
Physiological responses						
$\dot{V}O_2$	*Euhydration CPET-1*	0.52 ± 0.19 ^{bcde}	1.66 ± 0.36 ^{acde}	2.20 ± 0.39 ^{abde}	2.59 ± 0.33 ^{abce}	3.03 ± 0.36 ^{abcd}
(L·min^{-1})	*Euhydration CPET-2*	0.69 ± 0.44 ^{bcde}	1.71 ± 0.34 ^{acde}	2.11 ± 0.31 ^{abde}	2.69 ± 0.33 ^{abce}	3.12 ± 0.63 ^{abcd}
	Dehydration CPET-1	0.47 ± 0.14 ^{bcde}	1.68 ± 0.35 ^{acde}	2.17 ± 0.36 ^{abde}	2.63 ± 0.40 ^{abce}	2.72 ± 0.73 ^{abcd}
	Dehydration CPET-2	0.61 ± 0.29 ^{bcde}	1.79 ± 0.44 ^{acde}	2.31 ± 0.48 ^{abde}	2.69 ± 0.33 ^{abc}	2.93 ± 0.55 ^{abcd}
$\dot{V}CO_2$	*Euhydration CPET-1*	0.51 ± 0.19 ^{bcde}	1.42 ± 0.32 ^{acde}	1.99 ± 0.42 ^{abde}	2.51 ± 0.47 ^{abce}	3.77 ± 0.65 ^{abcd}
(L·min^{-1})	*Euhydration CPET-2*	0.55 ± 0.30 ^{bcde}	1.35 ± 0.27 ^{acde}	1.81 ± 0.35 ^{abde}	2.54 ± 0.43 ^{abc}	3.40 ± 0.65 ^{abc†}
	Dehydration CPET-1	0.48 ± 0.18 ^{bcde}	1.40 ± 0.31 ^{acde}	1.97 ± 0.43 ^{abde}	2.59 ± 0.56 ^{abce}	3.93 ± 0.86 ^{abce}
	Dehydration CPET-2	0.50 ± 0.24 ^{bcde}	1.37 ± 0.40 ^{acde}	1.95 ± 0.53 ^{abde}	2.60 ± 0.69 ^{abc}	2.99 ± 0.77 ^{abc†}
$\dot{V}_E/\dot{V}O_2$	*Euhydration CPET-1*	32 ± 10	24 ± 5 ^{e}	23 ± 5 ^{e}	27 ± 8 ^{e}	35 ± 8 ^{bcd}
	Euhydration CPET-2	29 ± 12	21 ± 3 ^{de}	26 ± 7 ^{e}	27 ± 8 ^{b}	35 ± 8 ^{bc}
	Dehydration CPET-1	38 ± 16	21 ± 4 ^{e}	23 ± 5 ^{e}	26 ± 8 ^{e}	35 ± 6 ^{bcd}
	Dehydration CPET-2	30 ± 11	21 ± 5 ^{de}	23 ± 6 ^{e}	27 ± 5 ^{b}	29 ± 6 ^{bc}
$\dot{V}_E/\dot{V}CO_2$	*Euhydration CPET-1*	32 ± 6	28 ± 6	26 ± 4	28 ± 6 ^{e}	31 ± 6 ^{d}
	Euhydration CPET-2	29 ± 12	21 ± 3 †	26 ± 7	27 ± 8 ^{e}	35 ± 8 ^{d†}
	Dehydration CPET-1	35 ± 10	25 ± 4	25 ± 3 ^{de}	27 ± 6 ^{c}	30 ± 5 ^{c}

	Dehydration CPET-2	30 ± 11	21 ± 5 [†]	23 ± 6 [de]	27 ± 5 [c]	29 ± 6 [c†]
HR	*Euhydration CPET-1*	69 ± 13 [bcde]	105 ± 13 [acde]	121 ± 17 [abde]	138 ± 16 [abce]	164 ± 14 [abcd]
(beats·min^{-1})	*Euhydration CPET-2*	90 ± 19 [bcde†]	113 ± 18 [acde†]	130 ± 21 [abde†]	146 ± 16 [abce†]	165 ± 19 [abcd]
	Dehydration CPET-1	69 ± 12 [bcde]	103 ± 13 [acde]	120 ± 8 [abde]	136 ± 12 [abce]	163 ± 16 [abcd]
	Dehydration CPET-2	89 ± 16 [bcde†]	119 ± 14 [acde†]	137 ± 15 [abde†]	151 ± 12 [abce†]	159 ± 12 [abcd]
T_{core} (°C)	*Euhydration CPET-1*	37.0 ± 0.4	37.0 ± 0.5 [e]	37.1 ± 0.4 [e]	37.2 ± 0.5	37.5 ± 0.5 [bc]
	Euhydration CPET-2	37.7 ± 0.3[†]	37.6 ± 0.4 [e†]	37.6 ± 0.4 [e†]	37.7 ± 0.4[†]	37.9 ± 0.5 [bc†]
	Dehydration CPET-1	37.1 ± 0.4	37.1 ± 0.4 [c]	37.2 ± 0.4 [b]	37.2 ± 0.5	37.5 ± 0.5
	Dehydration CPET-2	38.1 ± 0.5[†]	38.0 ± 0.5 [c†]	38.1 ± 0.4 [b†]	38.0 ± 0.4[†]	38.2 ± 0.5[†]
SpO_2 (%)	*Euhydration CPET-1*	100 ± 1	98 ± 2	99 ± 2	98 ± 2	97 ± 2
	Euhydration CPET-2	100 ± 1	99 ± 2	99 ± 2	99 ± 1	97 ± 4
	Dehydration CPET-1	100 ± 1	99 ± 1	99 ± 1	98 ± 1	97 ± 4
	Dehydration CPET-2	100 ± 0	99 ± 2	99 ± 1	98 ± 1	99 ± 2

$\dot{V}O_2$, volume of oxygen uptake; $\dot{V}CO_2$, volume of expired carbon dioxide; HR, heart rate; T_{core}, core temperature; SpO_2, oxygen saturation; [a] $p < 0.05$ *vs* 0 min, [b] $p < 0.05$ *vs* 2 min, [c] $p < 0.05$ *vs* 4 min, [d] $p < 0.05$ *vs* 6 min, [e] $p < 0.05$ *vs* PEAK, [#] $p < 0.05$ *vs* euhydration at corresponding time point; [†] $p < 0.05$ *vs* CPET-1 at corresponding time point. *Note. For detailed statistical output see Appendix G.*

6.4.5.4 *Perceptual responses*

Table 6.8 displays perceptual responses during incremental exercise tests, detailed statistical analysis is presented in Table 9.2 (Appendix G). RPE progressively increased during CPET-1 in both conditions (all $p < 0.05$). During CPET-2, the increase in RPE with exercise intensity was no longer significant from 4 min onward in the dehydration condition, and from 6 min onward for the euhydration condition. During CPET-2, RPE at 4 min ($p = 0.034$) and 6 min ($p = 0.007$) was higher in the dehydration condition compared to the euhydration condition.

During CPET-1 in both conditions, breathing discomfort was only elevated at peak exercise compared to 0 min ($p = 0.021$). During CPET-2, breathing discomfort increased earlier, with a difference noted at 6 min ($p = 0.034$) and peak exercise ($p = 0.019$). Breathing discomfort was higher during CPET-2 compared to CPET-1 at 2 min ($p = 0.017$), 4 min ($p = 0.013$), and 6 min ($p = 0.046$), but hydration status did not influence the response. At peak exercise, no difference in breathing discomfort was noted between dehydration and euhydration, or between CPET-1 and CPET-2.

Table 6.8. Perceptual responses to incremental exercise in dehydration and euhydration conditions, before (CPET-1) and after (CPET-2) 2 hours of exercise in the heat, with (euhydration) or without (dehydration) fluid replacement (*n*=11).

Time		**0 min (rest)**	**2 min**	**4 min**	**6 min**	**Peak exercise**
Power Output		**0 ± 0 W**	**80 ± 26 W**	**130 ± 26 W**	**176 ± 26 W**	**254 ± 56 W**
Perceptual responses						
RPE	*Euhydration CPET-1*	6 ± 0 ^{bcde}	10 ± 2 ^{acde}	12 ± 2 ^{abde}	14 ± 3 ^{abce}	18 ± 1 ^{abcd}
	Euhydration CPET-2	6 ± 0 ^{bcde}	12 ± 1 ^{acde†}	13 ± 2 ^{abde†}	16 ± 3 ^{abc†}	18 ± 1 ^{abc}
	Dehydration CPET-1	6 ± 0 ^{bcde}	10 ± 2 ^{acde}	12 ± 2 ^{abde}	14 ± 3 ^{abce}	19 ± 1 ^{abcd}
	Dehydration CPET-2	6 ± 0 ^{bcde}	14 ± 2 $^{acde\#\dagger}$	16 ± 2 $^{abde\#\dagger}$	17 ± 2 ^{ab†}	19 ± 1 ^{abc}
Breathing	*Euhydration CPET-1*	0 ± 0 ^{e}	2 ± 2 ^{e}	3 ± 3 ^{e}	5 ± 3	7 ± 4 ^{abc}
discomfort	*Euhydration CPET-2*	0 ± 0 ^{de}	3 ± 3 ^{e†}	4 ± 3 †	5 ± 3 ^{a†}	7 ± 4 ^{ab}
	Dehydration CPET-1	0 ± 0 ^{e}	2 ± 2 ^{e}	3 ± 3 ^{e}	4 ± 3	7 ± 4 ^{abc}
	Dehydration CPET-2	0 ± 0 ^{de}	5 ± 3 ^{e†}	5 ± 3 †	6 ± 4 ^{a†}	7 ± 3 ^{ab}

RPE, rating of perceived exertion; $^{a}p < 0.05$ *vs* 0 min; $^{b}p < 0.05$ *vs* 2 min; $^{c}p < 0.05$ *vs* 4 min; $^{d}p < 0.05$ *vs* 6 min; $^{e}p < 0.05$ *vs* peak; $^{\#}p < 0.05$ *vs* euhydration at corresponding time point; $^{\dagger}p < 0.05$ *vs* CPET-1 at corresponding time point. *Note. For detailed statistical output see Appendix G.*

6.5 Discussion

6.5.1 Overview of results

The aim of the present study was to investigate the effects of exercise-induced moderate dehydration on resting pulmonary function and on the ventilatory response to incremental exercise in healthy physically-active individuals. Through this research, the presence of a robust phenomenon of dehydration-induced pulmonary alteration has been confirmed, characterised by negative alterations in resting pulmonary lung volumes (increased RV) and capacities (decreased FVC and increased FRC) in healthy young adults. Further, at matched submaximal exercise intensities, ventilatory response and operating lung volumes were not altered by moderate dehydration. At peak exercise, the reduction in $\dot{V}_E$ of approximately 30 $L \cdot min^{-1}$ in the dehydrated state was associated with a reduced power output, but no change in operating lung volumes (i.e., no sign of dynamic hyperinflation) or in respiratory discomfort. Overall, the small, but consistent alterations in pulmonary lung volumes induced by dehydration do not seem to modify the breathing response to exercise in healthy physically-active adults.

6.5.2 Moderate dehydration and resting pulmonary function

The present study demonstrates that systemic water loss induced by exercise impairs resting pulmonary function in physically-active individuals. This finding is in line with previous work conducted in both athletes with asthma (Simpson et al., 2017), and in healthy normally active individuals (Chapter 5). In Chapter 5, a 2.6% body mass loss led to an average reduction in FVC of ~150 mL, and elevations in FRC and RV of ~130 mL and ~220 mL, respectively. In the present study, a greater magnitude of dehydration (i.e., 3.5% loss of body mass) led to greater pulmonary changes: i.e., ~210 mL decrease in FVC (+ ~ 60 mL *vs* Chapter 5), and ~255 mL and ~401 mL increases in FRC and RV (+ ~125 mL and ~180 mL *vs* Chapter 5, respectively). Further, moderate dehydration in the present study led to a reduction in FVC of >200 mL [which is commonly used for clinical significance (Pellegrino et al., 2005)] in 55% of participants, compared to just 30% following mild dehydration in Chapter 5. Altogether, this suggests that greater severities of systemic dehydration elicit greater alterations in airway function.

In the present study, moderate dehydration led to a significant increase in sRaw (0.11 ± 0.12 $kPa \cdot s^{-1}$), whilst a reduction in sRaw occurred in the euhydrated condition (-0.10 ± 0.14

$kPa \cdot s^{-1}$). This is the first study to show that dehydration may increase airway resistance. However, when IOS was used to provide an additional insight to changes in respiratory resistance and reactance, no significant alterations were noted. No previous work exists regarding the effect of dehydration upon IOS parameters. However, individuals with obstructive lung disease (especially those with asthma), commonly present increased reactance area [indicative of peripheral airway obstruction (Brashier and Salvi, 2015)], and increased resonant frequency and distal capacitive reactance (indicative of changes to elastic recoil at the peripheral airways (Brashier and Salvi, 2015; Cavalcanti et al., 2006). The recognised high-variability in IOS measurements, which has been shown to be ~5-15% between days in adults (Brashier and Salvi, 2015), may contribute to the difficulty in detecting small changes in airway function in intervention studies recruiting healthy population.

Reductions in FVC alongside elevations in RV and FRC are suggestive of impaired small airway function (McNulty and Usmani, 2014). Specifically, the elevated RV/TLC measured in the dehydration condition indicates potential gas trapping at the level of the small airway. This change, which appeared concomitantly to the elevated airway resistance, may physiologically be explained by the following mechanism. Small airway function is highly dependent upon lung compliance and elasticity; the surface tension of the airways is a fundamental component of its function and plays a key role in lung compliance and elasticity (Chen et al., 2019). The ASL plays a vital role in the maintenance of normal airway surface tension; alterations to the volume of this surface liquid can consequently alter surface tension and function (Macklem et al., 1970). In this study, the progression to moderate dehydration (compared to the previously induced mild dehydration state, Chapter 5) may have caused a greater magnitude of intracellular dehydration (Costill et al., 1976). This increased magnitude of fluid loss was associated with significant elevations in P_{osm} (13 ± 5 $mOsm \cdot kg^{-1}$ in this study compared to 6 ± 4 $mOsm \cdot kg^{-1}$ in Chapter 5). It is therefore likely that the elevation in P_{osm} contributed to the alterations to composition and/or content of ASL and, in turn, to airway instability and airway collapse (Macklem et al., 1970). Further, although the duration of experimental visits within the present study prevented pulmonary function tests from being performed at the same time of day within visits, reproducibility data presented within Chapter 4 demonstrated that spirometry and whole body plethysmography had ‘very good’ reproducibility when performed in the morning and afternoon of the same day. Time of day can therefore be excluded as a confounding factor

for the findings of the present study. Taken together, these data suggest that P_{osm} is a fundamental mechanism underpinning the pulmonary function changes that occur during systemic dehydration.

6.5.3 Ventilatory and perceptual response to dehydration during CPET

Based on the presented results, systemic dehydration does not appear to influence the ventilatory response to submaximal or maximal exercise in healthy adults. $\dot{V}_E$ at peak exercise was 26 ± 25 $L \cdot min^{-1}$ lower when participants were dehydrated compared to euhydrated. However, rather than a direct effect of dehydration on ventilatory regulation, this change is more likely to be explained by premature onset of fatigue, as supported by the average reduction in PO_{max} of 40 W in the dehydrated state. Surprisingly, whilst $\dot{V}_E$ was significantly reduced following dehydration, neither f_b nor V_T displayed statistically significant alterations. However, following dehydration, V_T was 0.3 ± 0.6 L lower compared to pre-dehydration, which may indicate that the changes in V_T primarily drove the reduction in $\dot{V}_E$. Beyond f_b and V_T, four principal variables are known to influence $\dot{V}_E$: *i)* inspiratory volume-time profile, *ii)* inspiratory duration, *iii)* expiratory volume-time profile, and *iv)* expiratory duration (Younes and Remmers, 1981). Whilst inspiratory volume-time profile and inspiratory duration reflect changes in EILV, expiratory volume-time profile and expiratory duration reflect changes in EELV (Tipton *et al.*, 2017). In the present study, EELV at peak exercise was not different between dehydration and euhydration conditions, and the reduction in EILV at peak exercise between the dehydration and euhydration conditions (0.4 ± 0.8 L) was likely not large enough to be the main contributor to the reduction in $\dot{V}_E$. Whilst it is possible that V_T and EILV were, in part, responsible for the change in $\dot{V}_E$, it is unclear what the main driver of this change was. It should be considered that variability of these parameters may explain the lack of significance.

During exercise, an elevation in EELV above baseline usually indicates occurrence of dynamic hyperinflation (Ferguson, 2006), which can be used as indirect marker for ventilatory limitation (Milne et al., 2020). Ventilatory limitation during exercise can lead to an increased work of breathing (O'Donnell et al., 2001) and enhanced perception of breathing discomfort (Iandelli et al., 2002). Previously, elevated EELV, and thus the presence of dynamic hyperinflation, has been reported in some well-trained adults (McClaran et al., 1999; Taylor et al., 2013). In the present study, no sign of dynamic

hyperinflation was noted in either condition (EELV was not altered during any CPET) and, whilst $\dot{V}_E$ was reduced, breathing pattern (V_T and f_b) and operating lung volumes (EELV, EILV, IC) were not altered by dehydration. These findings indicate that the healthy pulmonary system was able to meet the ventilatory demand of exercise, irrespective of significant systemic fluid loss. It is important to consider, however, that the ventilatory system often becomes often limited in highly trained/elite athletes due to the extremely high ventilatory demand exceeding the capacity of the respiratory system (Wells and Norris, 2009). Whilst the participants in the present study were physically-active (achieving $\dot{V}_E$ of ~120 $L \cdot min^{-1}$), they were not 'elite' athletes; hence, they may have been able to withstand the demand placed upon the ventilatory system, even in a state of dehydration.

Alongside dynamic hyperinflation, pulmonary gas exchange impairment is another marker of ventilatory limitation during exercise (Phillips and Stickland, 2019). Assessment of SpO_2 was used in the present study to indirectly evaluate exercise-induced arterial hypoxemia, which is a common occurrence in endurance-trained athletes (Dempsey and Wagner, 1999). In the present study, SpO_2 was not influenced by hydration status. Since neither SpO_2, nor EELV changed following moderate dehydration, systemic fluid loss does not appear to cause ventilatory limitation during incremental exercise in healthy physically-active individuals. When combined with the resting data, the exercise data in the present study therefore suggest that the functional impact of dehydration-induced pulmonary alteration is minimal in healthy young adults. Considering that resistance at the level of the small airway represents only ~10-25% of total airway resistance (Macklem and Mead, 1967), the impact of dehydration on the pulmonary system is likely not large enough to alter breathing pattern and induce dynamic hyperinflation in healthy young adults.

6.5.4 Strengths and limitations of research

Comprehensive analysis of the ventilatory response to exercise-induced dehydration was performed for the first time in the present study by combining breathing pattern and operational lung volume assessment. Dynamic hyperinflation was assessed indirectly, via IC manoeuvres, which has previously been deemed a suitable method for its assessment (Yan et al., 1997). The addition of oesophageal (pleural) pressure could have provided additional validation for the quality of these measurements (Babb and Rodarte, 1993). However, this procedure is invasive and would have been intolerable for the entire duration of our experimental trials (which lasted ~5.5 h).

Whilst the present study showed an impact of exercise-induced dehydration on resting pulmonary function, it is likely that the 2 h exercise protocol used to induce dehydration caused significant fatigue and thereby influenced some of the study outcomes. In future work, alternative dehydration methods prior to exercise testing, such as prolonged fluid restriction (as employed in Chapter 5), may be used to investigate the ventilatory response to dehydration, independent of exercise-induced local muscle fatigue. Fluid restriction might however be favoured over passive heating, as the duration of passive heating required to induce moderate dehydration [> 4 h at a dehydration rate of 1.3 -1.4 % body mass loss·h^{-1} (Cian et al., 2001; Melin et al., 2001)] could lead to confounding changes to T_{core}. Tipton *et al.*, (2017) previously studied the independent role of both passive- and exercise-induced elevations in T_{core} on pulmonary function and reported a bronchodilator response when T_{core} was increased by ~1ºC. Since T_{core} recorded during the CPET performed before and after 2 h of exercise in the heat did not differ between conditions, T_{core} can be excluded as a potential confounding factor in these results.

6.5.5 Functional and clinical relevance and future research

Findings from the present study have relevance to physically-active individuals and those working in hot environments who are susceptible to dehydration (Sawka et al., 2007). Although the findings from the present study highlight a negative impact of dehydration on resting pulmonary function, this effect is unlikely to modify the ventilatory response to submaximal and maximal exercise in healthy individuals. In this study, breathing discomfort was not exaggerated in the dehydrated state. It is therefore unlikely that respiratory symptoms experienced by physically-active individuals during endurance exercise are primarily driven by systemic fluid loss.

Further investigation is now required to understand the influence of systemic fluid loss on individuals with higher susceptibility to ventilatory dysfunction. Patients with COPD can experience dynamic hyperinflation and breathlessness not only during exercise, but also when undertaking simple daily tasks (O'Donnell and Laveneziana, 2006), which can reduce exercise tolerance (O'Donnell et al., 2001) and negatively impact quality of life (Garcia-Rio et al., 2009; Zhao et al., 2016). It is currently unknown if/how hydration status may impact the presence of dynamic hyperinflation and symptoms of breathlessness in those

patients. An increase in serum osmolality (as occurs during dehydration) has been previously shown to be associated with reduced pulmonary function in COPD patients (Pogson et al., 2008). Understanding the functional implications of reduced resting pulmonary function, and the role of fluid loss upon that impairment could highlight the importance of hydration status upon pulmonary function in clinical populations.

6.5.6 Conclusion

In this study, exercise-induced moderate dehydration in healthy physically-active individuals led to significant negative alterations in resting pulmonary function (including a reduction in FVC and elevations in RV, RV/TLC, FRC and sRaw), suggesting increased small airway gas trapping and airway resistance in a dehydrated state. These findings are well aligned with results obtained after exercise- and fluid restriction-induced mild dehydration in normally active healthy young adults (Chapter 5) and after exercise-induced mild dehydration in individuals with asthma (Simpson et al., 2017). Dehydration-induced pulmonary alteration appears therefore as a universal phenomenon. Further, with this study, it was established that the resting alterations in pulmonary function induced by dehydration are unlikely to influence the ventilatory response to exercise in young active adults. Specifically, neither dynamic hyperinflation nor increased respiratory discomfort were noted at submaximal or maximal exercise intensities. Since populations with pre-existing respiratory conditions are more prone to ventilatory limitation (which can induce respiratory discomfort and negatively impact quality of life), future work should aim to understand the role of hydration status upon the ventilatory response to exercise in those vulnerable individuals.

Chapter 7 General discussion

7.1 Key findings

The three research studies presented within this thesis have generated novel data supporting the role of adequate systemic hydration in maintaining pulmonary function in healthy adults.

Experiment 1 (Chapter 4) was a methodological paper designed to ascertain the repeatability and reproducibility of pulmonary function data gained through spirometry and whole body plethysmography in healthy young adults. 'Excellent' repeatability and 'very good' reproducibility of pulmonary function data was demonstrated when testing was performed on multiple days and at multiple times within the same day (aligned with data collection times of later experimental studies). Spirometry and whole body plethysmography were therefore deemed appropriate for use in subsequent experimental chapters aiming to detect changes in pulmonary function in healthy young adults following dehydration/rehydration interventions.

Hypothesis 1: Spirometry and whole body plethysmography show good inter-day repeatability and intra-day reproducibility in healthy young adults: **accepted**.

The two key aims of experiment 2 (Chapter 5) were as follows: firstly, to investigate the impact of mild systemic dehydration on pulmonary function in healthy young adults at rest; secondly, to establish whether dehydration-induced changes in pulmonary function could be reversed by systemic and/or local airway rehydration. Participants in this experiment displayed negative alterations in resting pulmonary function following mild dehydration induced by *i)* 2 h of exercise in the heat and *ii)* 28 h of fluid restriction. The simultaneous reduction in FVC and increases in FRC and RV following both dehydration protocols suggest that pulmonary alterations were primarily localised at the level of the small airways. Further, systemic (oral fluid intake), but not local (nebulised isotonic saline) rehydration was able to rapidly restore pulmonary function. As only the former mode of rehydration (i.e., systemic rehydration) was able to normalise P_{osm}, and since significant correlations were noted between changes in pulmonary function and changes in P_{osm}, it is proposed that P_{osm} plays a key regulatory role in the maintenance of small airway patency.

Hypothesis 2: Pulmonary function is impaired following mild systemic dehydration induced by exercise in the heat and by a prolonged period of fluid restriction: **accepted**.

Hypothesis 3: Immediate systemic and/or local airway rehydration successfully reverses negative alterations in pulmonary function induced by dehydration: **accepted** for systemic rehydration, **rejected** for local airway rehydration.

Experiment 3 (Chapter 6) aimed to establish whether a more severe state of dehydration (i.e., moderate dehydration) would *i)* induce further alterations in resting pulmonary function and *ii)* alter ventilatory response to exercise in young, physically-active adults. The findings from this experiment suggest that dehydration-induced impairments in pulmonary function is a robust phenomenon that may vary in severity with the degree of dehydration. The observed alterations in resting FVC, RV, and FRC however did not translate in altered ventilatory response to exercise (as no sign of dynamic hyperinflation and no exacerbation of respiratory discomfort were noted in a dehydrated state during maximal incremental exercise). These findings suggest that, during episodes of significant fluid loss, the ventilatory response to exercise is preserved in healthy active young adults, despite the presence of small airway impairments at rest.

Hypothesis 4: Exercise-induced moderate dehydration leads to negative alterations in pulmonary function in healthy physically-active adults: **accepted**.

Hypothesis 5: Ventilatory response is negatively altered and breathing discomfort is increased when incremental exercise to exhaustion is performed in a state of moderate dehydration: **rejected**.

Overall, the findings from this thesis demonstrate the importance of systemic fluid balance in the maintenance of small airway patency in humans and confirm the existence of a robust phenomenon of dehydration-induced pulmonary alteration at rest in healthy young adults. That these changes can be reversed with systemic (oral) rehydration provides a novel insight into the role of body fluid balance and P_{osm} upon small airway function. Whilst statistically significant, the subtle alterations in resting small airway function induced by

dehydration did not translate to a compromised ventilatory response to exercise in healthy young adults.

7.2 Effect of dehydration on pulmonary function

Findings from Chapters 5 and 6 are in agreement and support the hypothesis that systemic dehydration leads to negative alterations in resting pulmonary function in healthy young adults. The degree of dehydration appears to influence the severity of pulmonary function impairment. Moderate dehydration (-3.5% body mass) led to greater pulmonary alterations than mild dehydration (-2.5% body mass), with reductions in FVC ~60 mL greater following moderate dehydration. Further, elevations in FRC and RV following moderate dehydration were ~125 mL and ~180 mL greater, respectively, than following mild dehydration. Whilst the 60 mL difference in FVC would not be classified as clinically significant (Pellegrino et al., 2005), the difference in FRC and RV are almost doubled when the severity of dehydration is increased by ~1%. Whilst the variability of FRC and RV measurements were shown to be slightly higher (Chapter 4.4), the magnitude of change is indicative of a role for dehydration severity in pulmonary function impairment. When compared to the existing literature, the findings presented within this thesis confirm the existence of a robust phenomenon, whereby systemic dehydration induced by exercise and fluid restriction leads to impairments in small airway function. Figure 7.1 presents a comparison of the five studies that have specifically investigated the role of systemic dehydration upon pulmonary function to date. Notably, the greatest drop in FVC (-300 ± 190 mL) was reported by Simpson et al., (2017) in individuals with asthma after inducing a state of mild dehydration (2.3 ± 0.8% body mass loss) via exercise in the heat. In comparison, the moderate states of dehydration induced by *i)* Javaheri *et al.*, (1987) through diuretic drug administration and *ii)* in this thesis (Chapter 6) through exercise in the heat, led to lesser changes or even improvements in FVC. The divergence in findings are likely attributable to two factors: the respiratory health of the participants assessed within each study (i.e., existence or absence of a pre-existing pulmonary condition) and the mode of dehydration.

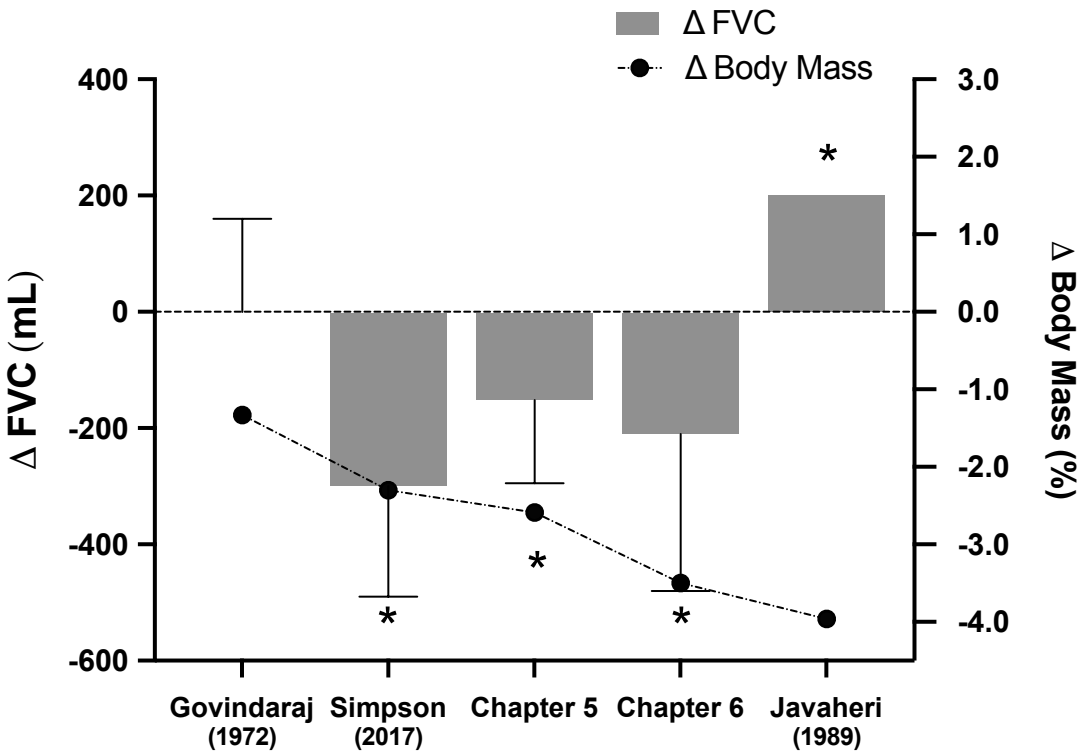

Figure 7.1. Impact of systemic dehydration upon pulmonary function (specifically FVC) based on the existing literature and experimental data from Chapters 5 & 6. *p<0.05 in FVC *vs* initial baseline value. *Note.* Javaheri et al. (1989) did not report SD of change. Dehydration was induced via: prolonged fluid restriction in the study by Govindaraj (1972) (16h) and in Chapter 5 (28 h), exercise in the heat by Simpson et al., (2017), and in Chapters 5 and 6; and via diuretic intake (chlorthalidone) by Javaheri et al., (1989).

Simpson et al., (2017) proposed that pulmonary function impairment following mild dehydration in individuals with asthma was due to a decrease in airway stability, following changes in content and/or composition of the ASL. Many individuals with asthma develop bronchoconstriction during and/or shortly after completing strenuous exercise (so-called EIB) (Aggarwal et al., 2018), and an 'osmotic theory' has been proposed to explain the occurrence of EIB. According to Anderson et al., (1982), airway water loss/airway drying is induced by high ventilatory rates during exercise, which subsequently leads to alterations in ASL osmolality and release of bronchoconstrictive mediators by the infiltrated pro-inflammation cells. Further, the severity of EIB response is associated with the volume of water lost from the airways (Anderson et al., 1982). Park, Stafford and Lockette, (2008) also confirmed the key role that water is playing at the airway surface in individuals with EIB. In a study of 22 healthy athletic participants with suspected EIB, a significant drop in

FEV_1 was observed following a methacholine challenge test, which was associated with diminished pilocarpine-induced sweat secretion. As sweat secretion can be used as proxy for fluid secretion at the level of the airways, these results suggest that a deficiency in body fluid secretions contributes to EIB, and highlight an important role for water at the airway surface for normal pulmonary function.

Following on from the clinical population previously studied by Simpson et al., (2017), data presented in Chapters 5 and 6 of this thesis show that, in healthy young adults (with no history of respiratory condition), resting small airway function can be compromised by mild and moderate systemic dehydration (Figure 7.1). In Chapter 6, healthy adults experienced moderate dehydration, yet did not reach the same magnitude of pulmonary function impairment as presented previously in asthmatic patients (Simpson et al., 2017). One explanation for the lesser impact of dehydration upon the healthy compared to the asthmatic airways is the likely impaired regulation of airway water secretion in individuals with asthma (Park et al., 2008). Healthy airways may be able to tolerate higher severities of dehydration before a clinically significant change in pulmonary function [>200 mL (Pellegrino et al., 2005)] is experienced, as water movement is more efficiently regulated.

In Figure 7.1 it is apparent that results from the work of Javaheri et al., (1987) are divergent to the ones presented in this thesis (with an increase in FVC noted in a state of moderate dehydration in healthy young adults). However, Javaheri and collaborators (1987) used a different approach to induce dehydration, i.e., they administered 50 mg of chlorthalidone every 12 hours (total 200 mg) over 48 hours. Diuretic-induced dehydration is known to cause isotonic-hypovolemia, which leads to little change in P_{osm} (Cheuvront et al., 2013). This is different to hypertonic-hypovolemia, as induced by exercise and fluid restriction in this thesis, which was associated with an increase in P_{osm}. As discussed below (see section 7.3.2), P_{osm} could play a key regulatory role in the maintenance of small airway patency, which may help to explain the discrepancy in the findings from Javaheri et al., (1987) in comparison to the work conducted within this thesis.

7.3 Mechanistic overview

Through this thesis, it has been established that dehydration-induced pulmonary alteration is a robust phenomenon that is present: in healthy populations; in various states of

dehydration (mild and moderate); and following various modes of dehydration (exercise in the heat and prolonged periods of fluid restriction). Through the use of divergent dehydration and rehydration interventions, insights regarding the potential mechanisms involved in the reported pulmonary function changes have been obtained.

7.3.1 Hydration status of the airway surface

Maintaining airway hydration is fundamental for the normal functioning of the pulmonary system. Dehydration of the ASL is implicated in disease states, such as cystic fibrosis (Boucher, 2007b) and EIA/EIB (Anderson and Daviskas, 1997, 2000), and associated with pulmonary dysfunction and epithelial damage (Tavana et al., 2011; Widdicombe and Widdicombe, 1995). Fluid delivery to the airway stems from the bronchial circulation, which is directly supplied by the systemic circulation (Flieder, 2018). With systemic fluid loss, the delivery of water to the bronchial circulation and subsequently to the airways could therefore be reduced. Epithelial cells line the respiratory tract and are responsible for controlling the volume and composition of the ASL through secretion and absorption mechanisms (Grotberg, 2001). These mechanisms allow the movement of water and ions across the epithelial barrier to regulate the volume and composition of the ASL (Boucher, 1999). Pulmonary surfactant is an important component of the ASL that reduces surface tension in order to stabilise the airways (Veldhuizen and Haagsman, 2000). This stabilisation prevents airway collapse by maintaining airway patency (Figure 7.2a-b) (Tavana et al., 2011). When water availability to the airways is reduced (as may occur during exercise in the heat and prolonged periods of fluid restriction), surfactant deficiency may follow, leading to increased surface tension, airway instability and subsequent airway collapse (Figure 7.2c). Further, with ASL dehydration the epithelial layer could become damaged (Kalhoff, 2003); this would further perturb secretory and absorptive ion mechanisms. Together, this supports the idea that systemic dehydration induces alterations in small airway function via perturbations in ASL volume and content.

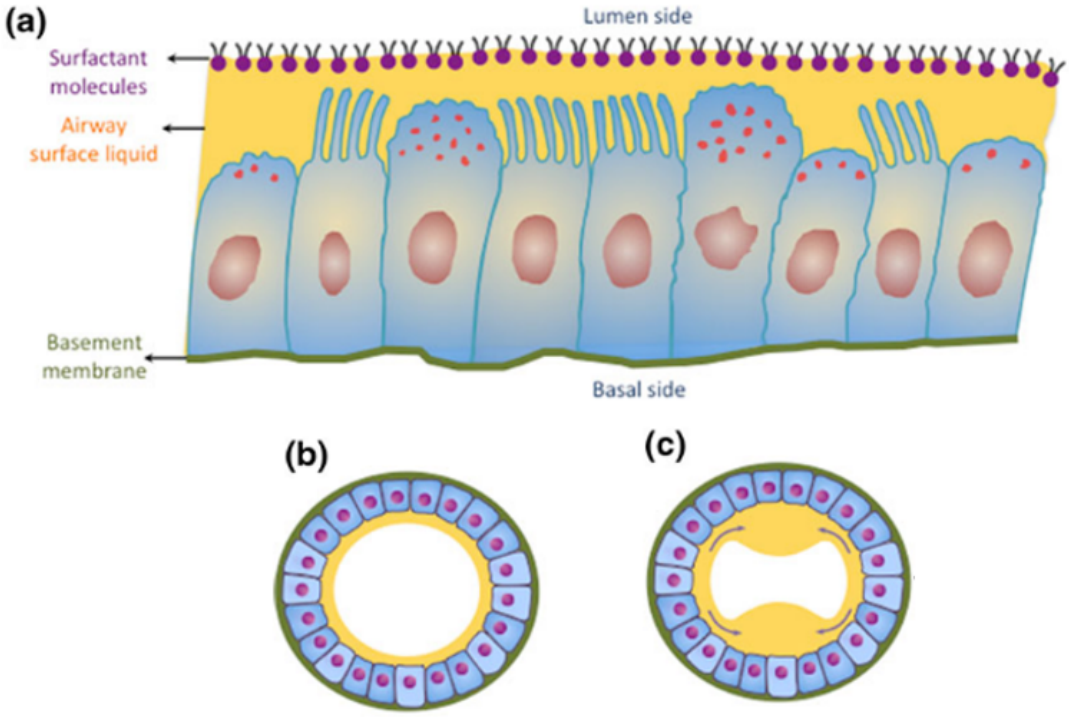

Figure 7.2. Schematic of a-b) normal peripheral airways with airway surface liquid homeostasis and stabilised airway surface liquid, and c) airway closure due to the instability of ASL. Adapted from Tavana et al., (2011).

7.3.2 Plasma osmolality

The findings from this thesis suggest an important regulatory role for P_{osm} in the maintenance of small airway patency. In Chapter 5, following exercise in the heat and a prolonged period of fluid restriction, concomitant to the alterations in pulmonary function (incl. FVC, RV, FRC and RV/TLC), changes in P_{osm} were noted (with an average increase of 7 ± 4 $mOsm \cdot kg^{-1}$). When the impairment to pulmonary function were subsequently reversed (following oral fluid intake, i.e., systemic rehydration), P_{osm} was also restored. On the other hand, the selective rehydration of the airways (achieved via isotonic fluid nebulisation) did not modify P_{osm}, nor pulmonary function. Together with the associations observed between P_{osm} and changes in FVC, RV and FRC (Chapters 5 and 6), the results from this thesis support the idea that changes in P_{osm} may influence airway fluid balance, and thereby, the opening of the small airways.

The proposed process of dehydration-induced pulmonary alteration that involves P_{osm} is presented in Figure 7.3; the figure presents a conceptual model linking systemic water loss to impaired small airway function.

Exercise and prolonged fluid restriction both induce hypertonic-hypovolemia, which is associated with a significant increase in P_{osm} (Cheuvront et al., 2013). In a normal euhydrated state P_{osm} is ~292 mOsm·kg^{-1}. Elevations of P_{osm} above 300 mOsm·kg^{-1} are often used as a diagnostic threshold for dehydration (Cheuvront et al., 2010a). In Chapters 5 and 6, P_{osm} was 300 ± 4 mOsm·kg^{-1} and 304 ± 4 mOsm·kg^{-1} following mild and moderate dehydration, respectively. When P_{osm} increases, it is thought that epithelial cells in the airway detect the change in osmolality (Bartoszewski et al., 2017), which may subsequently alter the water supply to the airway surface. The exact mechanisms that regulate the cellular response to hyperosmolarity in the airways are not currently known. In individuals with EIB, it is thought that dehydration of the airways causes the epithelial cells to shrink, which initiates cellular signalling and activates movement of water from the airway epithelia across an osmotic gradient to restore ASL volume (Hallstrand et al., 2013). In the healthy airway, the exact mechanisms causing the activation of epithelial cells in response to systemic dehydration remains in question, further investigation is required to shed light on the exact role of P_{osm} on airway function.

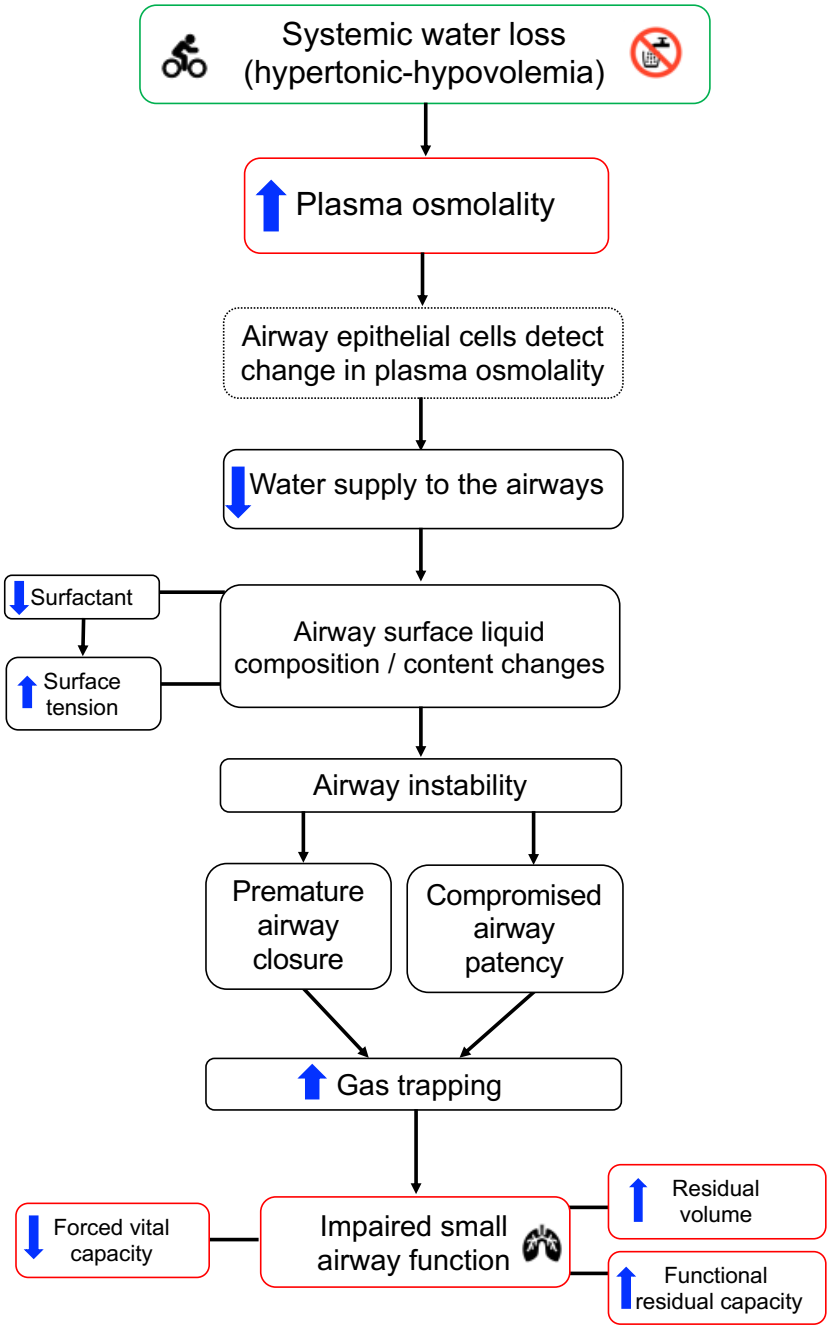

Figure 7.3. Model representation of the proposed mechanism of dehydration-induced pulmonary alteration. The green box represents factors implemented and the red boxes represent factors directly measured within this thesis. The increased plasma osmolality induced by systemic water loss is detected by the epithelial cells. This, in turn, alters water supply to the airways and modifies airway surface liquid composition and content. Changes to airway surface liquid have previously been implicated in airway instability and premature airway collapse. Therefore, small airway function is likely to get compromised, as demonstrated by reductions in FVC and elevations in RV and FRC in this thesis.

7.4 Functional implications for exercise

Once a robust phenomenon of dehydration-induced pulmonary alteration was established at rest, it was of interest to understand the functional implications of the observed dehydration-induced pulmonary alterations. To do so, exercise was used as the main physiological stressor, and comparisons were made between the ventilatory response to exercise in a dehydrated *vs* a euhydrated state. In Chapter 6, neither ventilatory responses, operational lung volumes nor breathing discomfort were significantly affected by moderate dehydration. This therefore suggests that the ventilatory response to exercise is not compromised in a state of dehydration in young healthy physically-active adults. Whilst participants exercised to volitional exhaustion, following the dehydration protocol (i.e., 2 h of cycling in the heat), most of them (80% in the dehydration and 40% in the euhydration trials) terminated exercise prematurely compared to the baseline CPET (prior to completing 2 h exercise). The premature onset of fatigue meant that the absolute 'maximal' intensity was significantly different between the initial CPET and the post-dehydration CPET, and that the ventilatory demand during the post-dehydration CPET was reduced. It therefore cannot be excluded that, in conditions of higher ventilatory demands, the ventilatory response may become compromised in a dehydrated state. Future work should consider implementing an alternative passive dehydration method, such as prolonged fluid restriction (as employed in Chapter 5), to induce dehydration prior to CPET without inducing additional/premature fatigue.

Respiratory symptoms during exercise are commonly experienced by athletes (Boulet, 2012; Kippelen and Anderson, 2012), individuals undertaking endurance exercise (Smoliga et al., 2016) and individuals participating in winter sports [i.e., cross country skiing and snowboarding (Kennedy and Faulhaber, 2018; Lennelöv et al., 2019)]. Winter-based athletes commonly train and compete in cold/dry air conditions. Prolonged exercise in these conditions can lead to local (airway) dehydration, as a result of ventilation at high volumes in cold air (Kippelen et al., 2012). Since it was proposed in Chapter 5 that systemic hydration impacts on airway hydration, it was of interest to investigate whether systemic fluid loss is associated with the onset of respiratory symptoms. In addition, as respiratory symptoms commonly occur concurrently with ventilatory limitation (Eltayara et al., 1996; Jetmalani et al., 2015), it was postulated that systemic dehydration would be associated with both ventilatory limitation and increased respiratory discomfort. However, in Chapter

6, moderate dehydration did not lead to an increase in respiratory symptoms during exercise. This suggests that dehydration is not the primary trigger of these symptoms. Whilst the modified Borg scale used for assessment of breathing discomfort in the present work has previously been validated (Kendrick et al., 2000; Wilson and Jones, 1989), it may be of interest for future work to implement a more comprehensive analysis of respiratory symptoms [such as a multidimensional profile for assessment of breathing discomfort (Banzett et al., 2015)]. The use of a more comprehensive tool may help further our understanding of respiratory symptoms during exercise-induced dehydration.

7.5 Methodological considerations

7.5.1 Assessment of small airway function

Within this thesis, assessment of pulmonary function, and specifically of small airway function, was conducted via a combination of techniques. Combining multiple tests allowed for a more comprehensive analysis of airway function and enabled the assessment of deeper zones of the airways compared to the use of spirometry alone. The pulmonary function tests used in these experiments include spirometry, whole body plethysmography, and IOS. All of these tests are highly standardised and have previously been deemed suitable and implemented for the assessment of small airway function (Anderson et al., 2012; Gibbons et al., 1996; Jain et al., 2013; Sorkness et al., 2008). It is important, however, to acknowledge limitations of these tests: firstly, spirometry manoeuvres are effort dependent, and, whilst the confounding effects of potential variability can be excluded (Chapter 4), it is possible that fatigue induced by exercise could have impaired the performance of the maximal manoeuvre. Indeed, evidence of respiratory muscle fatigue has been reported previously during high-intensity sustained exercise in both trained and untrained individuals (Aliverti, 2016; Johnson et al., 1993; Mador et al., 1993). In the present work, similarity in PEF (an effort dependent variable) between tests provides confidence that participants were able to reproduce the maximal effort of the spirometry manoeuvre, but this is an important factor to consider when designing future work. Secondly, whilst IOS provides a non-invasive measure of pulmonary resistance and reactance, this method has been less commonly implemented within experimental research, and thus direct comparisons between other studies are difficult. Further, the application of this technique to future work is limited by the requirement of specialist equipment that is less widely available than spirometry or whole body plethysmography. An alternative functional test

that could have been used as part of this thesis alveolar nitric oxide, which has been shown to be closely related to parameters of peripheral airway dysfunction, particularly in patients with asthma (van Veen et al., 2006). However, this method was not available for inclusion within the present work and would not have provided any additional/alternative insight compared to the findings reported from the PFTs implemented.

Imaging techniques are a useful tool for the analysis of small airway function, airway obstruction and gas trapping (King, 2012). Specifically, high resolution computed tomography can quantify changes at the level of the small airways (Hansell, 2001; King et al., 1999; Okazawa et al., 1996). Whilst this tool is capable of determining gas trapping and identifying small airway obstruction, it is extremely expensive, highly specialised, and carries the risk of radiation exposure (McNulty and Usmani, 2014). Taken together these issues mean repeated imaging for assessment was not a viable option within this thesis. Future work should consider how the use of imaging techniques alongside functional tests may be best used to evaluate the extent of dehydration-induce small airway alterations

7.5.2 Exercise protocol

In Chapter 6 the impact of dehydration-induced pulmonary alteration on ventilatory responses to maximal incremental exercise was examined. Whilst the protocol and magnitude of fluid loss was closely controlled between participants, it should be acknowledged that the premature termination of exercise following 2 hours of exercise in the heat was a limitation within this study. Whilst participants exercised to volitional exhaustion in all CPET (see Section 3.5.4 for criteria), 80% terminated exercise earlier in the CPET performed after 2 hours of exercise in the heat (CPET-2) compared to the initial CPET (CPET-1) (see Section 6.4.5). As a result of this earlier onset of fatigue, assessment of the maximal ventilatory response to exercise in a dehydrated state was not possible. This premature termination of exercise may have been associated with dehydration-induced changes to cardiovascular function – such as impaired cardiac output (González-Alonso et al., 1997) - and/or reduced muscle blood flow. Changes to cardiovascular function can lead to localised muscle fatigue (González-Alonso et al., 1998). In the present work, this could have prevented participants from reaching the same intensity as the baseline CPET. It would be of interest to revisit this research question using: *i)* a protocol with increments of specific percentages of WR_{max} [i.e., at 0, 20, 40, 60, 80 and 100% WR_{max}, as employed by Trangmar *et al.*, (2014)], which would allow for direct comparisons to be made at matched

intensities, or *ii)* using a protocol which can stress the pulmonary system to its limit whilst removing the confounding effect of peripheral muscle fatigue induced via exercise. For example, respiratory muscle endurance testing (i.e., a voluntary hyperventilation test) using the maximal incremental load testing method requires a stepwise protocol of increasing $\dot{V}_E$ (+8% maximal voluntary ventilation every 3 min) until task failure (ATS/ERS, 2002; Laveneziana *et al.*, 2019) and is able to induce maximal ventilatory stress upon a participant without the need for exercise.

7.6 Directions for future research

The novel research conducted within this thesis has generated additional questions and opens new avenues for future investigation.

In this thesis, the population studied in all experiments comprised of healthy young adults. This population was specifically selected to explore the impact of dehydration upon the healthy human lung. The findings reported in Chapters 5 and 6 have reproducibly established that dehydration can negatively impact pulmonary function of healthy young trained and untrained (i.e., normally active) adults. Since this phenomenon has been observed in healthy young adults (Chapters 5 and 6), and in individuals with asthma (Simpson et al., 2017), it would next be of interest to examine how this phenomenon may impact older adults, with or without compromised pulmonary function. Older adults are more vulnerable to dehydration due to age-related changes in fluid balance and perception of thirst (Kenney and Chiu, 2001), and dehydration has been associated with hospitalisation and mortality rates in this population (El-Sharkawy et al., 2017). Older adults experience compromised pulmonary function, including respiratory muscle atrophy and reduced force production, which increases the risk of ventilatory limitation during physical activity (Roman et al., 2016; Sharma and Goodwin, 2006). However, it remains unknown whether fluid loss could contribute further to pulmonary function impairment in older adults. Research is therefore required to expand our understanding of the phenomenon of dehydration-induced pulmonary alteration in older age. Furthermore, whilst the healthy population examined within this research did not display dynamic hyperinflation, nor respiratory symptoms when undertaking exercise in a dehydrated state, individuals with COPD often experience dynamic hyperinflation during periods of exacerbations (O'Donnell and Laveneziana, 2006) and when performing simple everyday tasks, such as

walking up the stairs (Castro et al., 2012; Silva et al., 2015). It is possible that dynamic hyperinflation in those populations could be exaggerated in a state of dehydration, since Pogson, McKeever and Fogarty, (2008) previously demonstrated an association between elevated P_{osm} and impaired pulmonary function (reduced FEV_1 and FVC) in COPD patients. Whether dehydration has negative functional implications, such as exacerbating dynamic hyperinflation, ventilatory limitation and/or respiratory symptoms during activities of daily living or exercise in COPD patients warrants further investigation.

Further, research presented within this thesis has suggested that the severity of dehydration is associated with the magnitude of pulmonary function impairment. It would be of interest for future research to investigate whether a direct relationship exists between magnitude of dehydration and magnitude of changes in pulmonary function. Performing PFT at regular progressive dehydration severities (i.e., from euhydration to mild, moderate then severe dehydration) could provide a greater understanding of the pulmonary response to body fluid loss and would enable the determination of the thresholds at which dehydration-induced pulmonary alteration occurs.

Understanding the functional implications of dehydration-induced alterations to small airway function is now warranted. In particular, future work should consider whether alterations to small airway function in a dehydrated state impacts significantly upon pulmonary gas exchange, and thereby possibly compromises blood gases homeostasis during exercise.

Within the present research, only two modes of dehydration (i.e., exercise in the heat and fluid restriction) were used, with both causing hypertonic-hypovolemia. Whilst hypertonic-hypovolemia is a form of dehydration commonly experienced by athletes (Casa et al., 2010; Kenefick and Sawka, 2007; Sawka et al., 2007, 2015), by individuals working in hot conditions [i.e., the military and firefighters; (Carter et al., 2005; Lucas et al., 2014)], and by the elderly (Allison and Lobo, 2004; Bennett et al., 2004), it is important to recognise that this is not the only type of dehydration. With diuretic intake (Caldwell et al., 1984; Watson et al., 2005), or secretory diarrhoea and vomiting (El-Sharkawy et al., 2015), individuals can experience isotonic-hypovolemia (also known as 'salt-depletion dehydration'). Since isotonic-hypovolemia leads to greater reductions in plasma volume but little change to P_{osm} (Cheuvront et al., 2013), it would be of interest to directly compare

the effects of hypertonic and isotonic-hypovolemia on pulmonary function. As mentioned before, such comparison would help to elucidate the role of P_{osm} on pulmonary function in healthy and clinical populations, and could offer further mechanistic insights in the pulmonary changes reported in this thesis.

7.7 Conclusion

The findings from this thesis provide novel information regarding the role of systemic hydration on normal pulmonary function and ventilatory response to exercise. In particular, the experiments performed within this thesis have demonstrated that: exercise in the heat and a prolonged period of fluid restriction cause negative alterations in resting pulmonary function in healthy young adults (with alterations primarily localised at the level of the small airways); oral fluid intake, but not local airway rehydration, is able to rapidly restore dehydration-induced pulmonary alterations. Resting pulmonary function impairments induced by dehydration do not compromise the ventilatory response to exercise, nor exacerbate respiratory discomfort in healthy active young adults. Since oral but not local rehydration also restored P_{osm} to baseline levels, the results from this thesis suggest that composition of fluid at the level of the airways is an important modulator of the observed response, perhaps associated with the movement of water across an osmotic gradient to restore ASL volume/composition.

Overall, the experiments conducted within this thesis have furthered our understanding of the effects and implications of dehydration upon pulmonary function and have provided some insight into the potential underlying mechanisms of dehydration-induced alterations in pulmonary function. It is now of particular interest to investigate whether the pulmonary changes observed in the population studied in the present work (i.e., young healthy adults) are exaggerated in older adults and/or in those presenting an already compromised pulmonary function at rest. Understanding the role of systemic dehydration could pave the way for the development of hydration strategies that may prevent further illness in older individuals and contribute to the management of respiratory diseases in clinical populations.

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Chapter 9 Appendices

9.1 Appendix A

Ethical approval for Studies 1 and 2:

University Research Ethics Committee
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

11 September 2018

LETTER OF APPROVAL

Applicant: Miss Hannah Marshall

Project Title: Effects of dehydration on lung function

Reference: 6639-A-Sep/2018- 14084-1

Dear Miss Hannah Marshall,

The Research Ethics Committee has considered the above amendment application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed amendment. Approval is given on the understanding that the conditions of approval set out below are followed:

- Please add a statement to the additional paragraph in the PIS to clarify that the optional visits are entirely voluntary.
- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Kind regards,

Professor Peter Hobson

Chair, University Research Ethics Committee

Brunel University London

Ethical approval for Study 3:

University Research Ethics Committee
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

3 July 2018

LETTER OF CONDITIONAL APPROVAL

Applicant: Miss Hannah Marshall

Project Title: Effects of moderate dehydration on the lungs

Reference: 11730-TISS-Jun/2018- 13047-1

Dear Miss Hannah Marshall,

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- On your PIS, please change details of the REC to the University Research Ethics Committee (and complaints to the UREC Chair).
- Please add a statement to the Consent Form to the effect that withdrawal from the study will not result in any disadvantage or penalty.
- Your Participant RA currently mentions the use of Virkon in relation to disinfecting equipment; please amend to a more suitable solution in line with your Health and Safety RA. This procedure should include immersion in Cidex OPA for 45 minutes at room temperature, rinse with 70% isopropanol and drying.
- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Kind regards,

Professor Peter Hobson

Chair, University Research Ethics Committee

Brunel University London

9.2 Appendix B

Health Questionnaire

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. The questions below are designed to identify whether you are able to participate now or should obtain medical advice before undertaking this investigation, Whilst every care will be given to the best of the investigators ability, an individual must know his/her limitations.

Subject name: ..... DOB: ..... AGE.....

Emergency Contact Name & Number: .....

	YES	NO
1. Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations or tightness of the chest?	☐	☐
3. Do you have known high blood pressure? If yes, please give details (i.e. medication)	☐	☐
4. Do you have low blood pressure or often feel faint or have dizzy spells?	☐	☐
5. Do you have known hypercholesteremia?	☐	☐
6. Have you ever had any bone or joint problems, which could be aggravated by physical activity?	☐	☐
7. Do you suffer from diabetes? If yes, are you insulin dependent?	☐	☐
8. Do you have any lung/chest disease (Incl. asthma, bronchitis, emphysema)?	☐	☐
9. Do you suffer from epilepsy? If yes, when was the last incident?	☐	☐
10. Do you have any kidney disease?	☐	☐
11. Are you taking any medication? (If yes, please specify).	☐	☐
12. Have you had any injuries in the last year? E.g. Muscle strains, back problems	☐	☐
13. Do you have any history of heat illness/ intolerance?	☐	☐
14. Are you currently enrolled in any other studies?	☐	☐
15. Do you have any history of infectious diseases (e.g. HIV, Heb B)?	☐	☐
16. **I have recently participated in a blood donation program**	☐	☐
17. Are you a smoker?	☐	☐
18. Do you exercise on a regular basis (at least 3 times a week)?	☐	☐

19. Describe your exercise routines (mode, frequency, intensity/speed, race times):

20. Provide detail of any other reasons that would affect your ability to take part in this experiment:

If you feel at all unwell because of a temporary illness such as a cold or fever please inform the investigator. Please note if your health status changes so that you would subsequently answer YES to any of the above questions, please notify the investigator immediately.

I have read and fully understand this questionnaire. I confirm that to the best of my knowledge; the answers are correct and accurate. I know of no reasons why I should not participate in physical activity and this investigation and I understand I will be taking part at my own risk.

PARTICIPANT'S NAME AND SIGNATURE: _____ DATE: _____

INVESTIGATOR'S NAME AND SIGNATURE _____ DATE:_____

9.3 Appendix C

Informed consent: Studies 1 and 2

College of Health and Life Sciences
Department of Life Sciences

CONSENT FORM

Effect of dehydration and rehydration on lung function in healthy young adults

The participant should complete the whole of this sheet

	Please tick the appropriate box	
	YES	NO
Have you read the Research Participant Information Sheet?		
Have you had an opportunity to ask questions and discuss this study?		
Have you received satisfactory answers to all your questions?		
Who have you spoken to?		
Do you understand that you will not be referred to by name in any report concerning the study?		
Do you understand that you are free to withdraw from the study:		
• at any time?		
• without having to give a reason for withdrawing?		
Do you agree to take part in this study?		
Name of participant:		
Signature of Research Participant:		
Date:		

Researcher name:	Signature:
Supervisor name:	Signature:

Informed consent: Study 3

College of Health and Life Sciences
Department of Life Sciences

CONSENT FORM

Effect of moderate dehydration on respiratory responses at rest and during exercise

The participant should complete the whole of this sheet

	Please tick the appropriate box	
	YES	NO
Have you read the Research Participant Information Sheet?		
Have you had an opportunity to ask questions and discuss this study?		
Have you received satisfactory answers to all your questions?		
Who have you spoken to?		
Do you understand that you will not be referred to by name in any report concerning the study?		
Do you understand that you are free to withdraw from the study:		
• at any time?		
• without having to give a reason for withdrawing?		
Do you agree to take part in this study?		
Name of participant:		
Signature of Research Participant:		
Date:		

Researcher name:	Signature:
Supervisor name:	Signature:

9.4 Appendix D

Breathing discomfort scale (Mahler and Horowitz, 1994):

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (almost max)

9.5 Appendix E

RPE Scale (Borg, 1982):

6	
7	Very, very light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

9.6 Appendix F

Table 9.1. Detailed statistical output from the 3-way repeated measure ANOVA performed in Chapter 5 for hydration parameters, spirometry, and whole body plethysmography.

Variable	Main effect of Dehydration method		Main effect of Rehydration Method		Main effect of Time		Interaction: Dehydration method x time		Interaction: Rehydration method x time		Interaction: Dehydration method x Rehydration method x time	
	F value	***P value***	***F value***	***P value***	***F value***	***P Value***	***F value***	***P value***	***F value***	***P value***	***F value***	***P Value***
Hydration parameters												
Body mass (kg)	1.437	0.261	2.445	0.152	183.181	0.000	0.705	0.507	126.616	0.000	0.202	0.819
P_{osm} ($mOsm \cdot kg^{-1}$)	0.005	0.943	9.721	0.012	55.316	0.000	0.592	0.564	52.758	0.000	0.292	0.750
U_{osm} ($mOsm \cdot kg^{-1}$)	25.030	0.001	11.990	0.007	124.481	0.000	23.894	0.000	12.744	0.000	3.847	0.041
Haemoglobin ($g \cdot L^{-1}$)	0.060	0.811	0.655	0.439	3.262	0.062	1.596	0.230	4.652	0.024	0.203	0.818
Haematocrit (%)	0.014	0.907	5.717	0.040	0.233	0.794	0.078	0.925	3.725	0.044	0.274	0.764
Plasma Volume (%)	1.050	0.332	3.866	0.081	10.566	0.001	4.414	0.028	2.708	0.094	2.373	0.122
Spirometry												
FEV_1 (L)	1.754	0.218	0.814	0.390	0.776	0.548	0.637	0.640	0.258	0.903	1.306	0.286
FVC (L)	8.347	0.018	0.17	0.69	17.519	0.000	2.329	0.142	4.537	0.005	1.713	0.168

FEV_1/FVC (%)	49.772	0.000	1.465	0.257	22.584	0.000	2.964	0.032	6.687	0.000	2.457	0.063
PEF ($L \cdot s^{-1}$)	4.227	0.070	0.038	0.849	2.325	0.075	2.436	0.065	1.253	0.306	1.000	0.420
Whole body plethysmography												
TLC (L)	1.051	0.332	1.524	0.248	3.246	0.063	0.288	0.753	0.230	0.797	0.443	0.649
FRC (L)	5.880	0.038	1.798	0.213	12.237	0.000	0.197	0.823	4.413	0.028	1.797	0.194
RV (L)	8.233	0.018	11.055	0.008	25.388	0.000	2.308	0.128	4.463	0.056	0.207	0.815
RV/TLC (%)	6.551	0.028	8.311	0.015	28.436	0.000	4.437	0.023	4.059	0.064	0.762	0.461
ERV (L)	0.383	0.552	0.976	0.349	5.329	0.015	4.059	0.035	0.942	0.408	1.840	0.187
sRaw ($kPa \cdot s^{-1}$)	0.044	0.839	0.879	0.373	1.037	0.375	1.149	0.339	0.908	0.421	0.024	0.976

ANOVA factors: Dehydration method: exercise vs fluid restriction, rehydration method: systemic vs local rehydration, time: baseline, dehydration, rehydration (15 min, 35 min, 60 min). P_{osm}, plasma osmolality; U_{osm}, urine osmolality; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; PEF, peak expiratory flow, TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; ERV, expiratory reserve volume; IC, inspiratory capacity; IVC, inspiratory vital capacity; sRaw, specific airway resistance.

9.7 Appendix G

Table 9.2 Detailed statistical output from the 3-way repeated measure ANOVA performed in Chapter 6 for ventilatory, physiological, and perceptual responses to CPET in the dehydration and euhydration conditions.

Variable	Main effect of Condition		Main effect of Test		Main effect of Time		Interaction: Condition x test		Interaction: Condition x time		Interaction: Test x Time		Interaction: Condition x Test x Time	
	F value	***P value***	***F value***	***P value***	***F value***	***P Value***	***F value***	***P value***	***F value***	***P value***	***F value***	***P Value***	***F value***	***P Value***
Ventilatory and physiological responses														
$\dot{V}_E$ **(L)**	2.556	0.154	2.332	0.171	78.244	0.000	3.694	0.096	3.282	0.025	8.952	0.000	4.977	0.004
V_T **(L·min^{-1})**	0.079	0.785	11.436	0.010	21.952	0.000	2.006	0.194	0.854	0.386	14.025	0.014	1.508	0.708
f_b **(breaths·min^{-1})**	0.675	0.435	28.662	0.001	42.86	0.000	3.252	0.109	0.975	0.435	2.778	0.043	0.915	0.467
T_i **(s)**	0.243	0.635	14.298	0.005	20.629	0.000	0.248	0.632	0.727	0.580	0.879	0.448	0.696	0.600
T_{tot} **(s)**	0.145	0.713	13.172	0.007	40.502	0.000	1.498	0.256	0.64	0.638	3.457	0.019	0.631	0.644
T_i/T_{tot}	0.781	0.403	0.520	0.491	11.493	0.000	2.290	0.169	0.760	0.735	0.092	0.983	0.393	0.968
$\dot{V}O_2$ **(L·min^{-1})**	1.484	0.254	11.397	0.008	322.011	0.000	1.798	0.213	3.354	0.020	0.648	0.632	1.009	0.415
$\dot{V}CO_2$ **(L·min^{-1})**	0.297	0.988	5.773	0.040	143.076	0.000	1.904	0.201	0.517	0.724	5.637	0.001	2.282	0.079
$\dot{V}_E/\dot{V}O_2$	0.066	0.805	2.524	0.156	30.642	0.000	0.173	0.690	11.813	0.013	6.574	0.013	18.501	0.400
$\dot{V}_E/\dot{V}CO_2$	0.360	0.567	8.602	0.022	6.541	0.001	0.002	0.967	3.367	0.017	3.526	0.019	1.583	0.206
HR	0.005	0.984	43.897	0.000	158.981	0.000	0.201	0.668	1.687	0.181	9.819	0.000	1.356	0.274

(beats·min^{-1})														
T_{core} (°C)	0.893	0.360	36.599	0.001	12.219	0.000	0.064	0.808	3.055	0.036	2.735	0.052	0.42	0.793
SpO_2 (%)	0.336	0.578	4.630	0.064	5.226	0.002	0.005	0.947	0.276	0.892	2.423	0.068	1.15	0.351
Perceptual responses														
RPE	4.578	0.070	17.113	0.004	166.622	0.000	9.677	0.017	2.339	0.080	13.719	0.000	6.042	0.001
Breathing discomfort	2.355	0.169	11.397	0.012	16.556	0.000	3.569	0.101	0.903	0.476	6.559	0.001	2.378	0.076

ANOVA Factors: Condition: euhydration *vs* dehydration; test: CPET-1 *vs* CPET-2, time: 0 , 2 , 4 , 6 min, and peak exercise. $\dot{V}_{E,}$ minute ventilation; $V_{T,}$ tidal volume; $f_{b,}$ breathing frequency; $\dot{V}O_2$, volume of oxygen uptake; $\dot{V}CO_{2,}$ volume of expired carbon dioxide; T_i, inspiratory time; T_{tot}, total time of breath; T_i/T_{tot}, inspiratory duty cycle; HR, heart rate; T_{core}, core temperature; SpO_2, oxygen saturation, RPE, rating of perceived exertion.